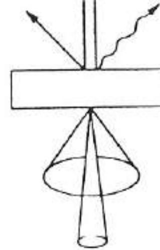
14-5-93

SEMT

Society of Electron Microscope Technology



Affiliated to the Royal Microscopical Society

PROBLEMS AND SOLUTIONS - a multi-technique approach

Friday 14 May 1993

at

Imperial Cancer Research Fund
Lincolns Inn Fields London W C 2

PROGRAMME

2.00 **Cracking Brittle Bone - a multi-technique approach**
John-Paul Casella and Yousef Ali (Institute of Orthopaedics, Stanmore)

2.40 **A fish's itch - functional biology of fish lice**
Karen Gresty (Natural History Museum, London)

3.20 TEA

3.40 **101 ways of immunogold labelling**
John Chandler (Biocell Research Laboratories, Cardiff)

4.20 **Immunocytochemical techniques for labile and fixation sensitive antigens**
Chris Hawes (Oxford Brookes University)

....

To the Secretary :

Dr Jill Lewis
19, Bellfield Avenue
Harrow Weald
Middx HA3 6ST

I hope to attend the meeting on 14 May 1993

Name.. Telephone.................

Address.......................................

....

SEMT Meeting: 14 May 1993

ABSTRACTS

Cracking brittle bone: a multi-technique approach.

John-Paul Cassella and S. Yousuf Ali

No single microscopic or analytical technique will provide all the information necessary for the interpretation of a complex biological system. The mineralisation of bone is no exception, especially in a pathological situation.

Osteogenesis imperfecta (OI, commonly called brittle bone disease) is an hereditary pathological disorder of connective tissue. Molecular biological analyses have demonstrated mutations in the type I collagen gene, but there is little or no correlative evidence using microscopy to visualise such changes in the type I collagen, cells or the mineral formed in association with this collagen.

Using a Philips CM12 TEM and Quantimet image analysis system, Type I collagen fibrils in the bone osteoid were histomorphometrically analysed and determined to be larger than in normal bone; such changes may be a result of the altered packing of mutated collagen molecules. Using an EDAX 9800 microanalysis system with the TEM, the calcium to phosphorus molar ratio, an indication of the type of mineral present in bone, was found to be lower in OI than in normal bone, indicating a change in composition of the bone mineral.
Analytical techniques, including Fourier transform infra-red spectroscopy, X-ray powder diffraction, electron diffraction, 31-phosphorus nuclear magnetic resonance spectroscopy and infra-red spectroscopy did not unequivocally demonstrate differences in the crystal lattice structure, resonances of phosphorus nuclei or group vibrations in OI bone mineral when compared with normal.
Numerous LM and TEM ultrastructural changes were observed in OI bone, including the presence of microfibrils associated with the type I collagen and mineralised mitochondria in osteoblast-like cells. These changes may be a result of the altered functioning of osteoblastic cells resulting from the poor secretion of abnormal collagen. The alteration in the calcium to phosphorus molar ratio of the apatite bone mineral may be a result of an altered nucleation and growth environment on the collagen fibrils. These changes may increase the risk and frequency of bone fracture in this disease.

JP Cassella and S Yousuf Ali (1992) *Abnormal collagen and mineral formation in osteogenesis imperfecta.*
Bone and Mineral **17** 123-128.

A fish's itch:- functional biology of fish lice.

Karen Gresty

Fish lice are highly pathogenic crustacean ectoparasites of both freshwater and marine fish. This talk will concentrate on two such parasites; the freshwater branchiuran *Argulus japonicus* (the fish louse) found mainly on Koi carp in Britain and the marine copepod *Lepeophtheirus salmonis* (the salmon louse) found on salmon and sea trout. Both these parasites are of great importance due to the commercial value of their respective hosts. Koi carp can command several thousand pounds a fish in the ornamental carp industry but if a fish is damaged due to parasitic activity then its commercial value will accordingly drop. Wild salmon are naturally exposed to the salmon louse at low levels. However, in farmed situations this parasite can run rife, completely decimating the entire stock.

To have either of these parasites on your fish is obviously a costly business. Therefore increasing amounts of money have been spent on research to examine the biology and to investigate possible control methods to reduce their effect. In this study, electron microscopy has been employed to determine the fine structure and function of the pre-oral spine of *Argulus*, an organ which inflicts a large amount of damage, during parasitic feeding, on the fish's skin. The sensory biology of the free-living stages of *Lepeophtheirus* has also been examined, to determine how the parasite finds a host and maintains its position in the water column. Techniques used include scanning and transmission EM, also ion-beam etching and freeze fracture. Fish lice are relatively small creatures and their tough crustacean skeletons makes normal histological techniques difficult. This talk demonstrates how EM techniques allow a unique insight into their biology.

One hundred and one ways to do immunogold labelling.

John Chandler.

It is now over 50 years since fluorescent labels were attached to antibodies for the detection of antigens in tissues in light microscopy. Since that time enzyme labels have been introduced and have dominated the LM detection of antigens for permanent recording. In 1972 gold labels were first introduced as antigen markers in EM, replacing the somewhat equivocal ferritin labels. Since then, gold has become the marker of choice in the EM and is an important alternative and complimentary technique in LM when coupled with silver enhancing. Sensitivity and resolution of detection is greater
when using gold and silver together in the LM than alternative techniques with enzyme or fluorescent labels.

The success or otherwise of immunogold labelling depends on a number of parameters, the most important of which are the specimen preparation schedules and the incubating steps. The quality of the immune reagents greatly affects the results but reliable results will always depend on a good understanding of the effects of specimen exposure during preparation procedures and the environment of the specimen during subsequent incubations.

Specimens may be examined in the LM and EM by both the pre-embedding and post-embedding methods. In the pre-embedding technique the antibody incubations are performed first in the tissue and the specimen subsequently processed for microscopy. In the more popular post-embedding technique the specimen is first prepared for microscopical examination and the incubations performed on the tissue sections or cells.
The choice of gold label is important in determining the sensitivity of the incubation. The correct choice of primary or secondary antibodies or other binding proteins can greatly affect the results. Small gold particles attached to antibodies will give higher labelling intensity than large gold particles due to the steric hindrance of the latter. Small gold particles are, however, more difficult to see. Silver enhancement allows the small gold particles to be used and provides an easily visible signal in both EM and LM. Epipolarised light, used in the LM, also gives a spectacular and very sensitive signal for antigens that are difficult to locate with other labelling methods.
This presentation will deal with the wide range of applications that can be solved with immunogold labelling together with methods for getting the best results.

Immunocytochemical techniques for labile and fixation-sensitive antigens.

Chris Hawes

Most immunogold labelling methods involve a compromise between maintaining sufficient antigenicity to give a good signal from the label and achieving an acceptable level of ultrastructural preservation of the tissue. Thus, in most cases conventional aldehyde/osmium fixation followed by epoxy resin embedding protocols cannot be employed. As a result of this a whole range of different techniques have been devised for the immunolocalisation of cellular antigens. Recently cryo-fixation has been shown to be an excellent way of preserving antigens and is often used in combination with freeze-substitution and low temperature embedding. However, in this talk I will describe the potential of controlled ultra-low temperature freeze-drying as an alternative technique for preserving both the antigenicity and the spatial organisation of antigens whilst permitting good structural preservation. Initial results from the use of this technique compared with other more conventional immunocytochemical techniques for the study of the endomembrane system in plant cells will be presented.