SOCIETY OF
ELECTRON MICROSCOPE
TECHNOLOGY

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MICROSCOPY OF THE NATURAL WORLD

Wednesday 8th December- 2.00 p.m. 1999

LONDON SCHOOL OF PHARMACY
Brunswick Square

2.00 **Epithelial integrity; cell loss and cell death in vertebrate intestine**

Prof. Terry Mayhew (Faculty of Med., Queen's Medical Centre, Nottingham)

2.30 **Microscopy and show business**

David Spears (Science Pictures Ltd., Hitchin, Herts.)

3.00 **TEA**

3.30 **The secret life of plants**

Kim Findlay (Dept. Cell Biol., John Innes Centre, Norwich)

4.00 **Twenty questions: animal, vegetable or mineral ?**

Chris Jones and Alex Ball (EMU, Natural History Museum, London)

4.30 **SHERRY and MINCE PIES** followed by the **ANNUAL GENERAL MEETING**

Members and others interested are very welcome to attend this meeting

RSVP to the Secretary by Monday December 6th. **PLEASE**

Comparative studies on epithelial cell death and extrusion in the small intestine

Terry M. Mayhew
School of Biomedical Sciences, Queen's Medical Centre, University of Nottingham

Intestinal epithelium must reconcile two potentially conflicting properties: it is a tight and continuously renewing epithelium. The first property depends on the presence of intercellular tight junctions (TJs) which bind adjacent cells to each other, partition their plasma membranes into apical and basolateral domains, and help to regulate transepithelial solute transport. The second depends on clonogenic cells which proliferate within crypts and send their progeny on a 2-6 day trip of differentiation and death. Cells migrate out of the crypt, on to the villus and towards the villus tip where they die and are extruded into the lumen.

Recently, the different mechanisms of epithelial cell loss occuring in vertebrate small intestine have been re-examined by ultrastructural and other techniques and information is now available for various mammalian and other types. Mechanisms can be divided into two main classes according to whether they [a] maintain epithelial integrity by preserving TJs throughout the extrusion process or [b] compromise integrity by allowing breaches of TJ continuity. Both types of cell loss are associated with non-epithelial cells found in the epithelium (intraepithelial lymphocytes, IELs) or underlying lamina propria (mononuclear phagocytes, LPMPs, and lymphocytes). IELs are involved in enterocyte targetting and killing whilst LPMPs sequester cell debris.

Where epithelial integrity is maintained, two types of loss can be distinguished because either complete cells (type 1; seen in hamster, human, mouse, rat) or only anucleate apical cell fragments (type 2) pass into the lumen. Variants of type 2 loss depend on the fate of nucleated basal fragments and the role of IELs. One variant (type 2a; cattle, guinea pig, horse, monkey, penguin, reindeer) involves creating large basal intercellular spaces extending from the preserved apical fragment down to the basal lamina. From these spaces, debris is removed by LPMP phagocytosis. Type 2b (pig, reindeer, seal) involves gradual shrinkage and degeneration of cells within increasingly narrower intercellular spaces. Type 2c (piglet, pig) involves IELs forming a physical barrier between apical and basal fragments. Cell death by apoptosis accompanies both type 1 and type 2 extrusion.

In contrast, type 3 loss (chicken, human, mouse, penguin, piglet, pig, rat, reindeer, seal) involves ultrastructural changes reminiscent of necrosis including cell swelling, total or subtotal degeneration of organelles and membranes, and disruption of TJs. The latter causes breaches of epithelial integrity. It ends in loss of either an abnormal cell apex (with spillage of degraded cell contents into the lumen) or a complete cell remnant (extruded into the lumen before total breakdown of plasma membranes). Naturally, there are functional implications to the occurrence of extrusion mechanisms which affect TJ integrity. Most solutes cross the epithelium by transcellular or paracellular routes. The epithelium is permeable to small molecules (ions, amino acids, monosaccharides) but relatively impermeable to large molecules. Mechanisms of cell death and loss which preserve TJs would maintain these transport routes. However, some macromolecules can leak across the epithelium in small quantities and this could be explained by the localised disruption of TJs which accompanies necrotic cell death. This method of loss seems to be more widespread than previously thought.

References

Mayhew TM et al (1999) Histol Histopathol 14, 257-267
Myklebust R, Mayhew TM (1998) Cell Tiss Res 291, 513-523.

Acknowledgements

I am grateful to Lakshmi Mahendran, Andrea Whybrow, Chris Ching, Robert Jenkins, Reidar Myklebust and Barry Shaw for their contributions to these studies.

Microscopy and show business

David Spears (Science Pictures Ltd & Science Footage Ltd;
www.science-pictures .ltd.uk)

The secret life of plants

Kim Findlay (Dept Cell Biology, John Innes Centre, Norwich)

A light-hearted, illustrated talk on the life-cycle of higher plants, using light and electron micrographs to demonstrate various aspects of structure and function of different cell types. Topics will include cell division, growth and development, coping with environmental stresses, wounding and disease.

This selection of purely whimsical, along with serious, scientific images come from a varied collection of micrographs taken by me over the last ten years whilst working on a variety of research programs at the John Innes Centre.

Twenty questions - animal, vegetable or mineral ?

Chris Jones and Alex Ball

Electron Microscope Unit, The Natural History Museum (London)

The tremendous complexity of natural samples has always necessitated detailed description and methods of illustration.

The trustees of The Natural History Museum are the custodians of the nation's natural history collections, comprising nearly seventy million specimens or objects. The collections include fifteen kilometres of shelving for fish specimens, more than ten million molluscs, half a million rocks and minerals and nearly three and a half thousand meteorites. The Museum employs three hundred and fifty scientists in five research departments. Scientists are engaged in programmed systematic and taxonomic research and curation, largely using the collections. Fields of research cover all the natural sciences and some extraterrestrial material, contributing to around four hundred peer reviewed publications every year.

The collections continue to glow as scientists carry out fieldwork in locations as exotic as Belize, Africa, Australia and Southend.

The Museum's Electron Microscope Unit applies many techniques for imaging such a wide range of samples, utilising one transmission and three scanning electron microscopes. The scanning electron microscopes comprise a conventional microscope for imaging fixed and coated samples, a low vacuum microscope for imaging uncoated and hydrated samples and a field emission microscope for high resolution work. We seem to do more low magnification imaging than high at present, a situation the microscope manufacturers find hard to understand! Other instruments available within the department include analytical SEM, microprobe and laser confocal microscopy.

Today's talk will illustrate some of the work recently carried out in the Unit and will help to show the diversity of samples prepared for electron microscopy. We look at literally anything animal, vegetable and mineral.

Microscopy of the Natural World - SEMT 8 December 1999

Epithelial Integrity; cell loss & cell death in vertebrate intestine
Prof. Terry Mayhew

Some mechanisms of cell death: the villi are normally tongue-like, with tall columnar epithelial and goblet cells and others with brush border + glycocalyx and digestive enzymes. It is "tight" epithelium; most molecular movements are trans-cellular - because there is an occluding tight junction by the border. The basal lateral membrane is contorted, and may have an ion pump. The turnover of epithelial cells is 2 - 6 days. Clonogenic cells are found at the base of the crypts, moving to extrusion of old cells at the tips of the villi; this would allow leakage because the tight junction is broken. The whole cell may be lost (Type i); or just the tip of the cell (Type 2); or there may be breaches of epithelial integrity.

Lymphocytes and macrophages as associated with the basal lamina, and send processes to the epithelium. Cells may swell; or shrink and separate into basal and nuclear particles, associated with lymphocytes. Necrotic swelling is usually single cells, but may be several together; the contents are extruded to the lumen. The cell swell and becomes paler; the microvilli become vesicular. Nucleated fragments move into the lumen.

I. Loss of complete nucleated fragments; tight junction migrates to the surface.

II. Apical fragments migrate to the lumen; macrophages remove basal particles.

III. Necrotic cells migrate to the lumen.

In studying the process, we need to harvest the luminal contents as well as the cells.

Microscopy and Show Business
David Spears

They are a production company for programmes and films. The public prefer cuddly creatures - owl more than heron. It is usually difficult to give a good idea of the size of the creatures; so now a micro-motion control has been develope, which can zoom in on people.

They use a Jeol 430 SEM; with bore-scope for gut, which has a great depth of field - they don't know why.
Bacteria in sweat produce the "smell of fear".
They also make programmes for schools.

Twenty Questions: animal, vegetable or mineral ?
Chris Jones & Alex Ball

The Natural History Museum has TEM, low-vacuum SEM, & confocal.
The Robinson detector, using elemental contrast, is used for Bruozoans not visible by light microscopy.

The secret life of plants
Kim Findlay

A leaf loses up to 80% water through the stomata. Plasts have no centriole. In the tobacco leaf, cell division stops when it is 1/5 of the final size; growth is then only by increase in cell size, unless the elaf is wounded.

Twenty questions - animal, vegetable or mineral?

Chris Jones and Dr Alex Ball

Electron Microscope Unit, The Natural History Museum.
(E-mail cgj@nhm.ac.uk or a.ball@nhm.ac.uk).

The tremendous complexity of natural samples has always necessitated detailed description and methods of illustration.

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SEMT Meeting 08.12.99

List of Registrants

Chris Andrews	School of Pharmacy, Queen's University Belfast
Alex Ball	EMU. Natl History Museum
Andrea Boyd	Oral Med and Pathol., Guy's Hospital
John Bredl	Electron Microscope Unit, Royal Veterinary College
Bill Clark	Agar Scientific. Stansted, Essex
Terry Cooper	Taab Laboratories, Aldermarston, Berks
Don Claugher	Surbiton, Surrey
Heather Davies	EMU, Biol. Sci., Open University, Milton Keynes
Barry Dowsett	CAMR, Porton Down
Anne Drewe	Microbiology, Charing Cross & Westminster Med School
Kim Findlay	Dept of Cell Biology, John Innes Centre, Norfolk
Sara Fletcher	Royal Vet College
Carol Furness	Royal Botanic Gardens, Kew
Tanya Hopcroft	Histology Dept, Royal Veterinary College, London
Chris Jones	EMU, Natl History Museum
Lynne Joyce	Agar Scientific Ltd., Stansted, Essex
Mike Kaiser	Inst Orthopaedics, RNOH, Stanmore
Gill Lewis	EMU, Eastman Dental Inst
Patricia Lovell	Dept Histopathology, Royal Marsden Hospital, London
Anna Lynch	Royal Botanic Gardens, Kew
Prof Terry Mayhew	Queen's Med Centre, Univ of Nottingham
David McCarthy	EMU, School of Pharmacy
Maria McCrossan	Dept Histopathol., London School of Hygiene
Nicky Mordan	EMU, Eastman Dental Hospital
Chrissie Prychid	Royal Botanic Gardens, Kew
Padmini Sarathchandra	Dept Surgical Research, NPIMR, Harrow
Catherine Sarraf	Dept Histopathol., Royal Postgraduate Med. School
David Spears	Science Picture Ltd., Hitchin, Herts
Larry Stoter	Jeol UK Ltd., Welwyn Garden City
Rosemary Suswillo	Bone Unit, Royal Veterinary College, London
Wendy Tynan	London School of Pharmacy
Chris Walker	FEI-Philips Electron Optics, Cambridge