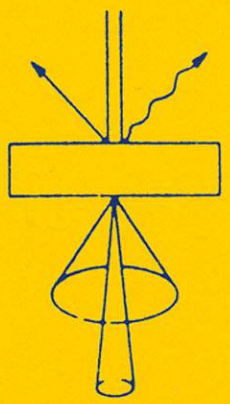
SEMT

Society of Electron Microscope Technology



Affiliated to the Royal Microsccpical Society

Annual Award

for

Young Researchers

Imperial Cancer Research Fund
Lincoln's Inn Fields London WC2
2.0 p.m.

Friday, 4 December 1992

Guest Speaker:Dr Lesley Coggins
Beatson Institute for Cancer Research, Glasgow

"Preparation of Nucleic Acids for Electron Microscopy"

4-12-92

2.50 Tea

Novice Electron Microscopists Presentations

3.15 **L. Babaei-Mahani**
at Department of Experimental Pathology
Institute of Orthopaedics, Stanmore

A microanalytical study of bone mineral from mice with partially deleted genes for human procollagens

3.30 **Simon Levy**
Department of Histopathology
Charing Cross & Westminster Medical School

The corneal endothelium in the iridocorneal-endothelial syndrome: an ultrastructural and immuno-electron microscopical study

3.45 **Tareq Ansari**
EMU
Royal Postgraduate Medical School

Comparison of a variety of immunocytochemical techniques at electron microscope level to demonstrate the morphological distribution of a regulatory peptide in damaged human intestinal epithelium

4.00 **John Giannios**
Centre for Drug Delivery Research
School of Pharmacy

Interaction of liposomes with blood plasma: an SEM study

4.15 **Philip Wilby**
Department of Earth Sciences
The Open University

EM techniques and the investigation of exceptionally well-preserved fossil material

4.30 **T V Anilkumar**
Department of Histopathology
Royal Postgraduate Medical School

An ultrastructural analysis of stem cell differentiation in the rat liver

4.45 **Carl Bryer**
Department of Earth Sciences
The Open University

Boring diatoms ? Observations of an antarctic bivalve

5.00 Buffet

5.30 **The Judges' Decision and Award Ceremony to the**

SEMT Novice Electron Microscopist 1992

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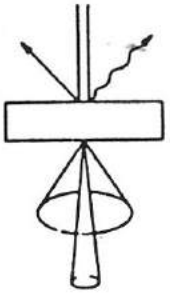

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PREPARATION OF NUCLEIC ACIDS FOR ELECTRON MICROSCOPY

Lesley W. Coggins
Beatson Institute for Cancer Research, CRC Beatson Laboratories, Garscube Estate, Switchback Road, Glasgow G61 1BD.

Electron microscopy provides a rapid means of investigating naturally-occuring nucleic acids and molecularly-cloned DNA [1]. DNA or RNA is mixed with cytochrome c, spread on the surface of a liquid to form a monomolecular film, and adsorbed to a collodion-coated grid. After staining and 'shadowing' by low-angle evaporation of Pt-Pd, the preparation is examined by TEM.

The form of individual DNA molecules (eg single- or double-stranded, supercoiled, circles or linear molecules, chi-shaped recombinant molecules etc) can be determined. More information can be obtained by first denaturing and reannealing the DNA and observing the resulting hybrid molecules by E.M. Heteroduplexes are formed by hybridising two different but related DNA sequences, to reveal regions of sequence homology, from 50 bp up to 100 kb long (the degree of homology can be ascertained from the hybridisation conditions). Hybridisation of DNA and mRNA maps exons and introns in genes, and reveals alternatively-spliced mRNAs.

Inverted repeat sequences can be mapped by the formation of stem and loop structures in single-stranded DNA. Minisatellite arrays are tandem repeats of short (10-50 bp) DNA sequences which are usually G-rich and biased to purine bases on one strand. We have found that, after denaturation and reannealing, such arrays form knot-like structures as a result of misaligned hybridisation of tandem repeats [2].

[1] L.W. Coggins (1987) In Electron Microscopy in Molecular Biology: A Practical Approach. Ed J. Sommerville and U. Scheer. IRL Press.
[2] L.W. Coggins, M. O'Prey and S. Akhter (1992) Intrahelical pseudoknots and interhelical associations mediated by mispaired human minisatellite DNA in vitro. Gene (in press).

A MICROANALYTICAL STUDY OF BONE MINERAL FROM MICE WITH PARTIALLY DELETED GENES FOR HUMAN PROCOLLAGENS.

*L. Babaei-Mahani, (as part of an intercalated BSc project in collaboration with), + JP Cassella and S. Yousuf Ali.

* St Bartholemews Medical School, Charterhouse Square, London E1.
+ Department of Experimental Pathology, Institute of Orthopaedics (University of London), Royal National Orthopaedic Hospital, Brockley Hill, Stanmore Middlesex, HA7 4LP.

Osteogenesis Imperfecta (OI) is a heritable disorder of connective tissue presenting clinically as bone fragility with frequent fractures. Using molecular biological techniques, mutations in the type I procollagen gene have been detected in a number of patients with OI.

Previous studies on human OI bone have shown ultrastructural changes at the TEM level and changes in the mineral composition (Cassella and Ali, 1992). However, no study has been performed on transgenic mice with collagen mutations to determine if generated collagen abnormalities have an effect on the mineral formed.

Electron probe x-ray microanalysis has been performed on bone to quantitatively analyse the calcium to phosphorus ratio (Ca/P) from two groups of transgenic mice. One group had a mutation of the type I procollagen gene resulting in a phenotype closely resembling OI. A second group of transgenic mice had a mutation in the type II procollagen gene resulting in a skeletal dysplasia affecting cartilage.

Distal femurs were embedded in resin and viewed uncontrasted in a Philips CM12 TEM. The bone mineral was analysed using an EDAX 9800 microanalysis system. All measured Ca/P values were corrected using a standard graph according to Cassella and Ali (1992).

Preliminary results indicated that the Ca/P ratio in transgenic mice with the type I collagen abnormality was *lower* than normal controls. The transgenic mice with the type II collagen abnormality, appeared to have a Ca/P ratio similar to normal controls.

Data from the mice with the type I collagen abnormality correlated well with the data from OI patients (Cassella and Ali, 1992). This suggests that an alteration in the structure of type I collagen fibrils has an effect on the mineral nucleation sites, with a subsequent effect on the composition of the apatite mineral. Alteration in type II collagen structure does not appear to have such an effect. Future study will confirm these preliminary results; these transgenic mice are proving to be of considerable use as models for OI and skeletal dysplasias.

Cassella JP, Ali S. Yousuf (1992)
A Microanalytical Study of Bone Mineral in Osteogenesis Imperfecta.
Micron and Microscopica Acta
23 (3) 351-352.

Acknowledgement The transgenic mice were kindly donated by Professor Darwin Prockop, Jefferson Institute of Molecular Medicine, in a collaborative project with JPC and SYA.

TITLE
CAPITAL LETTERS

THE CORNEAL ENDOTHELIUM IN THE IRIDOCORNEAL-ENDOTHELIAL SYNDROME: AN ULTRASTRUCTURAL AND IMMUNO-ELECTRON MICROSCOPICAL STUDY.

AUTHOR

Mr Simon G. Levy M.R.C.P, F.R.C.S(ED), D.O(LONDON), F.C.OPHTH

INSTITUTION

Honourary Ophthalmic Research Registrar, Charing Cross and Westminster Hospital Medical Schools.

The Iridocorneal-endothelial syndrome (ICE syndrome) is a rare disease of the anterior ocular segment. It manifests by a unique cluster of clinical signs - glaucoma, corneal failure preceded by clinically apparent thickening of Descemet's membrane (the basement membrane of the corneal endothelium) and spectacular iris atrophy. It may be uni- or bilateral and affects both sexes and all races equally. Onset is usually between thirty and forty years of age.

In vivo specular photomicroscopy, and histopathological case reports of corneas removed at the time of penetrating keratoplasty, have suggested that the condition is associated with changes in the corneal endothelium, the cellular monolayer lining the internal surface of the cornea.

We have assembled a large series of ICE syndrome corneas by collaborating with corneal surgeons at several centres in the United Kingdom. Normal corneas judged unsuitable for use in transplantation were used as controls.

The ultrastructural appearance of the corneal endothelium in the ICE syndrome was examined by transmission and scanning electron microscopy. This study demonstrated the presence of an abnormal cell type, alongside residual normal endothelial cells. Abnormal cells were distinguished by their larger size, surface microvilli and "blebs", complex intercellular junctions and greater number of intermediate filaments. Correlation with specular microscopical studies suggests that this cell type is of central importance in this disease.

In addition, we observed in the ICE syndrome specimens, a previously undescribed helicoidal structure for which we have suggested the term "spiral form".

Ultrastructural examination of Descemet's membrane revealed gross thickening due to the abnormal presence of three connective tissue elements: interstitial collagen, microfibrils and long-spacing collagenous aggregates. Electron microscopic immunocytochemistry was used to identity these elements. Corneas were fixed in paraformaldehyde and either frozen for cryo-ultramicrotomy or embedded in Lowicryl K4M resin. A two stage immunocytochemical technique was used with colloidal gold as an electron-dense marker.

These studies showed the abnormal connective tissue to consist of a complex mixture of several collagen types, laminin, fibronectin and tenascin. Information on the effects of these substances on corneal endothelial cells *in vitro*, suggests that these abnormal components of Descemet's membrane may exert a significant influence on disease evolution.

TITLE
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COMPARISON OF A VARIETY OF IMMUNOCYTOCHEMICAL TECHNIQUES AT ELECTRON MICROSCOPE LEVEL TO DEMONSTRATE THE MORPHOLOGICAL DISTRIBUTION OF A REGULATORY PEPTIDE IN DAMAGED HUMAN INTESTINAL EPITHELIUM

AUTHOR

Tareq Wiqar Ansari

INSTITUTION

Electron Microscopy Unit, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Doad, London W12 ONN

The regulatory peptide, pS2, occurs in an ulcer associated cell lineage (UACL) in gastrointestinal ulceration for example in Crohn's disease. Immunocytochemical localisation of pS2 at electron microscope level presents some difficulties due to low concentration of the peptide on cytomembranes, and the high electron density of endocrine granules, where the peptide occurs in greater concentration. Three different immunocytochemical techinques have been used to demonstrate the presence of pS2 at different locations . Packed in the granules of gastrointestinal endocrine cells, pS2 was demonstrable with an immunogold technique, and its presence in the Golgi apparatus and RER of goblet cells neighbouring the ulcer associated celi lineage was demonstrable using a simple peroxidase ABC technique. In the Golgi apparatus of epithelial cells of the UACL, pS2 in low concentrations was demonstrable with the use of peroxidase ABC technique in conjunction with silver enhancement of the enzyme final reaction product.

TITLE
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INTERACTION OF LIPOSOMES WITH BLOOD PLASMA:A SEM STUDY

AUTHOR John N. Giannios and Gregory Gregoriadis

INSTITUTION Centre for Drug Delivery Research,School of Pharmacy,University of London, 29-39 Brunswick Square,London WCIN IAX.

Liposomes are vesicles consisting of one or more phospholipid bilayers alternating with aqueous compartments within which lipid-soluble and water-soluble drugs can respectively be accomodated (1). They have been used extensively as a delivery system for drugs and vaccines both experimentally and clinically (2). It is well known (3) that in the presence of blood in vitro and in vivo, liposomes are attacked by plasma high density lipoproteins (HDL). These remove phospholipid molecules from the vesicle bilayer which, as a result, is destabilized (with concomitant drug leakage) and eventually disintegrated. The presence of excess cholesterol in liposomes prevents or delays HDL action on the bilayers to an extent that is dependent on the amount of cholesterol and the type of phospholipid (unsaturated or saturated) used (3). We have investigated this phenomenon of plasma-induced vesicle destabilization by scanning electron microscopy (SEM). Changes in vesicle stability were ascertained by SEM images of external liposomal morphology. Liposomes were of the multilamellar type (multilamellar vesicles;MLV) and composed of egg phosphatidylcholine (PC) alone or supplemented with cholesterol. Incubation of PC MLV with phosphate-buffered saline, pH 7.2 (PBS) at 37°C for 30 min did not alter the original appearance of vesicles which was identical whether cholesterol was present or not. However, exposure of PC MLV to mouse blood plasma at 37°C for 30 min led to stuctural changes. Images obtained are difficult to interpret but suggest that part of the vesicular structure has been removed. Incorporation of some cholesterol in PC MLV (PC:cholesterol molar ratio of 1:0.5) helped to preserve some of the stuctural integrity of the vesicles. On the other hand, structural integrity was maintained fully when the PC:cholesterol molar ratio was increased to 1.0. These results present for the first time morphological evidence of plasma-induced liposomal structural changes.

1.Gregoriadis, G.(Ed.) Liposome Technology,CRC Press, Vols I-III,Boca Raton,1992.

2.Gregoriadis, G.(Ed) Liposomes as Drug Carriers:Recent Trends and Progress, Wiley, Chichester,1988.

3.Gregoriadis, G. The Physiology of the Liposome, News in Physiological Sciences 4,146-151, 1989.

TITLE
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EM TECHNIQUES AND THE INVESTIGATION OF EXCEPTIONALLY WELL PRESERVED FOSSIL MATERIAL

AUTHOR

PHILIP WILBY

INSTITUTION

DEP'T OF EARTH SCIENCES, THE OPEN UNIVERSITY

Most processes of fossilization preserve only the skeletal elements or 'hard parts' of organisms. In a few specific sedimentary deposits however, soft tissues are preserved. These permit palaeontologists to comment with more confidence on the biology, evolutionary relationships, and interactions of the organisms. One such deposit is the 100 million year old Santana Formation of NE Brazil. Here, the soft tissues of pterosaurs (flying reptiles), primitive fish, and crustacea (Wilby and Martill, 1992) are replaced by Ca-phosphates with such precision that subcellular details are clearly recognisable. For example, the banding of striated muscle fibres, and the plasma membrane, nuclei and mitochondria of most cells are perfectly preserved. However, despite the exceptional nature of this material, virtually nothing is known of the processes involved in its fossilization. A variety of EM techniques are helping to clear up three of the principle problems:

1) **Process:** SEM and TEM examination of the fossil soft tissues has demonstrated that the Ca-phosphate crystallites nucleated directly onto the organic substrates in a manner comparable to that of biomineralization in living tissues. Indeed, micro-electron diffraction analysis of ultrathin sections of fossil dermis suggests that crystallite nucleation was similar to that in bone.

2) **Phosphate source:** Elemental mapping (Martill and Wilby, 1992) of sectioned fossil fish has demonstrated that clear gradients in the abundance of phosphorus exist from high at the periphery just beneath the dermis to low at a greater depth (supported by ZAF correction analysis). This suggests the source of phosphate ions for fossilization was external to the decaying carcasses and therefore diagenetic (from the sediment) in origin.

3) **Timing of mineralization:** a detailed comparison of the fossil soft tissues of the fish with those of progressively decayed extant fish in TEM, indicates (contrary to popular view) that the organisms were fossilized within 55hrs of death!

TITLE
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AN ULTRASTRUCTURAL ANALYSIS OF STEM CELL DIFFERENTIATION IN THE RAT LIVER

AUTHOR

Mr. T.V. Anilkumar

INSTITUTION

Department of Histopathology, Royal Postgraduate Medical School Hammersmith Hospital, London W12, UK.

Oval cells are nondescript cells located in the portal space which may have stem cell properties, being able to differentiate into various cell lineages in the liver. The ultrastructural features of these cells are virtually unknown. The present study employed extensive electron microscopic techniques to analyse the origin and fate of oval cells in the rat liver.

Oval cells were generated in a rat liver model of regeneration in which proliferation of normal hepatocytes was prevented by using acetylaminofluorene, a carcinogen. The presence of oval cells was confirmed under the light microscope by a specific immunohistochemical marker, the OV-6 antibody, which identifies a 56000 kd cytokeratin in oval cells. Viewed by electron microscopy, the oval cells had a large vesicular nucleus and few cytoplasmic organelles indicative of their low level of differentiation. They initially appeared in the periportal area but migrated into the parenchyma. The proliferation and subsequent migration of cells was traced by employing an immunogold technique for detecting the marker nucleotide, bromodeoxyuridine. The migrated cells formed small islands and retained the capability for proliferation. Later they acquired cellular features characteristic of either hepatocytes or bile duct cells. Those cells differentiating into hepatocytes permeated into the hepatic plates while those forming ducts developed a microvillus border on the apical membrane bordering the lumen. Clearly, oval cells deserve the appellation of **potential stem cells.**

TITLE
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BORING DIATOMS? OBSERVATIONS OF AN ANTARCTIC BIVALVE...

AUTHOR

CARL BRYER

INSTITUTION

DEP'T EARTH SCIENCES, THE OPEN UNIVERSITY.

The Antarctic bivalve *Yoldia eightsi* plays an important role in the soft bottom benthos of a great part of the Antarctic/subantarctic. It mainly occurs in in the top 4cm of the sediment and within a depth range of 5m-700m. The species is mainly a surface / sub-surface deposit feeder with the ability to filter feed, and maintains a horizontal feeding position when compared to its more northerly relatives. Diatoms both benthic pennate and centric diatoms have been found in the stomachs of *Yoldia* and are thought to be its primary food source.

Upon examination many adult specimens reveal erosion of both the protective outer organic layer (the periostracum), and the aragonitic the shell material at the hinge region or umbo. This must in part be due to the movement of the animal in the sediment, but SEM examination of juveniles 0.8mm in length, (with larvae settling at 0.36mm) showed erosion at the umbo. In addition adults showed pitting and discolouration of the shell and periostracum across a large proportion of the shell and in an arc around the umbo.

The shells of several adult specimens were dissolved in acid leaving the organic periostracum intact, which was then freeze substituted critically point dried (FSCPD). Several adult animals had the periostracum removed, leaving a bare shell and a number of juveniles were FSCPD.

Structures resembling diatoms were found on portions of the eroded shell and in many of the pits previously covered by the periostracum. Visual identification of several diatom species, and X-ray maps of Ca and Si, confirmed the diatomaceous nature of these structures. The prodissoconch (larval shell) of the juveniles were partially covered by large aggregations of diatoms, with disorganisation of the periostracum and erosion of the shell being evident. The FSCPD periostracum had numerous diatoms attached, with some appearing to be either enveloped by / or boring into the periostracum.

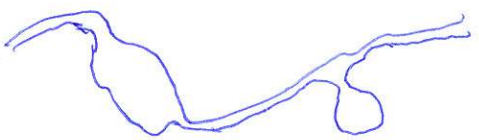
This apparent 'boring' suggests an almost parasitic behaviour for these previously innocuous diatoms. Those diatoms belonging to the genera *Nitzschia* can live heterotrphically and may be using the organic material of the shell and periostracum as a carbon source. The diatoms may just be agglutinated to the shell and periostracum, as is the case for coccoliths found on pectenids from hydrothermal vents, but the concentration around the prodissoconch suggests that diatoms are sticking to the pelagic larvae, and colonizing them thereafter.

Preparation of Nucleic Acids for EM

Dr. Lesley Coggins

Plasmid DNA can be characterised by EM. The DNA^+ is mixed with cytochrome c^- which coats the DNA strands and makes them thicker. A slide is dipped into ammonium acetate or Tris (etc) in a plastic dish; then pipette the DNA mixture down the slide and it will spread to a monomolecular layer on the slide. (Sprinkle talc on the slide to see this - the talc is pushed back as the DNA spreads). Pick this up with a (collodion-coated) grid, stain with uranyl acetate, dry with alcohol, shadow in a rotary evaporator with Pt/Pd at low angle.

Plasmids are circular DNA, and may be supercoiled. The length of the loop depends on the number of base pairs etc. The addition of formamide extends these to single-stranded loops. Restriction enzymes cut the DNA to facilitate plasmid inclusion. The DNA is denatured; then re-annealed. If the pairs do not match exactly (hetero-duplex), you have a loop where the plasmid has been inserted or delated, and therefore the base pairs are not in common.

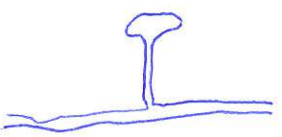


More formamide gives more mis-matches; 40% - 80%.

Bovine papilloma virus may cause cnacer.

Θ loop, or "mouse ears", from slipped-strand hybridisation, is always at the same distance from the end, and the same size; it maps in Variable Tandem Repeat.

Inverted repeats give snap-back structures; e.g. transposon which transfers resistance to antibiotics.



RNA can be hybridised onto DNA.

A microanalytical study of bone mineral from mice with partially deleted genes for human procollagens

L. Babaei-Mahani

Skeletal dysplasia is a genetic condition. A mouse model for Ca:P in bone mineral can be made by inserting human collagen gene. The result is osteogenesis imperfecta, and chondroplasia. Type 1 - 0.1 collagen mutation has a lower Ca:P ration; type 2 mutation has normal ratio. Type 1 is found in bone, Type 2 in cartilage; Type 1 is between the fibrils, as centre of mineral nucleation. Teeth are also affected.

The corneal endothelium in the iridocorneal-endothelial syndrome: an ultrastructural and immuno-EM study

Simon Levy

The cornea in this syndrome is grossly abnormal, the 'ICE' syndrome. Normally the cornea has a smooth surface; ICE cells have microvilli, numerous desmosomes, and intermediate keratin filaments - similar to a corneal epithelium. Immunological studies using LRGold and Lowicryl, were done for fibronectin and Type 1 collagen.

Comparison of a variety of immuno-cytochemical techniques at EM level to demonstrate the morphological distribution of a regulatory peptide in damaged human intestinal epithelium

Tareq Ansari

(very nice low-power pictures!) Crohn's disease; initially an attempt was made to produce an antibody in-house, but commercial antibody was better. Silver enhancement of the gold helps to avoid false positives.

Interaction of liposomes with blood plasma: an SEM study

John Giannios

Liposomes can be used as carriers for drugs internally, also for viruses and many other things, in the lipid or the aqueous phases. A variety fo routes into the body are used. ? opsonins released ?

EM techniques and the investigation of exceptionally well-preserved fossil material

Philip Wilby

Fossilised fish muscle etc; treatment with weak acid showed the structure of the gills, gut epithelium with microvilli, eye of shrimp in stomach. Comparison with fresh muscle showed that these specimens had been fossilised within 55 hours of death. Phosphate ions were derived from the outside, not from the fish itself. If bacteria were present around the muscle, the preservation was poor. If apatite crystals were present, preservation was excellent. A phosphate-rich environment is very important; possible after a storm? The fossila re

found within calcium carbonate concretions.
Soft tissue fossils were found from a variety of animals, including from Pterodactyls, but never from squids, perhaps because of the ammonia concentration.

An ultrastructural analysis of stem cell differentiation in the rat liver

T.V. Anilkumar

Liver can regenerate a great deal. Experimentally, there was a mass proliferation of "oval" cells around the hepatic ~~xxxx~~ space.
It was possible to demonstrate migration through the liver, so as to be able to differentiate the cells. These were probably stem cells for the liver.
Oval cells stain less, possibly because of the metabolic rate and immaturity.

Boring diatoms? Observations of an antarctic bivalve

Carl Bryter

The specimens were taken in the South Orkneys. At a 5 metre depth, an 80-year old shell is 30 mm long; eroded at the hinge. The bivalve gets an incrustation of diatoms on the juvenile shell; soak in hydrogen peroxide for about 40 minutes to remove these. XRMA etc shows the silicon from the diatoms, at the bottom of pits in the bivalve shell. The diatoms are evenly spread over the shell.

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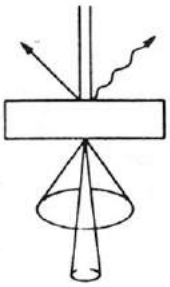

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REGISTRATIONS FOR SEMT MEETING - 4 Dec 1992

T V Anilkumar	Dept of Histopathology, RPMS
Tareq Ansari	EMU, Royal Postgraduate Medical School
Paul Ansell	Hitachi Scientific Instruments
L Babaei-Mahani	Institute of Orthopaedics, Stanmore
Pauline Barber	EMU, Inst. of Dental Surgery
Sue Barnes	Natural History Museum
Richard Blackburn	Environmental Sciences, Univ of Greenwich
Lynda Bonner	Dept of Plant Sciences, Univ of Reading
John Bredl	Royal Veterinary College
Carl Bryer	Dept of Earth Sciences, Open University
John-Paul Cassella	Inst. of Orthopaedics, Stanmore
Don Claugher	Surbiton, Surrey
Lesley Coggins	Beatson Inst. for Cancer Research, Glasgow
Heather Davies	Biology Dept, Open University
Barry Dowsett	CAMR, Porton Down
Anne Drewe	Charing Cross & Westminster Hospital
John Giannios	School of Pharmacy
Karen Gresty	Natural History Museum
Gisele Hodges	Imperial Cancer Research Fund
Chris Jones	Natural History Museum
Louisa Jones	Natural History Museum
Lynne Joyce	Agar Scientific Ltd.
Mike Kelly	London Hospital Medical College
Simon Levy	Charing Cross & Westminster Hosp. Med School
Jill Lewis	EMU, St Bartholomew's Hosp Med Coll
David McCarthy	School of Pharmacy
Maria McCrossan	Dept of Histopathology, LSHTM
Paul Monaghan	Inst. of Cancer Research, Sutton
Nicola Mordan	Inst. of Dental Research
Jenny Plummer	Royal Veterinary College
Catherine Sarraf	Dept of Histopathology, RPMS
Rosemary Suswillo	Royal Veterinary College
Chris Walker	Philips Analytical
Philip Wilby	Dept of Earth Sciences, Open University
Naomi Williams	Open University