SEMT

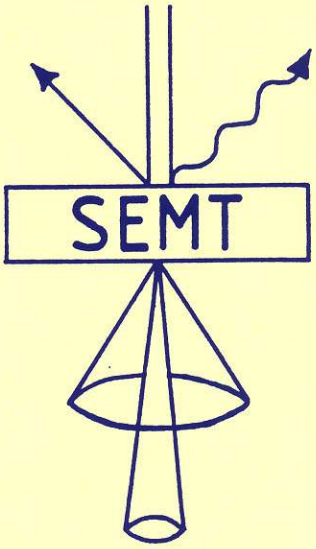
One Day Meeting, 1992

Friday, 9th October, 1992

Institute of Dental Surgery

Gray's Inn Road

London, WC1



THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

Prospective members should contact Jill Lewis (the Secretary), Electron Microscope Unit, St Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ for an application form. Current members of the committee are listed below, and are available for further information.

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The SEMT wishes to express special thanks to:

The Institute of Dental Surgery, as hosts and caterers;
Leica UK Ltd, for supplying the folders and drinks;
and to the following companies for attending the trade exhibition:

(in alphabetical order)

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The SEMT hopes that you will find the One Day Meeting interesting and informative, and that you will be able to attend our forthcoming meetings. The next meeting of the Society will be the Annual Award for Young Researchers, and will take place at 1400 on Friday, 4th December, 1992 at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London.

WELCOME TO THE ONE DAY MEETING

Friday, 9 October, 1992

Conference Centre
Institute of Dental Surgery
Gray's Inn Road
London WC1

PROGRAMME

0930	Registration and Coffee
1030	Introduction by Professor Thorogood *(Chairman, Division of Oral Pathology)*
1040	Quantitative XRM: Why Bother? *Alice Warley (St Thomas' Hospital)*
1120	Microanalysis of Fossils and Archaeological Bone *Terry Williams (Natural History Museum)*
1200	EM in Investigative and Therapeutic Studies of Hyperpigmented Skin Disorders *Professor A.S. Breathnach (St Thomas' Hospital)*
1240	Poster Display and Trade Exhibition Buffet Lunch
1415	Things That go Bump in the Dark: Meteorites, Minerals and Misunderstood Microscopes *Anton Kearsley (Oxford Polytechnic)*
1455	A Beginner's Guide to High-Pressure Freezing *Paul Monaghan (Institute of Cancer Research, Sutton)*
1525	Tea, followed by discussion of Poster Communications
1600	Sherry, followed by The Annual General Meeting

ABSTRACTS OF SPEAKERS' PAPERS

Quantitative X-Ray Microanalysis - Why Bother?

A. Warley (Division of Physiology, UMDS, St Thomas' Hospital Campus, Lambeth Palace Road, London, SE1 7EH)

Although x-ray microanalysis has been used for the study of biological specimens for some considerable time now, it is still carried out routinely in only a small number of laboratories. The reason for this is not clear, but may be because quantification is perceived as being difficult, and perhaps not necessary. However, in the study of diffusible elements full quantification of spectra is required for several reasons.

Quantification is necessary when comparing results, since it is impossible to determine by eye the extent of changes which have occurred as a result of experimental intervention. Quantification also allows comparison of results from x-ray microanalysis with those from other techniques, and enables x-ray microanalysis to be regarded as an established technique for physiological investigation. The unique ability of x-ray microanalysis is the ability to determine element concentration at cellular an subcellular levels. The usefulness of this information is lost if quantitative results are not obtained. The benefits of quantitative x-ray microanalysis will be illustrated with results obtained from various different mammalian tissues.

Acknowledgement: The author would like to thank the Wellcome Foundation for financial support.

Microanalysis of Fossil and Archaeological Bone

T. Williams (Natural History Museum)

The number of chemical studies of fossil and archaeological bone have increased enormously over the last 10-15 years, and information from the chemical composition of "old bones" have been used in many scientific disciplines. Archaeologists obtain relative and absolute dates; palaeoanthropologists attempt to gain information of the diet and health of past populations; palaeontologists and sedimentary geochemists obtain information of the processes of fossilisation and on the palaeoenvironment; geologists have used such information as an exploration tool.

Until recently, however, information sought by some of the different groups of scientists have been based on diametrically opposed assumptions involving the chemical changes to bone during fossilisation processes. For many palaeoanthropologists, the lure of chemical studies of fossil bone has been the reconstruction of dietary habits of past populations. Palaeodietary (or palaeonutritional) studies are based on the knowledge that the concentrations of some elements in living bone reflect the diet (or health) of that organism. Major problems that palaeoanthropologists have to address is that of post-depositional alteration (or diagenesis) of the bone, the effect this has on the *in vivo* biogenic element signal, and how best to identify and overcome these effects. Some sedimentary geochemists, however, use those same bone chemical changes that occur in bone during diagenesis to provide information on the palaeoenvironment.

Often, such compositional changes occur at levels below the detection limit of electron beam techniques. However, some examples of the microanalysis of fossil and archaeological bone from

laboratory and field-based studies will be presented using electron probe microanalysis, together with other examples involving proton-induced, synchrotron radiation-induced and neutron-induced microanalysis.

Electron Microscopy in Investigative and Therapeutic Studies of Hyperpigmented Skin Disorders

Professor A.S. Breathnach (St Thomas' Hospital)

The ultrastructure of the melanocyte and the melanin granule was first examined by Barnicot and Birkbeck (1956-59), and their studies led directly to the concept of the melanosome as the sub-cellular site of melanin synthesis (Seiji, Fitzpatrick *et al* 1961-3). Electron microscopy also established the fact that the Langerhans cell is not related to the melanocyte (Birbeck *et al* 1961); Breathnach *et al* 1961-70; Wolff *et al* 1966-70). These studies were fundamental for understanding of the basic biology of the melanocyte and provided for the more extensive analysis of clinical pigmentary disorders and for monitoring results of *in vitro* experimental studies.

This presentation will show how electron microscopy contributed to understanding of the biological properties and clinical application to hyperpigmentary disorders of a naturally-occurring, simple, saturated fatty acid, azelaic acid. It was essential in monitoring results of biochemical, radioautographic and tissue culture experiments, and in establishing the fact that azelaic acid has a biological effect on the hyperactive and abnormally proliferative melanocyte, and therefore potential as a therapeutic agent. Typical results of clinical application will be shown, including a surprising one which emerged during the investigations.

Things That go Bump in the Dark: Meteorites, Minerals and Misunderstood Microscopes

A. Kearsley (Oxford Polytechnic)

Why study meteorites?

They provide the only clear evidence for the early history of our solar system, they show what asteroids are made of - and they are fascinating objects!

Meteorites also provide many excellent examples of problems which face the geologist in interpretation of the composition of minerals and their textures. The traditional research tool of the petrologist has been the polarising, transmitted light microscope, used for visual and photographic imaging of rock thin sections. Until the advent of microanalysis by electron probe the compositional interpretation of rocks and minerals relied upon bulk chemical analysis (largely by titration). The introduction of sophisticated electron scanning imagery has revealed a new scale of structure, and has explained the apparently fluctuating compositional analyses as reflecting fine scale mineral intergrowth and exsolution textures. Indeed, the complexity and range of mineral textures can be so bewildering that they can lead one to wish for a return to the innocent ignorance of the thirty micron "thin" section!

Backscattered electron images (BEI) and digitised X-ray emission maps (DXRM) show details of fine textures in a wide range of meteorites. In Allende (carbonaceous chondrite) the relatively primitive textures of olivine and pyroxene chondrules (fluid droplets?) contrast with zoned calcium-aluminium rich (CAI) inclusions of high temperature gaseous paragenesis. Iron-rich chondrule rims and Fe, Ni, Cr metal/oxide spherules can be easily distinguished, at a scale impossible by earlier optical methods.

The stony iron (pallasite) meteorite Imilac contains olivines set in a matrix of Fe/Ni. Kamacite and taenite lamellae (produced during cooling over many millions of years) can be seen in both BEI and DXRM, but the backscattering intensity reveals that images need to be interpreted with great care, *structural* as well as compositional contrast can be important!

More familiar mineral growth textures can be seen in Millbillillie (calcium-rich achondrite), which contains ophitic feldspar/orthopyroxene intergrowths, very similar to those in terrestrial igneous rocks. These reflect crystal growth in a hot fluid, derived from melting of components at depth within a body of asteroidal dimensions. Later textures reveal impact between bodies (creating fractures and faults) and frictional heating during brief atmospheric transit (creating a vesicular ablation crust of glass).

Thus scanning electron microscope studies can be used to document processes which occurred from condensation more than four and a half billion years ago; though aggregation, heating, melting, segregation and cooling of asteroids over hundreds of millions of years; to the surface melting and vaporisation which produces spectacular shooting stars and fireballs.

Finally, there may well be a more sinister side to these extraterrestrial uninvited guests ..... remember the dinosaurs?

A Beginner's Guide to High Pressure Freezing

P. Monaghan (Institute of Cancer Research, Sutton)

Aldehyde primary fixation has become the mainstay of almost all biological electron microscopy preparation methods. There are, however, occasion where an alternative method of stabilisation of biological samples is required. Rapid freezing has proved successful in this context and may be followed by a range of techniques including direct visualisation of thin frozen films, freeze-fracture, ultrathin cryosectioning and freeze-substitution.

Rapid freezing may be achieved by plunging into liquid nitrogen cooled propane or ethane, jet freezing, or impact freezing against a liquid nitrogen cooled copper block. The aim of all these methods is to provide a fast enough cooling rate (10^6 °C/sec) to prevent ice crystal formation in the sample and if achieved leads to the formation of vitreous ice. For solid tissues, this can be achieved to a maximum depth of 10-15μ or about one cell thickness. The limitation is in the rate at which the sample itself can be cooled and at ambient pressure cannot readily be improved.

At increased pressure, the physical characteristics of water (e.g. maximum supercooling temperature and viscosity) change such that cooling rates lower than those needed at ambient pressure will still provide freezing with minimal ice crystal formation. The concept was described over 20 years ago by Riehle and Moor in Zurich and high pressure freezing has been developed and used with considerable success by Muller in Zurich. A commercial instrument is made by Balzers to their design, and pressurises samples to 2,100 bar prior to freezing with liquid nitrogen. Samples up to 300μm have been vitrified by this system. High pressure is not yet a routine freezing method, but holds enormous potential when followed by freeze-substitution for immunocytochemical studies.

ABSTRACTS OF POSTERS

Age Related Changes in Renal Basement Membrane Morphology in Aves

J. Bennett, D. Prashad and R. Blackburn (University of Greenwich, Deptford, London, SE8)

It has long been established that the efficiency of glomerular filtration is moderated by physiological and haemodynamic factors, such as those which influence glomerular capillary pressure and viscosity of the blood. More recently, it has been shown that microanatomical changes may play a complementary role in influencing filtration parameters.

We have investigated glomerular basement membrane thickness during post-hatch renal maturation in aves (3 week old), treated with small amounts of cadmium. The control group showed a highly significant ($p < 0.001$), linear, age-related increase in basement membrane thickness. In cadmium treated pullets, the basement membrane thickness was found to be highly significantly ($p < 0.001$) increased over that found in control animals. These findings suggest that in control animals the age-related increase in thickness of the glycoprotein barrier may retard filtration; this might be compensated by either hypertrophy of glomeruli or an increase in their numbers (Wideman, 1989). However, such increase or hypertrophy is unlikely to occur in cadmium intoxicated individuals. This is currently being investigated in our laboratory.

Preliminary Studies on Distal Tubular Morphology in Normal and Cadmium Treated Pullets

J. Bennett, D. Prashad and R. Blackburn (University of Greenwich, Deptford, London, SE8)

Cadmium induced ultrastructural changes in proximal tubules have been extensively investigated, however, only rudimentary attention has been directed towards changes in distal tubular cells. Preliminary studies in our laboratory have revealed the presence of unusual structures ("multivesicular bodies") in distal tubular cells. The frequency with which these bodies occur seems to decrease with increasing age of the normal animal; however cadmium intoxicated individuals show larger numbers of the multivesicular bodies than is found in control animals, and remains constant with increasing age. At present, the importance of these bodies, appearing exclusively in the distal tubular cells, remains unexplored.

Wideman, R.F. (1989). Maturation of glomerular size distribution profiles in domestic fowl (*Gallus gallus*). J. Morph. *201* 205-213.

Localization of Surface Associated Material in *Porphyromonas gingivalis*

P. Barber, M. Wilson, S.J. Challacombe[1] and H.N. Newman (Institute of Dental Surgery and UMDS[1], London)

Recently we have isolated a surface associated material (SAM) from *P. gingivalis* which is soluble, stimulates bone resorption, inhibits bone collagen synthesis and has a deleterious effect on fibroblasts, polymorphs and keratinocytes. The aim of the present study was to verify at the ultrastructural level the cellular location and morphology of this SAM using immunogold labelling (IGL). Pure cultures were grown under anaerobic conditions for 48 hours at 37°C and harvested by centrifugation. One half was then subjected to a mild saline extraction to remove the SAM. Primary antiserum was raised

same fibre. This study demonstrates that the application of IGL to tissues *in situ* is an effective method for the identification of collagens. Such studies may help to resolve the question of the heterogeneity of collagen fibres and be a useful technique for the study of new tissue in wounds around implants.

Acknowledgement: Supported by SERC grant GR/56413.

Scanning Electron Microscopy of Uncoated Microfossils

C. Jones and J. Whittaker (Natural History Museum, London)

The need to coat specimens with a conducting surface layer has always been a drawback for material that should not be prepared in any way, such as type and figured material. This poster describes a low vacuum (environmental chamber) SEM system in routine operation at the Natural History Museum for examining uncoated specimens and shows some of the micrographs obtained. The microscope can also be used to successfully image fresh plant material and other partially hydrated specimens with no preparation.

S.E.M.T. One-Day Meeting 9 October 1992

Quantitative XRMA: Why bother?

Alice Warley
St. Thomas's Hospital

1) What can be achieved 2) why
XRMA has been possible since 1951; the first biological papers appeared in 1962.

Diabetes

There is rapid weight loss at onset, mostly from loss of muscle; then this stabilises. The heart is largely protected, so its proportion of the body weight increases. The ability to develop tension in the heart decreases in response to adrenalin decreases dramatically - the "fight or flight" response - because of alterations in membrane permeability and increase in intracellular calcium. Analysis of myofibrils shows differences, mainly increase in sodium and sulphur, but the increase in calcium is not significant. It is possible that calcium is being sequestered within the cell. There is loss of contractile force because cells are dying, and the potassium-to-sodium ratio is reduced in individual cells. There is no evidence for increased uptake of magnesium. Analysis can be carried out of individual cells, at sub-cellular level, and of a number of elements simultaneously.

The spectra alone can be misleading; we do need quantitation for reliable results.

A tissue-culture medium rich in sodium chloride was used. The cells were seeded onto gold grids; then frozen with liquid nitrogen. Acetate or sucrose will remove the excess sodium chloride of the medium; ammonium acetate removes less sodium, phosphorus and chloride than does the sucrose, but more potassium because the ammonium substitutes for it. They have standardised on a sucrose wash.

Different cell types may behave differently; but only one type divides especially in the pathogenic situation. Quantitative XRMA can locate this.

The studies are done on cryo-sections. The peak/continuum ratio is measured, to compensate for differences in section thickness.

Increased sulphur concentration was found; reason unknown. Usually the sulphur in the cell is bound to proteins; this may be related to the acetyl co-enzyme A.

Microanalysis of fossils & archaeological bone

Terry Williams
Natural History Museum, London

Parameters studied include the Paleodiet - strontium and zinc; health - antimony & lead; environment & ecology - cerium & uranium - stable isotopes; age dating.

Impure hydroxycarbonate apatite has as its major components hydrogen, carbon, oxygen, phosphorus, calcium; minor components sodium, magnesium, sulphur, aluminium; trace elements fluorine, silicon, chlorine, manganese, iron, copper, zinc, strontium.
During fossilisation, hydrogen, carbon & nitrogen are lost, but some other elements gained. The bone also beomes more porous, and there is increase of crystallisation.

The Olduvai gorge is important, and a different environment for fossilisation. Rare earths are detectable on the outer edge of bone, e.g. cerium when bone is exposed in oxidising environments. This gives information on

the redox conditions of the fossil beds. Sections across the bone show that europium is concentrated at the edge, and uranium is lost.

Levels of exposure to lead are in the ratios of Neolithic 1 : Mediaeval 13 : modern 4; but lead can be mobilised during fossilisation. In some bodies from Spitalfields, lead and tin have been incorporated from the lead coffins. Similarly from iron coffins. Strontium is lower in carnivores than in browsers on plants or molluscs; and also was low in the Spitalfields bodies. Strontium is very high at Olduvai; in recent material it is high at the edge but diffuses in relatively rapidly, from the edge and through the osteone channels. Zinc and manganese diffuse in from the edge, and are not paleodiet indicators.

Modern bone should be embedded and 30 µm slices cut for microanalysis, then polished for XRMA; otherwise it will peel off the su;port.

EM in Investigative & Therapautic Studies of Hyperpigmented Skin Disorders

Prof. A.S. Breathnach
St. Thomas's Hospital

Vitiligo = patchy loss of pigmentation. Tyrosine is a substrate for melanin. Langerhans cells are not dead melanocytes; pigmented granules of melanosomes are found in the cytoplasm.of the melanocyte; there is a protein matrix on which the melanin is deposited.

In Pityriasis versicolor, there is de-pigmentation because of the Pityrosporum. The official theory is that this happens because the UV is filtered out; the fungus uses the lipids in the skin. The mitochoindria in the melanocytes are damaged or dead. The fungus can be cultured in unsatureted fatty acids, oxidising them to dicarboxylic acids which inhibit enzymes. This involves azeleic acid, NADH dehydrogenase, succinic dehydrogenase, tyrosinase.

Autoradiography of cultured melanocytes in dodecanoic acids shows the radio-isotopes in the mitochondria and nuclei; not in the endoplasmic reticulum, GERL, Golgi, or melanosomes.

Cloasma, a patchy pigmentation on women, is removed in 4 months by application of azeleic acid cream. Similarly there is a cream which removes lentigo maligna in 4 - 6 months; but clinical colleagues have not taken this up. The cream is effective even if the cells have already become malignant.

Topical and oral azeleic acid destroys the affected malenocytes, and the patient returns to normal conditions, and quality of life is improved. Even if disseminated melanoma has developed, ther is a positive effect; it is hoped to use this as an adjuvant to treatment, he does not (yet) say that it is a cure.

In squamous cell carcinoma, azelaic acid has an effect on the mitochoncria; & clinically does have an effect on solar keratosis. Azelaic acid is a specific selective cytotoxic agent for atypical melanocytes in malignant melanoma; but this has not been taken up clinically.

Azelaic acid also helps acne vulgaris, leaving very little scarring, and can be used in recurrences. Acne is an increase in the activity of the sebaceous glands, increased keratinisation, and increased microbial activity. The anti-inflammatory action is by scavenging hydroxyl radicals in vitro, and inhibition of oxy-radical toxicity in cell cultures.

Azelaic acid does not affect normal melanocytes, e.g. freckles, tanning. In the abnormal melanocytes, it penetrates into the mitochondria three times as fast; in normals, it enters at a rate at which it is normally metabolised. Probably the levels in the abnormals cells inhibits the mitochondrial oxidation/reduction reactions.
For acne, azelaic acid has been available on prescription since Sept. 10, 1992.

Melanosomes are "handed over" to the keratinocytes, by dendrite penetration, nipped off, and the membranes then disintegrating.

Azelaic acid is not toxic; it is a natural substance, found in rancid butter. After one week's application there may be a little redness; in this case, give the treatment a rest for one week, then re-apply once a day.

Things that go Bump in the dark
Anton Kearsley
Oxford polytechnic

The brightness of the backscattered image is related to composition; but it is essential to have XRMA. Feldspar is important in petroleum geology; cathodoluminescence is also important, but not much work has yet been done to determine the relevant controls.

Meteorites show fine textures which reflect the early history of the earth. Porbably many of them sample the asteroids, and so may have very different chemical compositions.

The meteorite Allende, in Mexico, has round carbonaceous chondryles; magnesium & calcium in iron-rich olivine, which may be oval. It also contains rounded droplets of iron sulphide. There are calcium- and aluminium-rich inclusions, and numerous volatile elements - the return of the extra-terrestrial laxative!

Holbrook - an ordinary chondrite.
Millbillie is a very pale conglomerate, similar to the asteroid Vesta.
Gas bubbles may be found in the outer layers because of passage through the atmosphere; this causes surface melting by friction.
Imilac, in Cuba, conytains metallic nickel and iron, and olivines.

A Beginner's Guide to High-Pressure Freezing
Paul Monaghan
Inst. of Cancer Research, Sutton

High-pressure freezing is useful for morphology, antigenicity, prevention of diffusion, time resolution.
Immuno embedding can be in LR White or Lowicryl immuno work can also be done on thawed cryosections; freeze and freeze-substituted material.

Hexagonal ice crystals are enormous; cubic are still bad. Vitreous ice is only found if freezing is at a rate of 10^6 °C/sec; but still is only found for 10-15 μm into the tissue, although deeper layers may look OK by light microscopy.

High-pressure freezing:
- lowers the supercooling temperature
- increases viscosity
- inhibits ice crystal formation

With experienced operators, 1 in 4 samples will show good morphology !!

Ice crystal damage can start some way from where the damage is seen, and it spreads and grows as it spreads.