SEMT

Society of Electron Microscope Technology

ONE DAY MEETING

Wednesday 22nd May 2002

School of Pharmacy
Brunswick Square
London WC1N 1AX

The Society of Electron Microscope Technology

Prospective members should obtain an application form from the Hon. Secretary
Mr. David McCarthy, School of Pharmacy, Brunswick Sq. London WC1N 1AX
Tel: 44 (0) 20 7753 5806; Email: David.McCarthy@cua.ulsop.ac.uk (The annual subscription is £10.00.)
Our web site is: www.semt.org.uk
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Acknowledgments:
The School of Pharmacy as host.
Jeol (UK) Ltd. for contributing Wine prior to conference dinner.
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Future Programme:
Visit : Institute of Oceanography. September 18th 2002
Half-Day Meeting : November 6th 2002.

The Society of Electron Microscope Technology

Programme

9.15 - 9.35	**Registration,** Tea/Coffee. Trade Exhibition.
9.35 - 9.40	**Introduction:** Chair, Heather Davies.
9.40 - 10.15	**Pre-embedding Immunolabelling Revisited.** David Robertson. Institute of Cancer Research, Sutton, Surrey.
10.15 - 10.50	**An Ultrastuctural and Immunocytochemical study of the Monocyte-Endothelial Cell Interaction on the Ex-Vivo Model of Hamster Atherosclerosis.** Elena Constantinescu. Institute of Cell Biology & Pathology, Bucharest.
10.50 - 11.20	**Coffee.**
11.20 - 11.55	**Chemosymbiosis in Marine Bivalve Molluscs.** John Taylor. Zoology Dept. The Natural History Museum, London.
11.55 - 12.30	**Morphological Basis of Neural and Synaptic Plasticity Mammalian CNS.** Prof. Mike Stewart. Head of Dept. of Biological Sciences, Open University, Milton Keynes.
12.30 - 14.20	**LUNCH**. Trade Exhibition
14.20 - 14.55	**Who Needs E.M. Anyway ?** Barrie Hartley. Histopathology Dept. St. Jamesí University Hospital, Leeds.
14.55 - 15.30	**Backscattered Electrons in S.E.M.** Iolo ap Gwynn. Institute of Biological Sciences, The University of Wales, Aberystwyth.
15.30 - 15.50	**Tea.**
15.50 - 16.25	**Microscopy of Apoptosis.** Jeremy Skepper. Multi-imaging Centre, University of Cambridge.
16.25 - 17.00	**Applications of E.M. in Forensic Science.** Robin Keeley. Forensic Science, EM division. Metropolitan Police, London.
17.00 - 19.00	**Close, followed by wine reception.**
19.00 - 22.00	**Conference Dinner**

Abstracts

Pre-embedding Immunolababelling Revisited

David Robinson. Inst.of Cancer Research, Sutton, Surrey.

Preparing cultured Cells for EM immunogold experiments is a time consuming and technically involved process usually requiring specialist pieces of equipment. In this presentation some experiments towards a pre-embedding immunogold labelling method are described.

AN ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDY OF THE THE MONOCYTE-ENDOTHELIAL CELL INTERACTION ON THE EX-VIVO MODEL OF THE HAMSTER ATHEROGENESIS

E. Constantinescu, D. Alexandru, V. Alexandru

We imagined an ex vivo atherogenic model consisting in hypercholesterolemic versus normocholesterolemic aortic arch rings or sigmoid valves incubated in cell culture conditions with human monocytes. Normal tissues did not show any attached monocytes. On the atherogenic aortic arch three different aspects were observed: a) there were no monocytes attached on a normal zone; b) many monocytes adhered to the endothelium on a thickened area, with intimal smooth muscle cells infiltration, and c) the greatest number of attached monocytes was seen in mechanically injured zones of the aortic arch, where the subendothelial area was totally exposed. Immunohisto- and immunocytochemistry for LFA-1 revealed the presence of this leukocyte integrin on monocyte plasma membrane. The labeled monocyte had an activated shape, with pseudopodes extended over the endothelial cells and the anti-LFA-1 antibody coupled with colloidal gold decorating areas apposing to a morphologically modified endothelium. In conclusion, the ex vivo model reproduced the in vivo situations where the monocytes adhere to the modified endothelium covering the thickened areas of hypercholesterolemic aortic wall; they express at least one of the adhesion integrins, namely LFA-1. This study intended to contribute, at least in part, to the understanding of some mechanisms governing the monocyte-endothelial cell interactions in hypercholesterolemia.

Chemosymbiosis in marine bivalve molluscs

John Taylor Department of Zoology, The Natural History Museum, London SW7 5BD

The discovery in the early 1980's of communities of animals associated with deep-sea hydrothermal vents caused a biological sensation when it was realised that these communities were based on geothermal hydrogen sulphide rather than photosynthesis. Many of the vent animals possessed a symbiosis with sulphide-oxidisng bacteria that live intracellularly in specialised organs. Subsequently, it was realised that sulphide-dependent animals were rather widespread and lived in all kinds of environments and could be found even in intertidal habitats of southern England. By far the most diverse group of molluscs possessing sulphide-oxidising bacterial chemosymbionts is the bivalve family Lucinidae with around 500 species worldwide. They often occur in organic-rich habitats such as mangroves and seagrasses and live buried at the interface between oxidised and anoxic sediment. Downwards probing and tunneling by the foot allows sulphide-rich interstitial water to be drawn from the sediment and passed over the gills. Here sulphide-oxidising bacteria are packed into special cells (bacterioctyes) within the modified gill filaments. In this talk I will show how we are using SEM and TEM to investigate the association between the bivalve molluscs and the bacterial chemosymbionts.

MORPHOLOGICAL BASIS OF NEURAL AND SYNAPTIC PLASTICITY MAMMALIAN CNS

Prof. Mike Stewart,Dept. of Biological Sciences, The Open University, Milton Keynes, MK7 6AA.

Information is stored when short term memory becomes consolidated into long term memory-when neural circuitry is modified - the Hebbian synapse strengthening idea. The hippocampus plays a crucial role in memory formation: e.g. studies on 'HM' & lesion experiment on animals. For morphologists, and in particular electron microscopists in essence therefore the question can be stated as: how do synapses change to permit neural circuitry to be modified after learning?

There are many paradigms used in such studies, (e.g.) synaptic changes are known to occur after rearing of rats in enriched environments, motor learning, but the evidence about ultrastructural changes in mammals after actual learning paradigms is contentious, - some data exist on spine changes after spatial learning; but synaptic data provides less clear evidence. Many examples have been given of alterations in synapses in mammals after stimulation e.g. long term potentiation (LTP) of the perforant path in the hippocampus -both in vitro and in vivo, which is not learning but which may induce the types of changes that underpin and are similar to those that occur after learning.

However, results vary - there is no absolute consensus as to how or what synaptic changes occur. Alterations in shape of synaptic elements, rather than synaptic numbers, appear indicative of the early (< 1-2 h) stage of LTP in perforant path synapses. The LTP phase which requires enhanced protein synthesis (both in vivo and in vitro) appears to begin 3-5 hours after potentiation is induced. Thus more prominent structural changes are likely to occur only then, after, that period - hence we have chosen 24 hours post stimulation of the perforant path (LTP) of rats to induce long term potentiation, and for comparison a shorter time, 45 minutes after initial stimulation. Morphological analyses of synapses in the molecular layer of the hippocampus using

unbiased stereological techniques (Sterio, 1994), demonstrated that there is a 30% increase in synapses density in potentiated hippocampal tissue compared to control, but no changes at 45 minutes post potentiation. (Stewart et al 2000).

In a second paradigm we have examined the effects of spatial learning on hippocampal synaptic and neuronal structure in male rats after training in a Morris water maze. Ultrathin sections were prepared for electron microscopy and images taken from the middle molecular layer of the dentate gyrus granule cell dendritic field. Consecutive images were used to estimate the synapse density (Nv) of excitatory synapses, again using the disector technique. Synapses were identified by the presence of a post-synaptic density and presynaptic vesicles, and were categorised as axo-dendritic or axo-spinous, and as perforated or non-perforated. Morphometric analyses were made 3, 9 and 24h after training.

A significant increase was noted in non-perforated axo-spinous (NAS) synapse density in rats 9 hours after training compared to two control groups, naive and ìswim onlyî animals. Conversely, mean height underwent a significant but transient decrease, the reduction being greatest at 9h. Synapse to neuron ratios were calculated and these also showed a significant increase 9 hours after training for non-perforated axo-spinous synapses, as compared to both controls.

These results from both LTP and water maze studies suggest that restructuring of synaptic circuits occurs as a result of experience of each paradigm. One major problem is that they rely on stereological analyses to extrapolate 3 dimensional structures from 2-dimensional measurements - based upon powerful mathematical theory However, such methods do not really give a true 3-Dimensional picture. Today with the increasing power of PCs at accessible prices, and freely available software, such 3D manipulations have become quite possible. Unpublished studies from our research using reconstruction of synapses after LTP will be described.

(1) Sterio, D.C. (1984 The unbiased estimation of number and sizes of arbitrary particles using the disector. J. Microscopy 134: 127-136

(2) Stewart et al 2000, Re-structuring of synapses 24 hours after induction of long-term potentiation in the dentate gyrus of the rat hippocampus in vivo.
Neuroscience. 2000;100(2):221-7
Supported by: BBSRC and The Wellcome Trust

Who needs EM anyway?

Barrie Hartley, Histopathology Dept. St.James' Univ. Hosp. Leeds.

There are two main areas where electron microscopy fits into the diagnostic strategy of interpreting renal biopsies.

a.Making up for limitations in light microscopy

Identifying very early membranous nephropathy.
Supporting a diagnosis of lupus nephritis.
Making it possible to diagnose IgA nephropathy if immuno is not available.
Distinguishing between MCGN, types I, II and III.
Distinguishing between recurrent/denovo MCGN and graft nephropathy.
Confirming a diagnosis of post infectious nephritis after the immuno ceases to be positive.

b.Situations where diagnosis can only be made by EM

Identifying thin basement membranes.
Identifying the changes of Alportís syndrome.
Diagnosing and distinguishing the fibrillary nephropathies.

Backscattered Electrons in SEM

Iolo ap Gwynn The University of Wales Bioimaging Laboratory, Institute of Biological Sciences, The University of Wales, Aberystwyth, Ceredigion, SY23 3DA, Wales
iag@aber.ac.uk

Elastically scattered electron have been the main signal utilised in most TEM work since they first became available. Imaging in the SEM has tended towards the use of the secondary emitted electrons (SE), resulting from inelastic scattering events. Backscattered (elastically scattered) electrons (BSE) do however usually make a significant contribution to the image displayed. The need to work at slow scanning speeds, because of the low level of BSE, when compared with SE, emission from the specimen has made this a less than popular mode of operation with tungsten gun SEMs. With the advent of the field emission SEM, along with the development of better signal detection methods, it has been possible to investigate the usefulness of the BSE signal. Examples will be shown of how the images derived from the BSE signal can provide much useful information from biological samples.

S. E. E. C. ROMANIA

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It is difficult to change the attitudes of a life time, and until 1989 Romania had been a communist country for 50 years. Now, thirteen years on, many (but not all) of those in position of authority still have the "old mentality", even in the scientific world, at times actively impeding contact with scientists and institutions in the west. How many of us have to get five different signatures before we can send one work-related fax ? The economy is still in a bad way; even a head of department receives only $100 a month, and the research institutions are similarly hard up.

WE CAN HELP, by sending recent books and runs of journals and equipment - even the humble beaker is in short supply, and microscopes (of all sorts, including low power stereo) PH meters, etc are especially welcome, but we can find a good home for almost anything in good working order and with a reasonable chance of obtaining spares and manuals. There are EU grants for multi-national projects, so if you are interested in finding Romanian partners, we may be able to put you in touch with people in the same field; many Romanian Scientists are not able to publish in Western journals, nor have easy access to the Internet. Most of all, SEEC Romania welcomes help with funding; to send a consignment of 200Kg costs over £1000.

There will be a poster up-date about our work to help scientists in Romania by sending books, journals and equipment and by forming professional links.

Jenny Anne Drewe will be available at the meeting to discuss any aspects of the work of this small group.

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