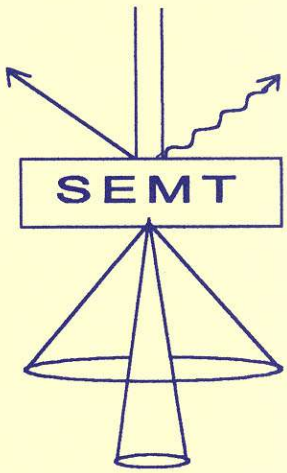
SEMT

ONE DAY MEETING

Wednesday 19 March 1997

Royal Veterinary College
Royal College Street
London NW1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should obtain an application form from
Jill Lewis (Hon Secretary), 19 Bellfield Avenue, Harrow Weald, Middx, HA3 6ST .
The annual subscription is £10.
Current committee members are listed below and are available for further information.

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ACKNOWLEDGMENTS

The SEMT wishes to express special thanks to :-

The Royal Veterinary College, London, as hosts
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The next SEMT meeting will be

RMS Beginners' Meeting - Wed 11 June, 2 p.m. Eastman Dental Institute
Prospective competitors should apply to the SEMT Secretary for an Abstract Form

Our Annual Excursion is to Science Pictures, Hitchin, on Wednesday, 7th May.

WELCOME TO THE ONE DAY MEETING

PROGRAMME

9.30 **Registration**
Coffee, Trade Exhibition

10.10 **Introduction**
Heather Davies (Chairman, SEMT)

10.15 **The human placenta as a barrier to materno-foetal infection**
Graham Burton (Dept of Anatomy, University of Cambridge)

11.00 **Cryo-preparation methods for immunogold labelling**
Wim Voorhout (Faculty of Veterinary Medicine, University of Utrecht)

11.45 **Visualising exocytosis to determine what is released, and where, when and why**
John Morris (Dept of Anatomy, University of Oxford)

12.30 **LUNCH**
Trade Exhibition

2.30 **Perspectives on metal pollution and inflammatory diseases from XRMA and immunocytochemical observations**
John Morgan (Dept of Pure & Applied Biol., Univ of Wales, Cardiff)

3.15 **Boxgrove man in close-up; new results from 1995-97 excavations**
Simon Parfitt (Natural History Museum, London)

4.00 **TEA.**

Abstracts of papers

The human placenta as a barrier to materno-foetal infection

Graham Burton (Dept of Anatomy, University of Cambridge)

The human placenta consists of a delicate array of villi which are bathed directly by the maternal blood. At the end of pregnancy a surface area of 10 - 14 m^2 is presented for exchange purposes. Whilst this extensive and intimate contact facilitates diffusive transfer of gases and metabolites, it also poses a risk for the transmission of infectious agents from mother to foetus. The outer covering of the villi, the syncytiotrophoblast, is a multinucleated syncytium and presents the first line of defence. We have investigated physical defects in this layer; their origin, their subsequent evolution and their significance in the vertical transmission of HIV. In all placentas examined defects were observed down to the level of the trophoblastic basement membrane. At these sites platelet activation leads to the deposition of a fibrin clot. Syncytiotrophoblast appears to migrate over the surface of this matrix, eventually re-establishing the epithelial cover. Despite these defects no transmission occurred in our study group. Therefore, the physical integrity of the trophoblast is not of paramount importance, and other factors, such as the underlying macrophage population may play a key role in limiting transmission. Hence the placenta should be viewed as an active rather than a passive barrier to infection.

Cryo-preparation methods for immunogold labelling

Wim Voorhout (Faculty of Veterinary Medicine, University of Utrecht)

The ideal preparation technique for immunogold labelling should fulfil several criteria: maintenance of antigenicity, immobilisation of antigens, preservation of overall structure and accessibility of antigens. For 'classical' embedded material (e.g. osmium fixed and epoxyresin embedded) preservation of antigenicity and accessibility have always been the biggest problems. Nowadays these deteriorating effects can be largely overcome by the application of cryopreparation methods such as cryoultramicrotomy or freeze-substitution. Due to the introduction of immunogold labelling on ultrathin cryoesections biological EM has received a tremendous impulse in the early eighties. Since that time many proteins have been localised with high resolution in various subcellular compartments. Especially for membrane proteins and structural proteins the combination of ultrathin cryosections and immunogold labelling has resulted in good structural preservation combined with accurate localisation. The success of this method is mainly due to the mild chemical fixation and the fact that no dehydration is involved.

However, several tissues like brain, lung, and plants do not give optimal results after cryosectioning due to bad structural preservation or difficulties with sectioning. Another important factor is that hydrophobic molecules such as lipids and glycolipids are either lost from ultrathin cryosections or are relocated. Freeze-substitution in combination with low temperature embedding has been shown to be a good alternative for the localisation of many molecules. Cells or tissues can be prefixed and cryoprotected before freezing or cryofixed by fast freezing methods. Afterwards the material is dehydrated at low temperature (-90°C) and embedded at low temperature in Lowicryl resin. Freeze-substitution in combination with Lowicryl HM20 embedding at low temperature is a powerful method for both ultrastructural preservation and for immunogold localisation of lipids and glycolipids, which are easily lost or relocated in ultrathin cryosections.

Visualising exocytosis to determine what is released, and where, when and why

John Morris (Dept of Anatomy, University of Oxford)

Peptides are widespread signal molecules produced by many types of cell including neurons and endocrine cells. Those that can be rapidly released from cells are released by exocytosis. However, because of the speed of exocytosis, this process has been difficult to visualise and therefore to localise with conventional microscopy. This has been a particular problem within the nervous system where it has been largely assumed that the peptides must be released from the axon terminals. However, in many cases there is a mismatch between the known distribution of receptors for the peptides and the distribution of the peptides in axonal terminals. A method of arresting and thereby visualising the exocytosis of the peptide was therefore needed. Rapid freezing can achieve this but only for very thin structures and is therefore not applicable to the brain.

Tannic acid, a polymer of gallic acid, has been widely used in fixative solutions for some time to enhance membranes. Tannic acid in secretagogue-containing physiological solutions does not cross plasma membranes or significantly interfere with the functioning of endocrine cells or neurons, but it does interact with the peptides in the exocytosing vesicles to chemically 'freeze' the exocytosing vesicle core, which can then be fixed by standard procedures. One particular advantage of tannic acid is that the immunoreactivity of the peptides is well preserved so that peptide can be immunoidentified and the precise site of the peptide release identified.

We have used this technique on both endocrine cells and neurosecretory neurons and in both cases have been surprised by the results. In the neurons, which are generally thought to be highly polarised in their release, we have located the exocytosis of peptides not only from the axon terminals, but also at sites far distant from the terminals, including undilated parts of the axons. Moreover we have identified the dendrites of neurosecretory neurons as an important site of peptide release in the hypothalamus, and can correlate this release with well-understood physiological mechanisms. By contrast, experiments on pituitary endocrine cells, in which we had expected exocytosis to occur at all parts of the membrane, revealed that exocytosis of the secretory granules is very largely polarised to the vascular border of the cells. In both systems, there was considerable heterogeneity among apparently similar cells in the extent of exocytosis in response to a stimulus.

Perspectives on metal pollution and inflammatory diseases from XRMA and immunocytochemical observations

John Morgan (Dept of Pure & Applied Biol., Univ of Wales, Cardiff)

The technique of electron probe X-ray microanalysis (EPXMA), including state-of-the-art quantitative mapping protocols, has a number of intrinsic advantages and disadvantages. Some of these issues will be discussed by reference to a case-history of essential and non-essential metal accumulation in earthworms inhabiting polluted soils.

Biochemical studies have revealed that Cd is sequestered in earthworm tissues by an inducible, highly-conserved, cysteine-rich protein belonging to the family of low molecular weight metalloproteins called metallothioneins (MTs). I shall demonstrate how these proteins have been localized by immunohistochemical (light microscope) and immuno-cytochemical (electron microscope) techniques in a number of metal-stressed and diseased vertebrate tissues, indicating that they are multifunctional. MTs probably play fundamental roles that are only sparsely understood in: (a) the homeostasis and sub-cellular distribution of essential metals such as Zn and Cu; the detoxification of non-essential metals such as Cd and Hg; (c) anti-inflammatory cytoprotective responses by scavenging highly-reactive and transient oxyradicals.

The contribution will end somewhat speculatively with a reflection on how the rather contradictory preparative demands of EPXMA and immunocytochemistry may be reconciled by using hyperbaric freezing as a launch pad.

Boxgrove man in close-up; new results from 1995-97 excavations

Simon Parfitt (Natural History Museum, London)

The Lower Palaeolithic site at Boxgrove, West Sussex has produced spectacular evidence for the earliest human occupation of the British Isles. Archaeological excavations at the site, undertaken by University College, London, and funded by English Heritage over a period of ten years, culminated in 1993 with the discovery of a human tibia. This find represents the oldest human fossil from the British Isles and is probably broadly contemporary with the lower jaw from Mauer, near Heidelberg in Germany, with an estimated age of about 500,000 years. In 1995 and 1996 renewed excavations at the site led to the recovery of a second hominid represented by two lower incisors. The Boxgrove site is situated in a quarry at the foot of the southern dipslope of the South Downs about 5 km East of Chichester and approximately 10 km North of the present day coastline of the English Channel. The geological deposits exposed in the quarry were formed during a warm interval (interglacial) when a rising sea level cut a cliff and deposited a beach and marine sands. Towards the top of the marine deposits there is evidence for falling sea level which led to the formation of extensive mudflats and a grassy plain and spring-fed ponds. These deposits are overlain by gravels deposited during an extremely severe cold period. Evidence for human activity is found throughout the geological sequence, and the preservation is such that short episodes of activity, including tool manufacture and butchery of large mammals are preserved in their entirety. Due to exceptional preservation, Boxgrove provides some of the best evidence we have for the behaviour of the earliest Europeans.

In this talk I will discuss the results of the archaeological work at Boxgrove, and outline the scientific analysis of the artefacts, human fossils and other biological evidence. Scanning EM using an ISIABT 55 (fitted with a variable pressure chamber) at the Natural History Museum has allowed the examination of large and fragile objects, such as stone tools, bones and the hominid fragments, without the necessity for coating the specimens. The development of the variable pressure chamber has transformed the analysis of archaeological and palaeontological specimens, and the application of the SEM to the Boxgrove site will be discussed.