SEMT

Society of Electron Microscope Technology

ONE DAY MEETING

Wednesday 16th April 2003

The School of Pharmacy
University Of London
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, Experimental Officer, Electron Microscope Unit
The School of Pharmacy, Brunswick Sq. London WC1N 1AX
Tel: 44 (0) 20 7753 5806; Email: David.McCarthy@ams1.ulsop.ac.uk
Our web site is: www.semt.org.uk
The annual subscription is currently free
Current committee members are listed below and are available for further information.

OFFICERS:

Chair Mrs.Heather Davies
Secretary Mr. David McCarthy
Treasurer Mrs. Nicola Mordan

COMMITTEE:

Ms. Ann Dewar
Mr. David Robertson
Mr.Terry Cooper
Mr. Chris Jones
Mr.Barry Dowsett
Miss Anne Drewe
Mrs. Trish Lovell
Mr. Derek Lovell

Honorary Advisors:

Mrs. Pauline Barber
Mr. Don Claugher
Mr. Chris Walker

Honorary Archivist:

Dr. Jill Lewis

Acknowledgments:

The SEMT wishes to express special thanks to The School of Pharmacy as host.

And to the following companies for supporting the Trade Exhibition :

Emitech Ltd.
Agar Scientific Ltd
Imaging Associates
Quorum Technologies Ltd.
Leo Electron Microscopy
Taab Laboratories Ltd
Leica Microsystems UK Ltd
Hitachi Scientific Instruments Ltd.
Jeol UK Ltd.
FEI UK Ltd
Oxford Instruments analytical Ltd
Edax UK Ltd
Princeton Gamma-Tech Ltd
I.S.S. Group services

Future Programme:

Half-Day Meeting: 29th October 2003

Programme

09.15-10.10	**Registration**
10.10-10.15	**Introduction:** Chair, Heather Davies.
10.15-10.50	**Elucidating the role of Potassium in the development of Apoptosis** A case for EPXMA Dr. Alice Warley. Rayne Inst. St. Thomas' Hospital, London.
10.50-11.25	**Applications of immunolabelling in the Electron Microscope.** Dr. Paul Monaghan. Inst. of Animal Health, Pirbright.
11.25-11.45	**Coffee.**
11.45-12.20	**Airborne particulate matter in the Gatwick area.** Dr. Tim Jones. Dept. Cell Biology, Cardiff University, Wales. (This talk will be introduced by; Cllr. Dr. Andrew Kent, Horley Town Council)
12.20-12.55	***In situ* Particle Analysis in Tissue Samples and its Applications.** Dr. Jonathan Powell. Dept. of Nutrition & Medicine, KCL, London.
12.55-14.30	**Lunch and Trade Exhibition.**
14.30-15.15	**RMS beginners Competition.** **1. The role of PECAM-1 (CD31) in mediating neutrophil transmigration through the perivascular basement membrane.** John Dangerfield, Cardiovascular Med.Unit, Nat. Heart & Lung Inst. Imperial College. **2. The dynactin complex : the keys to a molecular motor?** Carsten Peters, Biomedical Sciences division, Imperial College, London.
15.15-15.50	**Tea. Result of the RMS beginners competition.**
15.50-16.25	**"Unheavenly Rain- coping with what Bioterrorism may send us"** Prof. Dick Madeley. Stocksfield, Northumberland.
16.25-17.00	**Electron Microscopy in Forensic Science** Dr. Robin Keeley, Forensic Science Service, Metropolitan Police.
17.00-18.30	**Wine Reception.**
18.30	**Conference Dinner.**

ELUCIDATING THE ROLE OF POTASSIUM IN THE DEVELOPMENT OF APOPTOSIS: A CASE FOR EPXMA.

A. WARLEY[1]
(1) Electron Microscopy Unit KCL Dept of Ophthalmology, St Thomas Hospital Lambeth Palace Road, London SE1 7EH

There is considerable debate about the role that potassium plays in the progression of apoptosis. Early *in vivo* studies suggested that loss of potassium occurred as a late event during the shrinkage of pyknotic cells. Whereas, more recently, it has been suggested that loss of potassium plays a key role in the activation of effector caspases required for DNA degradation. In addition it has been suggested that high intracellular levels of potassium inhibit caspase activation through inhibiting assembly of the apoptosome.At present the time course of changes in the concentration of potassium, or of other elements, during the progression of apoptosis are not known. Neither is it known whether the changes are the same throughout all cellular compartments. We have used the technique of X-ray microanalysis to address these questions, since this technique allows the unequivocal determination of element concentrations in different subcellular compartments.

Apoptosis was induced by exposing U937 cells to UV-light, and aliquots of cells were taken at 0, 15, 30 60 and 90 minutes after exposure. The progression of apoptosis was monitored by exposing the cells to the fluorescent dyes Hoechst 33342 and propidium iodide. Element concentrations were measured using the technique of electron probe-X-ray microanalysis. Cells were concentrated by centrifugation, and drops of the pellet were cryofixed in liquefied propane. Cryosections 250 nm thick, were cut, placed on Pioloform-coated Ni grids and freeze-dried overnight under temperature-controlled conditions. X-ray microanalysis was carried out in a Zeiss EM 10C electron Microscope in STEM mode at ambient temperature. Spectra were collected with a Link EDS detector and processed using PGT Excalibur software. Quantification was achieved by the continuum normalisation procedure with reference to previously prepared gelatin standards.

The different subcellular compartments cytoplasm, nucleus, mitochondria and condensation of the chromatin were clearly seen in the cryosections. The concentration of K fell steadily throughout the period studied the decrease in the concentration of K was significant at 15 min after the onset of apoptosis. The concentrations on other cell compartments showed a similar progression.

The fall of K at 15 minutes occurs before apoptotic nuclei can be detected by Hoescht staining, and therefore occurs sufficiently early for this element to be important in the degradative process. However, in separate experiments, we have shown that treatment of U937 cells with K channel blockers is unable to prevent the development of apoptosis in these cells. Further studies are in progress to determine whether UV irradiation leads to the release of cytochrome C and thus apoptosome formation before potassium is able to exert its protective effect

In situ Particle Analysis in Tissue Samples and its Applications.

Dr. Jonathan Powell. Departments of Nutrition & Medicine. King's College London, Franklin Wilkins Building, 150 Stamford Street, London SE1 9NN.

In situ particle analysis of biological specimens generally refers to a range of microanalytical techniques that can be used to examine submicron sized particles in tissue samples. Typically, all techniques involve some form of microscopy as imaging is critical in differentiating genuine particle-cell interactions from *ex-vivo* tissue contamination - an issue that has been the subject of considerable debate (e.g. refs. 1 & 2).

In the first place it is important to consider the potential for particle-cell interactions. Arbitrarily, particles may be termed coarse (1-10 um diameter), fine (0.1-1 um) or ultrafine(<0.1 um). Both inorganic (e.g. soil particles) and organic particles (especially from combustion) are ubiquitous in the environment and many have active, charged surfaces carrying redox-active metal ions, allergens and antigens. Continued exposure for humans occurs at the lung and gastrointestinal mucosal surfaces and uptake occurs by constitutive pino- and endo- cytosis of ultrafine particles or responsive phagocytosis of fine particles. Small coarse and fine but not ultrafine particles cause cytoskeletal ruffling and impact upon immune activation associated with phagoytosis- a point often misunderstood when considering the relative impacts of these particles on disease. The finding that ultrafine particles are more 'toxic' than their fine counterparts in animal models may be an artefact with respect to human disease. Ultrafine particles certainly have much greater redox-potential but immunological mechanisms, which provide for signal amplification, are likely to dominate in human disease associated with exposure to ambient atmospheric
or dietary particles. In rare cases of abnormal particle load toxicity may occur. With the above in mind, the target particulates for analysis are of the order of 0.1-3 um diameter. The term microparticles has been adopted (3) and this talk will be restricted to inorganic microparticles, especially silicates and titanium dioxide. Such microparticles are ingested at levels of 1012-1014 per person per day in the Western world (3). These are scavenged by M cells overlying the intestinal mucosa and retained within mature (CD68+) macrophages. In spite of many promising techniques for the analysis of *in situ* microparticle-cell interactions a gulf exists between what can be done and what is presently done. The present methodology for determining the presence of microparticles in tissue specimens is outlined below and ends with a "wish list" for the future.

Ultra high magnification microscopy (ie electron microscopy) is not a practical tool for the screening of particles in tissue samples. Even with scanning imaging analysis (eg EELS or XRMA) sample coverage is time and labour consuming. Moreover scanning EM does not easily allow surface contamination to be precluded while transmission EM requires thin sections and particles may be refractory to cutting. Conventional light microscopy should be first employed, especially in the 'dark field' mode which readily identifies particle-containing cells. Birefringence etc may be used if the particles are responsive to polarised light. Stains (lakes or chelators) are rarely useful for inorganic particles as most cations are too well bound in the particle matrix and refractory to staining. Regular bright field imaging will indicate if the particle is intracellular or on the surface of the section. Only light counter staining is advisable. Confocal microscopy in reflectance mode and optical sectioning are especially useful to confirm the intracellular nature of the particles, their size and subcellular localisation (e.g. intralysosomal). In addition cellular antigens may be examined by antibody staining to determine cell phenotype etc. Sections may then be

appropriately re-processed for either scanning or transmission EM baring in mind the site and size of the particle(s). Inorganic particles are readily analysed by XRMA, which is widely available on electron microscopes, and gives qualitative and potentially quantitative (4,5) information on particle make up. EELS, in its variant forms, is less widely available but is a sensitive technique for particle analysis with electron microscopy. It is especially useful for image scanning analysis and for providing information on the lighter elements (eg carbon, oxygen etc) that, generally, XRMA is unable to detect. However, specimen preparation and particle thickness are important for EELS. Coupled with particle morphology these data should be sufficient to identify intracellular particle types. Proton or 'nuclear' microscopy is also extremely useful for such applications but the requirement of a proton particle accelerator limits its availability to a few specialised laboratories around the world. Despite this structured approach the above techniques are time consuming and require specialised knowledge. The advent of an imaging/analytical technique that allows large sections to be rapidly and easily scanned with submicron resolution would be a major advancement. As noted above, metal ions, allergens and antigens may accumulate on particle surfaces and the provision of a technique that allows for the detection and identification of such species would also be a major break through. New research groups, such as ISPAN at KCL, are considering these and other problems with renewed interest and new technologies may be on the horizon.

References.

1. Role for aluminium and silicon in the pathogenesis of senile plaques: studies in chronic renal dialysis..J.A. Edwardson, A.E. Oakley, G.A. Taylor, F.K. McArthur, M.K. Ward, H.P. Bishop, J.M. Candy. *Adv Neurol*. 1990, 51, 223-6.

2. Absence of aluminium in neuritic plaque cores in Alzheimer's disease. J.P. Landsberg, B. McDonald, F. Watt. *Nature*, 1992, 360, 65-8.

3. The role of dietary microparticles and calcium in apoptosis and IL-1B release of intestinal phagocytes. Evans S.M., Ashwood P., Warley A., Berisha F., Thompson R.P.H.. Powell J.J. *Gastroenterology.* 2002:123;1543-1553.

4. Analysis of Aluminosilicate Particles in Biological Matrices using Histochemistry and X-ray Microanalysis. Powell J.J. *Analyst.* 2002:127(6); 842 - 846.

5. Aluminium-Dependent Regulation of Intracellular Silicon in the Aquatic Invertebrate Lymnaea stagnalis Desouky M., Jugdaohsingh R., McCrohan C.R., White K.N., Powell J.J. *Proc. Natl. Acad. Sci.* 2002;99:3394-3399.

The role of PECAM-1 (CD31) in mediating neutrophil transmigration through the perivascular basement membrane.

John Dangerfield, Karen Larbi, Shijun Wang, Ann Dewar, Sussan Nourshargh. BHF Cardiovascular Medicine Unit, National Heart & Lung Institute, Faculty of Medicine, Imperial College London. Hammersmith Hospital, Du Cane Road. London W12 0NN

Infiltration of leukocytes from the vascular lumen to the extravascular tissue into sites of inflammation requires migration through venular walls. There are 2 distinct steps in this process. The first is migration through endothelial cells and there is evidence that molecules such as PECAM-1, JAM, CD99 and ICAM-2 may be involved in this process. The second step is migration through the perivascular basement membrane (PBM), a thin, tough, distensible mesh composed principally of laminin and collagen IV. Very little is known about the mechanisms by which leukocytes interact with the BM, although a number of molecules have been implicated in this process and include PECAM-1, proteases and the major leukocyte laminin receptor $\alpha_6\beta_1$. There is convincing published data to indicate separate roles for PECAM-1 and $\alpha_6\beta_1$ in leukocyte migration through the PBM but to date, there is no in vivo evidence demonstrating an association between PECAM-1 and $\alpha_6\beta_1$ in regulation of leukocyte transmigration through the PBM. The aim of the present study was to address the role of PECAM-1 as a regulator of $\alpha_6\beta_1$ expression during transmigration.

The functional role of $\alpha_6\beta_1$ in leukocyte migration through stimulated mouse cremasteric venules was investigated using the anti-α_6 integrins mAb GoH3 as observed by intravital microscopy, a technique allowing direct visualisation of leukocyte-endothelial cell interactions. GoH3 significantly inhibited neutrophil migration through IL-1B-stimulated venules (78%, $P < 0.001$). Transmission electron microscopy analysis of selected cremaster muscles treated with the anti-α_6 integrins mAb indicated that this inhibition was at the level of the PBM, with 3 times as many neutrophils trapped between the endothelial cell layer and the basement membrane compared to control-antibody treated mice. In PECAM-1-deficient mice (KO), a reduced level of neutrophil transmigration elicited by local IL-1B (4 hour reaction) was observed in both the cremaster muscle (55% inhibition, $P < 0.05$) and in the peritoneum (57% inhibition, $P < 0.01$) but GoH3 had no additional inhibitory effect on these responses. FACS analysis of transmigrated peritoneal neutrophils demonstrated increased expression of $\alpha_6\beta_1$, as compared with blood neutrophils, in wild-type (WT) but not KO mice. Analysis of purified and fixed mouse neutrophils, using a ZEISS confocal microscope indicated comparable levels of intracellular α_6 expression in neutrophils from both WT and PECAM-1-deficient mice.

The results demonstrate a functional role for α_6 integrins in neutrophil migration through the perivascular basement membrane in vivo. Furthermore the study provides direct in vivo evidence for the involvement of PECAM-1 in neutrophil transmigration and enhanced expression of $\alpha_6\beta_1$ on transmigrated neutrophils.

The Dynactin complex: the key to molecular motors?

Peters, C.[1], Steffen, W.[2], Kuznetsov, S.[3], and **Hodgkinson, J.[1]**

1 Imperial College School of Medicine, London, UK; 2 King's College, London, UK; 3 University of Rostock, Rostock, Germany

Dynactin, co-complexed with the molecular motor cytoplasmic dynein and kinesin II, is involved in a variety of intracellular dynamic processes. It is believed that one of the main functions of dynactin is to mediate the cargo binding of dynein and to ensure processive transport along microtubules. Little is known about how dynactin facilitates these functions due to the enormous size and complexity of the co-complexes. Dynactin was isolated from bovine brain and its function to mediate the dynein motor activity was tested in a microtubule-gliding assay by AVEC-DIC microscopy (Fig.1).

Comparing a non-specific surface with a dynactin pre-coated surface, only the latter is able to establish a dynein-dependent transport of microtubules. Insight into the structure of dynactin is required in order to understand how dynactin mediates dynein function. To elucidate the structure of dynactin electron micrographs of negatively stained dynactin were processed by single particle image analysis (IMAGIC). Seven thousand dynactin complexes were processed and grouped into classes of similar orientation (see Fig. 2a). Dynactin consists of a 37 nm long 2-stranded rope like filament made up of **A**ctin **R**elated **P**rotein 1 with additional sub-complexes (barbed-end and pointed-end) at either end. The ARP1 has a helicity very similar to that of filamentous actin (see Fig. 2b).

The number of ARP subunits is apparently 9-10 ARP1, plus one ARP11 sub-unit known to bind at the pointed-end of the complex. At the apex of the filament (barbed-end) an additional non-ARP mass is visible, most likely from CapZ a capping protein which can be seen to be strategically placed to block further binding of ARP sub-units to this end of the filament. In summary dynactin, a component of the microtubule transport system, has properties in common with the actin filament system and our structural data are consistent with recent models of the complex.

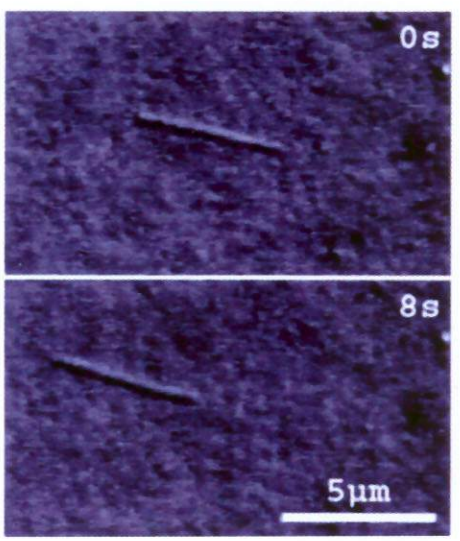

Figure 1: Movement of a microtubule in the motility assay.

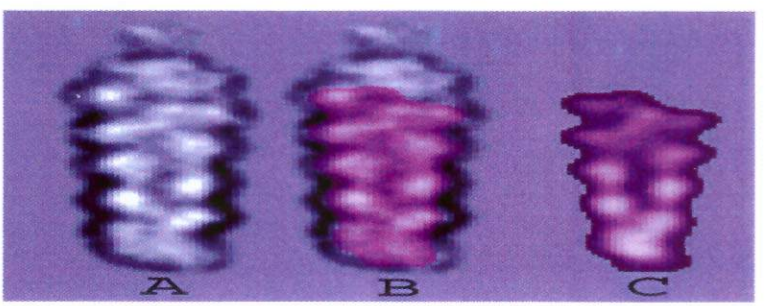

Figure 2a. A selection of class averages. **2b** Comparison of ARP1 filament with a short actin filament. A) class average of dynactin, C) 10 subunit model of F-actin [Holmes et al. 1990], B) Superposition of A and C. The barbed-end complex is at the top-end of the filament and the pointed end the bottom-end of the filament.

"Unheavenly rain - coping with what bioterrorism may send us"
Dick Madeley (Stocksfield, Northumberland)

Unexpected and/or unfamiliar illnesses may appear at any time. They may be due to a deliberate release of 'old' agents as a bioterrorist act or, much more likely, new variants of old organisms (such as influenza), or old organisms spreading to new areas as a result of population movements, climate change, etc. (such as arboviruses), or even something totally new, but occurring naturally. No matter what it is, it is essential to find out something about it so as to be able to measure the threat (if any) to the community. Electron microscopy, particularly using negative contrast, has a vital part in this because it can provide some of, and maybe all, the answer(s) to the question of what is causing the infection more quickly and with greater conviction than other methods.

Given the rapidity of modern communications, speed is vital in making the diagnosis. The result may be either to confirm the probable cause or, hopefully, to make an alternative identification that excludes the worst scenario. If this decision can be made the same day, it takes all the heat out of a situation in which to get it wrong (false positive or false negative) will be disastrous. EM can provide both a same-day answer and, at the same time, one with a degree of certainty that is almost unique.

These and other aspects of the appearance of something out of the ordinary will be discussed.

What is Forensic Science ?

Forensic science is simply the application of science to the law. In criminal cases forensic scientists are often involved in the search for and examination of physical traces which might be useful for establishing or excluding an association between someone suspected of committing a crime and the scene of the crime or victim. Such traces commonly include blood and other body fluids, hairs, textile fibres from clothing etc, materials used in buildings such as paint and glass, footwear, tool and tyre marks, flammable substances used to start fires and so on. Sometimes the scientist will visit the scene itself to advise about likely sequence of events, any indicators as to who the perpetrator might be, and to join in the initial search for evidence. Other forensic scientists analyse suspected drugs of abuse, specimens from people thought to have taken them or to have been driving after drinking too much alcohol, or to have been poisoned. Yet others specialise in firearms, explosives, or documents whose authenticity is questioned.

In civil cases forensic scientists may become involved in some of the same sorts of examinations and analyses but directed to resolving disputes as to, for example, the cause of a fire or a road accident for which damages are being claimed.

Forensic scientists can appear for either side - prosecution or defence in criminal matters, and plaintiff or defendant in civil ones. They tend to present their findings and opinions in written form either as formal statements of evidence or reports. Sometimes they are required to attend court to give their evidence in person.

Forensic Medicine and Forensic Dentistry

By analogy, this is the application of medical and dental knowledge to legal problems. Forensic medical examiners, who deal with the living and forensic pathologists, who deal with the dead, are qualified medical practitioners who, having completed their training as doctors, choose to specialise in either field. Forensic odontologists are qualified dentists who have undergone additional training and who provide expert evidence on dentistry.

Scientific Support within the Police Forces

Civilians are now employed by many police forces to provide a variety of technical services. These include photography, the collection and comparison of fingerprints, vehicle examination and the detailed examination of scenes of crime. Scene examiners, often referred to as SOCOs (Scenes of Crime Officers), will normally have some scientific training.

Career Opportunities

The majority of forensic scientists in the United Kingdom are employed by the Forensic Science Service (in England and Wales), by specific police forces (in Scotland), and by regional government (in Northern Ireland), and by private companies which also specialise in providing primary forensic science services to the police such as Forensic Alliance Limited and the Laboratory of the Government Chemist. Aside from these, there are a number of other organisations which focus on specific areas of forensic science such as fire investigation, questioned documents, and advising the defence.