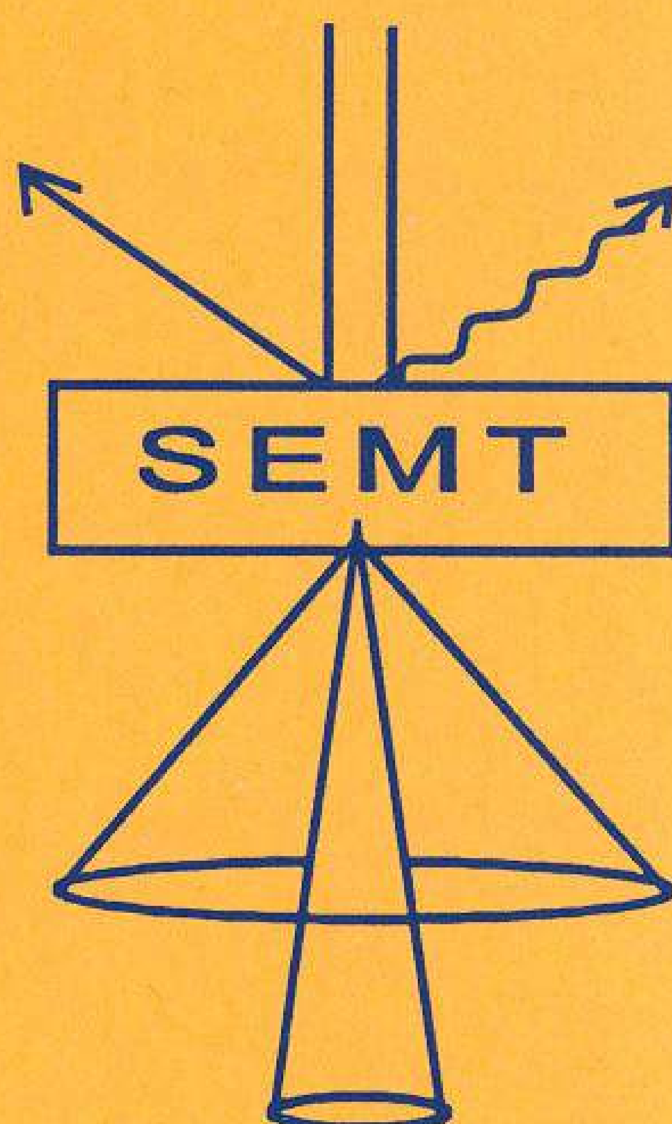
SEMT

MILLENNIUM MICROSCOPY 2000

Thursday, Friday 3-4 February

OPEN UNIVERSITY MILTON KEYNES



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

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FUTURE PROGRAMME

Wed 04 May	2.00 p.m.	**Tea and Tuition III**	School of Pharmacy
Wed 18 Oct		Excursion	(to be announced)
Wed 13 Dec	2.00 p.m.	**Multidisciplinary Approach**	School of Pharmacy

PROGRAMME

February 3, 2000

12.30 **REGISTRATION & BUFFET LUNCH; TRADE EXHIBITION**

1.45 Welcome by the Pro Vice Chancellor of the Open University
Professor Alan Bassindale

1.50 Introduction by Don Claugher

SESSION I Chair: Don Claugher

2.00 Dynamics of membrane traffic and protein targeting in eucaryotic cells
Dr John Lucocq (MSI/WTB Complex, Dundee)

2.30 Gilding neurones in the brain
Professor Peter Somogyi (Anatomical Neuropharmacology, Oxford)

3.00 The structure of articular cartilage
Dr Iolo ap-Gwynne (Inst of Biological Sciences, Univ of Aberystwyth)

3.30 **TEA**

SESSION II Chair: Pauline Barber

4.00 Cryo-EM of chaperonins and viruses.
Dr Alan Roseman (MRC Lab of Molecular Biol., Cambridge)

4.30 Biological X-ray microanalysis; as easy or as difficult as you make it
Dr Alice Warley (Dept. Ophthalmology, St Thomas's Hospital)

5.00 No you don't...O yes we do....the continuing need for diagnostic viral EM
Professor Dick Madeley (Stocksfield, Northumberland)

5.30 Trade Exhibition and Poster display

6.00 **Pre-dinner drinks sponsored by Jeol UK Ltd.**

7.30 **Transport to Conference dinner**

PROGRAMME

February 4, 2000

SESSION III **Chair: Barry Dowsett**

9.30 Imaging ions and dynamic events; correlating confocal microscopy with EDX
Dr Jeremy Skepper (Multi-imaging Centre, Univ. of Cambridge)

10.00 Imaging mitochondrial function in living cells
Professor Michael Duchen (Physiology Dept., Univ. College London)

10.30 Improvements in fluorescence laser scanning microscopy using multi-photon excitation
Dr Andrew Dixon (Bio-Rad Microscience)

11.00 **COFFEE**

SESSION IV **Chair: Jeremy Skepper**

11.30 Biological applications of scanning probe microscopy
Professor Martyn Davies (Pharmaceutical Sci., Univ. of Nottingham)

12.00 Application of focussed ion beams to the preparation of TEM samples and micromachining
Dr Richard Langford (Dept of Materials, Oxford University)

12.30 **LUNCH, DISPLAY OF POSTERS & MICROGRAPHS; TRADE EXHIBITION**

SESSION V **Chair: Heather Davies**

2.30 Environmental scanning EM; an update
Dr Chris Gilpin (School of Biological Science, University of Manchester)

3.00 Holey bone and the SEM
Prof Alan Boyde (Anatomy Dept., University College, London)

3.30 Martians under the microscope
Dr Monica Grady (Natural History Museum, London)

4.00 **TEA** **Prize presentations**

INTRODUCTION

Don Claugher

(Past Chairman of SEMT)

It gives me great pleasure to enter the millennium as a member of the Society, which I have been associated with for many years. I can honestly say that they have been the most fruitful and interesting years of my life.

The raison d'être for our Society dates back to the year 1933 when the electron microscope as we know it today was developed by Ernest Ruska in Berlin, who was working in a team headed by Professor Matthias investigating the nature of the electron beam. 1933 is also significant for two other events - it was the year that Adolph Hitler came to power and also the year that I was born.

The electron microscope was not an immediate success and Adolph Hitler effectively prevented further innovative work in Germany by his ambitions for global domination - microscopy it seems was not very high on his list of priorities.

The first practicable commercially available electron microscope was built by Metropolitan Vickers for Imperial College in 1936 with a grant from the Royal Society. The second World War interrupted much of the subsequent work on this new type of microscope, but when the war ended in 1945 scientists from all over the world were once again able to exchange ideas and to discuss problems freely. The result of this was a burgeoning of ideas associated with electron optics, and manufacturers were quick to grasp the new ideas that were being discussed.

AEI were in the forefront, and produced the EM6B which was to be (in this country at least) the workhorse of transmission microscopy for many years to come. One of the development team was a young Alan Agar, who many years later set up Agar Scientific and continues his interest in electron microscopy to this day.

France, Germany, America, Canada and eventually Japan all started to produce electron microscopes. It was an exciting and at times a confusing and conflicting scene. RCA had continued experimenting with electron optics during the war years, and so when it ended they were in a position to supply a fairly reliable electron microscope to the scientific community. Professor Wycoff who was attached to the American embassy in London imported and installed an RCA instrument in the embassy itself. Wycoff used to invite people of some scientific standing to come and see and use his instrument; one of these was Professor J.Z.Young.

I was fortunate to be in J.Z.Young's team at University College working on nerve structure. The invitation to examine some of our material using an electron microscope caused great excitement. We who were all proficient at cutting wax sections for optical microscopy were told that the sections required for the electron microscope had to be much thinner than the 7-10μm sections that we normally produced. This problem was eventually solved by fracturing plate glass to produce glass knives and selecting appropriate areas. The glass knife together with the specimen was attached to a small rotary microtome and the whole lot was cooled down in a freezer. When it was thought that the microtome was cold enough it was removed from the freezer and the handle rotated - as the metal of the microtome expanded to room temperature it provided the forward movement necessary to produce the ultra-thin sections.

I can well remember the excitement when a week or so later J.Z.Young came into the preparation room with a hand full of photographs. We all gathered around the large central table and watched as he made up a montage of a cell with a nucleus as big as a tennis ball, and other associated structures such as the Golgi apparatus. That was all 46 years ago. A lot has happened since then as you all know, but nothing to eclipse that first experience of seeing an electron micrograph that you had helped to make.

We the SEMT are not the oldest microscopical society - that distinction belongs to the Queckett; we are not the largest - the Royal Microscopical Society is. However I think that it would be true to say that we are the most diverse, covering more disciplines and methods of investigation than the other two put together and that makes us quite unusual.

Our society was founded by a group of enthusiastic young people such as Derek Lovell, Chris Walker, Steve Chapman, Pauline Barber and Bill Edwards and others. The object of their coming together over a period of time was to exchange ideas and communicate results informally - this informality continues and I think it is one of the great strengths of our society.

Through our various members and (needless to say) the microscope manufacturers we as a Society have kept abreast with the rapidly changing technology and I hope will continue to do so. Our strength does not lie in our size but in the minds of our members, many of whom have made significant contributions to the progress of instrumentation and scientific thought.

I earnestly hope that we all continue to pursue our philosophical and practical investigations and that we will grow in strength in the millennium.

ABSTRACTS

SESSION I

Dynamics of membrane traffic and protein targeting in eucaryotic cells

Dr John Lucocq (MSI/WTB Complex, Dundee)

Quantitative immunoelectron microscopy provides a high resolution overview of protein distribution and movement in eukaryotic cells and in particular has enhanced our understanding of membrane traffic and protein targeting pathways. The method of choice is immunogold labelling of Tokuyasu thawed frozen sections combined with stereology to estimate the sizes of reference compartments/structures. This approach has helped our laboratory to gain insight into how the secretory pathway is shutdown and modified structurally during animal cell mitosis and has produced results which challenge established dogmas of Golgi apparatus cisternal stack organisation. Recently quantitative immunoelectron microscopy has also provided insights into how insulin signalling is regulated at the plasma membrane. These developments will be reviewed.

Gilding neurones in the brain

Professor Peter Somogyi (Anatomical Neuropharmacology, Oxford)

The structure of articular cartilage

Iolo ap Gwynn (Institute of Biological Sciences, University of Wales, Aberystwyth)
e-mail: iag@aber.ac.uk
[Joint authors: S. Wade, M. J. Kääb, G. Rh. Owen, R.G. Richards, K. Ito]

By applying freeze-substitution based microscopic techniques, entirely new aspects to the structure of articular cartilage were revealed. Conventional fixation techniques destroy the structure and the reasons for this are discussed. Samples were prepared for SEM and TEM. Freeze-fractured, substituted and critical point dried tissue was examined using an FESEM. As a result of this work, we are proposing a radically new structural model that is consistent with all known properties of the tissue. A gel composed of protein-carbohydrate complexes, in association with water molecules, is contained radially in columns (or tubes), each with a diameter of 1-3μm, by a tightly packed matrix of collagen fibrils. Initial interpretation of the results suggested that the structures might have been segregation artefacts of ice-crystal formation. However, closer study revealed that this was not the case and that these were genuine structures. The destruction of this structure by chemical fixation techniques provides indirect evidence which may be of assistance in explaining the way in which the collagen fibrils associate with each other in this tissue. We propose that the load bearing properties of the tissue are explained by the directed flow of the interstitial fluid, modulated by the protein-carbohydrate complexes, along these collagen tubular structures.

SESSION II

Cryo EM of chaperones and viruses

Alan Roseman (MRC Laboratory of Molecular Biology, Cambridge)

Proteins in solution can be vitrified and subsequently imaged in a transmission electron microscope equipped with a cryo specimen holder. These cryo electron micrographs record projections of the macromolecules preserved in a frozen hydrated state, from which it is possible to calculate the 3D structure at a resolution of about 10 to 20 Å.

Transient states of molecules can be trapped by vitrification and mixtures of states separated by classification of images. Structures of the transient chaperonin complex GroEL-GroES-ATP and GroEL-GroES-ADP were calculated using this technique. GroEL is protein-folding-machine, that binds and releases GroES and non-folded proteins in cycles driven by ATP hydrolysis. The atomic structure of the substrate-binding domain was docked into the EM maps, which revealed that the binding site was more accessible in the GroEL-GroES-ADP complex. The crystal structure of the isolated terminal domain of clathrin was docked into a cryo EM map of a clathrin coat, giving more insight into its role in a transport vesicle.

Icosahedral viruses are ideal specimens for cryo EM imaging and 3D reconstruction, being large particulate protein complexes with 60-fold symmetry. Complexes of the hepatitis B core shell and antibody fragments were found to be heterogeneous, which was an obstacle to high resolution reconstruction. However, lower resolution maps (~20 Å) are consistent with the known epitope position and a model of the binding pattern could be deduced.

These examples have shown that cryo EM is particularly useful for probing transient or heterogeneous structures, but there is great potential to extend maps to higher resolution with suitable specimens.

Electron Probe X-ray microanalysis of biological systems - as easy or as difficult as you make it !

Alice Warley, (EMU, Dept of Ophthalmology, Rayne Institute, St Thomas Hosp. London)

Specimen preparation is probably the most important step in any biological study using the technique of electron probe X-ray microanalysis. In this paper I will review two methods in current use for the preparation of specimens. The first is for the study of total ionic content in whole cell preparations and can be used with either freshly isolated or cultured cells. The cells are cultured on, or allowed to adhere to, a plastic support film (either Pioloform or Formvar) supported on gold or titanium electron microscope grids. On completion of experimental procedures the cells are washed, by dipping rapidly into ice-cold distilled water, to remove the external medium and then freeze-dried before analysis. This very simple technique is in use in our laboratories to study the effects of parathyroid hormone stimulation on osteoblasts and in determining the effects of apoptosis in a monoblastoid cell line.

The study of ionic concentrations in intracellular organelles is more difficult. For such studies it is important that the specimen is cryofixed under known physiological/experimental conditions, and simple dissection of a tissue followed by cryofixation can not be used. It is often necessary to construct a specialised apparatus. Once cryofixed the specimen is cryosectioned to provide thin sections which are freeze dried before analysis. We have developed a cryoclamp for the fixation of papillary muscle preparations at known time points in the contraction cycle. This clamp is being used to study the development of intracellular ionic gradients in heart muscle cells.

"No you don't ... Oh, yes we do ..." - the continuing need for diagnostic viral EM

Dick Madeley, Stocksfield, Northumberland.

Pictures and other visual images are very much part of life now - we are constantly invited to recognise things we have seen before. Microscopy, and particularly EM, fits well into this format - recognition is almost instantaneous and frequently reliable. Given the constant structure of many viruses and that infection produces vast numbers of new viruses, EM is tailor-made for diagnostic purposes but has now become unfashionable. This comes from a failure to understand fully the arguments for and against its use. Its use is neither so expensive, nor so cumbersome, as some believe, but does depend on skill and judgement, both attributes that are also out of favour as being too subjective.

Seen from the point of view of the operator, diagnostic EM is enjoyable, rewarding and the work-load is manageable, if a trifle daunting at busy times. Administrators look for tests that are quick, cheap and reliable. The speed of EM is undeniable; compared with any other routine test, it is (wrongly) perceived as expensive because of the capital cost. Nevertheless, the running costs are low, and one EM can do the equivalent of 6 or 7 assays. Reliability depends on the quality of the operator -impossible to quantify, and hence unpopular with auditors.

Nevertheless, I believe very strongly that the benefits far outweigh the disadvantages - there is a lot of mileage yet in a most satisfying technique !

SESSION III

Imaging ions and dynamic events: correlating confocal microscopy with EDX

J.N.Skepper (Department of Anatomy, University of Cambridge)

The confocal microscope is an ideal tool for the study of dynamic events in live cells. It is possible to introduce fluorescent dyes into cells and to use such dyes to follow their responses to external stimuli such as the application of pharmacological or pathological agonists. We have a particular interest in the early events associated with apoptosis of human monocyte/macrophages. Recent studies have implicated changes in intracellular potasssium concentration as an early and pivotal event in the apoptotic pathway for monocyte/macrophages. The classical morphologic techniques used for the in vitro or ex vivo demonstration of apoptosis i.e. Hoescht staining (vital staining), nuclear pyknosis (histology), TUNEL (cytochemistry) or chromatin condensation (TEM) all demonstrate late/terminal stages in the apoptotic process. A more recent in vitro assay uses annexin-V-fluorochrome binding to translocated phosphatidylserine to identify apoptosis. Annexin-V will also stain necrotic cells but in a different time course and with a different staining pattern, thus enabling a clear discrimination between necrosis and apoptosis. In this study we have induced apoptosis by exposure to gliotoxin and used the confocal microscope to determine if early stages of apoptosis (reduced intracellular K) coincide with annexin-V binding.
Monocytes were seeded onto Formvar coated, gold, EM finder grids and viewed with a Leica TCS-SP-MP equipped with a temperature controlled, stage. Cells in identified grid squares, were followed through time, after exposure to gliotoxin, in the presence of annexin-V-FITC or annexin -V-alexa568 and propidium iodide or BOBO-1 respectively. The grids were quench frozen at identifiable times after exposure to gliotoxin, freeze-dried and analysed in a Philips XL30-FEG equipped with an Oxford Instruments GEM spectrometer. Cells that were unstained with Annexin-V or Propidium iodide showed high K/Na. Cells that stained with annexin-V only showed lower K/Na and cells stained with annexin-V and propidium iodide showed reversed K/Na.

Imaging mitochondrial function in single living cells.

Michael R Duchen (Dept of Physiology, University College London)

Mitochondria play a central role in the economy of almost all cells and tissues: they provide the major source of energy as ATP, they may accumulate calcium and so help shape calcium signals which play so central a role in cell function, they release factors which initiate the systematic destruction of the cell (programmed cell death or apoptosis) - a process central to normal development but also central to much disease when triggered inappropriately. Last but not least, they define the requirement of the organism for oxygen.

Understanding of the fundamental mechanisms that define the function of mitochondria was achieved between the 1950's through to the early 70s through studies of mitochondria isolated from tissues. However, a major challenge remained in the exploration of the functional interplay between mitochondria and their host cells. Until recently, we knew little of how mitochondria might respond to changes in cell signalling, exemplified by the response to changes in [Ca2+]i . Little is known about how mitochondrial function might be specialised in relation to the functional demands and requirements of different cell types within complex tissues. In terms of understanding pathophysiology and disease, little was known about how alterations in mitochondrial state - during episodes of oxygen deprivation, as a result of mutations, or in response to toxins - might influence the function of the cell.

I will describe how an array of fluorescence probes may be used to allow us to follow changes in mitochondrial physiology in real time, in living cells. Imaging epifluorescence microscopic systems, including confocal microscopy, has allowed us to ask exciting questions about mitochondrial responses to the routine events involved in cell physiology, and conversely, to explore the consequences for cellular function of alterations in mitochondrial state. I will try to illustrate how this approach is now helping us to understand the fundamental cellular processes that lead to cell death in some pathological states.

Improvements in fluorescence laser scanning microscopy using multi-photon excitation

Andrew Dixon (Bio-Rad Microscience)

Laser Scanning Confocal Fluorescence Microscopy is now established as a routine tool for biomedical research with several thousand instruments in use world wide. As with any powerful technique new instrumentation capabilities invite new applications. For the LSM these new applications are particularly in the study of living cells and tissue, a direction of development very much driven by the need to study the function of genes and proteins in real life. The study of living material puts new demands on the optical microscope. It must be able to look into tissue that may scatter light quite strongly and must be able to collect images over an extended period of time without toxicity. The development of Multi-photon Laser Scanning Fluorescence Microscopy goes a long way to meeting these requirements.

This talk will introduce the basic concepts of multi-photon microscopy and then, with real examples, explain how it allows imaging deeper into samples and imaging for longer than is typically possible by confocal microscopy.

Since multi-photon imaging is still a relatively new technique the pace of development in the instrumentation has been rapid. This talk will attempt to put these developments into context and describe some of the areas where there is need for further development.

SESSION IV

Biological Applications of Scanning Probe Microscopy

Martyn Davies (School of Pharmaceutical Sciences, University of Nottingham)

The last decade has seen a major growth in the application of scanning probe microscopy to study a wide range of biological systems. This talk will provide a personal perspective on the role of SPM for the study of biomolecules, and biomolecular assemblies and biomaterials. Molecular resolution imaging of biomolecules will be reviewed and the emerging role of SPM in the study of dynamic processes and molecular recognition events will be discussed. The examination of functional surface engineered biomolecular systems at biomaterial interfaces will also be discussed. The direct measurement of biomolecular forces using SPM suggests the technique will become a powerful biophysical tool. The study of dynamic processes such as hydration, degradation, adsorption and recognition at surfaces will also be highlighted. Finally, SPM can provide important information on the physicochemical properties of biomedical interfaces and the current status will be reviewed.

Application of focused ion beams to the preparation of TEM samples and micromachining

Dr Richard Langford (Dept of Materials, Oxford University)

Focused ion beam (FIB) systems are becoming increasingly used for the preparation of transmission electron microscopy (TEM) cross-sections because the TEM cross-sections can be positioned to within ± 50 nm, can be prepared with near parallel sides and can be prepared in under 2 hours.

Currently, there are two techniques generally used for the preparation of TEM cross-sections using FIBS, the lift-out and the trench technique. These two techniques are reviewed and their relative advantages and disadvantages are discussed.

The FIB thinning of the TEM cross-section damages and implants the milling species into the sidewalls of TEM cross-section. The depth of the damage in silicon is of the order of 20 nm for a 30 keV gallium ion beam. The effects of using low energy milling, gas assisted etching and broad ion beam milling to reduce the depth of the damage are discussed.

FIBs are also becoming used for a wide range of micro-machining applications. The areas in which FIBs are currently being used, such as ion beam lithography and modifying atomic force microscopy tips, are discussed.

SESSION V

Environmental scanning EM – an update

Chris Gilpin (Biological Sciences Manchester University)

Environmental scanning electron microscopy (ESEM) has been available as a commercial instrument for approximately ten years. The author's lab has had one of these microscopes for 9 years during which time a wide variety of samples have been examined. In addition to biological samples the microscope has been used for many other specimens from materials science, chemistry and pharmacy.

New instruments are now available which make use of a wider range of electron sources with tungsten, LaB6 and field emission guns all capable of being fitted to an ESEM. There remains some confusion as to how ESEM differs from other low-vac microscopes.
The aim of this presentation is to review a wide range of applications to which ESEM can be applied and to discuss where differences exist between ESEM and other technologies. In addition some comments on the likely future direction of this type of technology will be made.

Holey bone and the SEM

Alan Boyde (Department of Anatomy, University College London)

Mineral density distribution using backscattered electrons (BSE)

The 'density' of bone has different meanings to workers in various branches of applied osteology. We generally use the term to refer to the density at the scale of a volume of matrix smaller than that occupied by an osteocyte. At this scale, microradiography has insufficient spatial resolution. We have therefore developed methods for the quantitation of electron backscattering from topography free specimens. The mean degree of mineralisation of lamellar bone increases with age. Thus the reduced amount of bone tissue is expected to be stiffer (more brittle) due to the increased mineral packing density. Woven bone is more densely mineralised than lamellar bone in accordance with the greater water content of its matrix. However, microcallus, the type of woven bone deposited upon old lamellar bone trabeculae in crush fractured vertebral bodies, is, at least initially, poorly mineralised in comparison to lamellar bone.

Architectural changes in the ageing of trabecular bone.

Vertebral body spongiosa in younger, mature adults is evidently 'porous' when prepared as cleaned, macerated thick transverse sections (TSs). In either the more finely divided superior or inferior portions or in the more grossly partitioned central section of the body, many large tubular marrow spaces penetrate a 4 mm thick TS: However, 4 mm longitudinal sections will almost entirely obstruct the a beam of light or electrons. The bone resembles a honeycomb with less than perfect walls between cells. The same bone in an ageing individual is a latticework of rods, and the original curved plates are far less extensive. In bone from aged individuals, including those with relatively high volume fraction for their age, substantial direct light transmission can be seen through clearings in any direction of view through 4 mm square longitudinal beams. These architectural changes are associated with loss of tissue occupancy by bone, but the increased 3D extent of individual, line of sight pore channels is greater in proportion than the net loss of bone tissue per unit volume.

SEM of bone surfaces in ageing and osteoporosis

Current opinion regarding the sequence of events leading to the development of bone porosis is strongly influenced by the idea of the BMU and the activation, resorption and coupled formation hypothesis, which subscribes to the view that porosis is the end result of a large number of cycles in which a standard packet of resorption is replaced by an inadequate packet of repair. SEM study of bone surfaces cleaned of cells can locate newly deposited bone packets by their lower backscattering than surrounding surface bone, whether this is

fully mineralised or resorbed: such images refine the oversimplified models of trabecular bone remodelling which permeate the literature. The examination of trabecular surfaces in more porotic bone slices allows us to survey a very high proportion of surfaces more rapidly and efficiently than could be achieved with light microscopic sectioning methods. From such surveys, we can affirm that new bone packet deposition occurs at sites of prior resorption, but often elsewhere, and many resorbed sites remain unrepaired. This leads to a simpler explanation for osteopenic change than that currently favoured. When confirmed through more, and more quantitative, study, this conclusion should lead to a shift in emphasis in attempting to unravel the complex interactions of cells and cytokines in the control of bone remodelling.

Abnormal mineral fronts

Buried faults, undetectable by routine histomorphometric procedures, may be a part of the whole complex of changes which render bone incapable of withstanding loads which would otherwise not be dangerous. Bone surfaces cleaned of both cells and non mineralised matrix are at least superficially anorganic; the cleaning may be achieved by proteolytic enzyme treatment at alkaline pH, followed by hydrogen peroxide at neutral or slightly alkaline pH. In young mature adults, such treatment will leave a pattern at the bone surface indicating that the most superficial collagen fibres of bone matrix were mineralised A higher incidence of an abnormal mineral front is found in aged and particularly in osteoporotic individuals. One unrecognised fault in old bone may be that it accumulates imperfect resting cement lines, where poorly mineralised matrix is sandwiched between the old mineral front (deep to the surface prior to new bone deposition), and the newly initiated mineralisation process within the matrix of the new packet. The further occurrence of defective resting cement lines can be shown in the repair of crush fractures in vertebral cancellous bone. BSE SEM images show that the newly deposited, relatively poorly mineralised callus bone is separated by from the well mineralised, lamellar, cancellous bone upon which it is deposited by a poorly- or non-mineralised layer.

Where next?

Microscopic analysis of surfaces and volumes of ex vivo tissue will have to be employed to define, refine and confine that data which can be obtained in vivo, because bone density cannot be correctly determined in vivo. Even with the highest resolution commercially available x-ray microtomography (μCT: which is only possible with small laboratory species), the voxel size is big relative to the dimensions of trabeculae and soft tissue (cell) filled spaces and there are other reasons for uncertainties in the reconstruction. In understanding skeletal quality at the tissue level, composition, fabric organisation and orientation, anisotropy, and architecture are all of high importance. The definitions of bone histomorphometry leave out spaces within calcified bone tissue at any particular working scale (e.g. cell lacunae) and mineral packing density in the organic matrix, yet the last is of critical importance in the physical properties of elemental tissue volumes. Microradiography, scanning x-ray microscopy and μCT can allow the estimation of micro-scale mineralisation density. None of these compare favourably with the resolution of electron optical or even optical microscopy, but these cannot be applied to relevant samples in the living patient.

Martians under the Microscope

Monica M. Grady (Natural History Museum, London)

There are over 22,500 meteorites whose compositions have been studied; the overwhelming majority of these specimens originate from the Asteroid Belt, and formed as the Solar System accreted from the cloud of gas and dust that composed the pre-solar nebula. These "regular" meteorites have crystallisation ages corresponding to the formation of the Solar System, and so are around 4560 Myr. old.

There is, however, a group of (currently) 14 meteorites that are believed to originate from Mars, having been broken from the planet during a series of impacts over several million years. These meteorites are igneous rather than sedimentary in nature (i.e., they formed by crystallisation from molten material, not deposition from water), but they bear traces of low temperature aqueous processes that can be used to infer conditions on the martian surface. There is overwhelming evidence for the type of environmental conditions on Mars in which life could have survived in the past, when the planet had a thicker atmosphere and liquid water was stable at the martian surface. The martian meteorites have been altered by weathering, leading to the production of secondary minerals (clays, carbonates, sulphates), associated with which are low concentrations of martian organic material. It has thus been suggested that these meteorites might contain evidence for a martian biosphere.

Almost four years ago, a team of US scientists claimed to have found evidence for a primitive "fossilised martian biota" in carbonates within the ALH 84001 martian meteorite. SEM images of freshly-broken surfaces of the carbonate grains exhibit parallel ridge-like features. The features, about 200nm long, were too small for elemental analysis, but on the basis of morphology and association with organic material, were interpreted as microfossils, although the interpretation has not entirely been accepted by the scientific community. Building on results obtained from study of martian meteorites, an outline of what we can learn about Mars, and the possibilities for life on Mars will be presented at the meeting.

POSTERS

Postnatal development of the rat myenteric plexus

S. Jones and M.J. Saffrey, (Department of Biology, The Open University, Milton Keynes)

The myenteric plexus lies between the smooth muscle layers of the gastrointestinal (GI) tract and plays an essential role in regulation of GI motility. Postnatal development of the myenteric plexus is poorly documented so we employed a variety of techniques to investigate postnatal development in the rat ileum. Ultrastructural analysis revealed early postnatal (7 day) neuron and glial profiles to be relatively mature and exhibit a similar variety of shapes to that observed in the adult rat and guinea pig. The basal lamina which surrounds adult myenteric ganglia, was found to be developmentally acquired. The number of smooth muscle cells was found to increase until 21 days when their numbers stabilised and size appeared to increase. Immunohistochemistry for PGP 9.5 and correction for growth, revealed changes in the density of myenteric neurons during postnatal development, the most striking being a significant increase ($P<0.05$, ANOVA) in neuronal density between postnatal days (PND) 7 and 14, implying an increase in neuron number in the myenteric plexus. Neurons of the NADPH-diaphorase containing subpopulation were among those that increased in number during this time. Neuronal density decreased significantly ($P<0.05$, ANOVA) between PND 14 and 21, possibly indicating a period of naturally occurring cell death. There was no change in the density of the NADPH-diaphorase containing neurons during this time and total neuronal density appeared to stabilise at 35 days. In summary, although myenteric ganglia are relatively mature in early postnatal life, a number of important changes occur postnatally and maturity is not complete until after the first postnatal month of life.

Ionic changes in parathormone stimulated Saos-2 cells analysed by X-ray microanalysis

A.Warley, F.McDonald. GKT School of Medicine and Dentistry, London.

The aim of this investigation was to determine ionic changes in osteoblast-like cells (Saos-2) stimulated with parathyroid hormone (PTH).

Saos-2 cells were cultured in modified Eagles medium with 10% foetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B, at 370C in a humidified atmosphere of 5% CO2. Cells were then seeded onto Pioloform covered gold electron microscopy grids at a density of 40 x 10^3 per well. Twenty-four hours before the experiments were undertaken the foetal calf serum was removed. Grids with cells attached were exposed to 2.5 µM PTH for 0,5,15, 30 and 60 seconds, then washed in distilled water at 0°C for 10 seconds, dried and immediately immersed in liquid nitrogen. The grids were freeze dried overnight and carbon coated. Areas of cytoplasm and nucleus (2 µm2) were analysed for 60 seconds live-time using a Zeiss electron microscope with a Link AN 10,000 microanalysis system. The concentrations for Na, Mg, P, S, Cl, K & Ca were determined in control cells ($n = 15$) and agonist stimulated cells ($n = 15$).

There was an initial drop in element concentrations between 5 and 15 seconds of stimulation which was significant at $P < 0.001$ for the elements Na, K and Ca. The concentrations of these elements recovered almost reaching their initial concentrations by 60 seconds of stimulation. These findings imply volume changes possibly due to the activation of ion channels in the cytoplasmic membrane. To establish if these volume changes were G-protein linked we incubated cells in pertussis toxin (500 ng/ml) for 2-3 hours prior to stimulation and repeated the stimulation with PTH ($n = 15$). Complete elimination of the volume changes clearly demonstrated a strong G-protein control.

S.E.E.C. RUMANIA - UPDATE 2000

Jenny Anne Drewe

The Romanian economy is still in a very bad way, and unemployment is high.

The universities and research institutes are desperately in need of good equipment and recent books and journals, but items from abroad must be paid for with hard currency.

In 1993 a group of SEMT members set up S.E.E.C. Romania (Scientific, Environmental and Educational Collaboration with Romania) to help our fellow scientists, and we have been able to send equipment and books surplus to requirements here; to a wide range of institutes in Romania. An update is given about our achievements, with information about some of the institutes and projects which we have been able to assist.

If you would like further information or have items to offer, please contact Jenny Anne Drewe, 21 Westmore Court, Carlton Drive, London SW15 2BU; tel./fax. 020 8785 6911.

Surface Effects of Soft Drinks on Exposed Dentine Sections

D G Gillam, N Khan and N J Mordan

Concern has been expressed with regard to the frequent and excessive consumption in the general population of acid foods and drinks such as citrus fruits and fruit-based and carbonated soft drinks. Little is known regarding the erosive potential of these foods/drinks though evidence from child studies appears to demonstrate an association between erosion and frequency of consumption of acidic drinks. One reason for this may be that most soft drinks have a low pH (3-4) and high titratable acidity. The aims of the present study were to examine the effects of 11 soft drinks on the unetched dentine surface and to examine these effects at two time periods, 5 and 15 minutes.

Of the drinks tested, those termed 'sports drinks' were observed to etch the surface strongly. The majority of drinks containing fruit acids were moderate to severe in their ability to etch, including the BDA approved, so-called 'tooth kind' product. The drinks containing vegetable extracts were of moderate to low etching capacity. Most surprisingly the cola-based drinks appeared to have low etching ability and had the weakest capacity to remove the artificially created smear layer.

All the drinks contained citric acid and many also included other added acid components. However the cola drinks contained phosphoric acid which, although capable of etching, also has a precipitative effect. It is possible that the combination of increased awareness of oral hygiene and greater frequency of intake of carbonated drinks may, in the future, increase prevalence of erosion and dentine sensitivity in the general population.