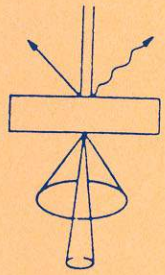
SEMT One Day Meeting 27th Oct. 1989



WELCOME TO THE **SEMT** ANNUAL ONE DAY MEETING

INDEX

The SEMT would like to express their special thanks to :-

The Eastman Dental Hospital.

Cambridge Instruments for their generous sponsorship.

All the other Companies who have attended the Trade Exhibition and generously sponsored us during the past year.

Copyprint Bureau Ltd. for producing this programme.

We hope you will find the meeting interesting and informative and that we will see you at future FREE afternoon meetings held at the ICRF, Lincolns Inn Fields, especially on Wednesday 6th December 1989 for the

R.M.S. Beginners' Meeting

with guest speaker Dr. Julian Beasley.

All members are invited to an informal sherry reception after today's meeting which will be followed by the Annual General Meeting.

An Introduction to

THE SOCIETY OF ELECTRON MICROSCOPE TECHNOLOGY

The society was formed in 1970 to promote the exchange of information and ideas in the field of electron microscopy. Membership is unrestricted and although mainly London based we have many members from further afield. By holding regular meetings with invited speakers and trade stands we aim to facilitate contact between all our members including instrument manufacturers and users. The current format includes a series of afternoon meetings, each concentrating on a specialist topic and an annual one day meeting with a broader based content. Our meetings are always well supported by the trade and information on the latest technology is usually on display. Forthcoming events are advertised in the Proceeding of the Royal Microscopical Society (to whom we are affiliated) and various technical publications.

Membership of the SEMT has many advantages. For a newcomer to the field it provides a point of contact with others who can give support and encouragement as well as practical assistance. For those with more experience, co-operation and sharing of ideas is as important as keeping abreast of the latest developments in techniques and equipment. For the trade, it provides a unique opportunity to advertize to a keenly interested audience and to obtain customer-reaction directly.

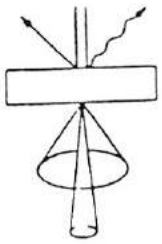
Prospective members should contact Jill Lewis (Secretary), Electron Microscope Unit, St. Bartholemews Medical College, Charterhouse Square, London EC1M 6BQ, for a membership form. An annual subscription, to cover postage and stationery costs, (£5) is due on the 31st October and cheques should be made payable to "SEMT". Current committee members available for further information are listed below.

Mrs Pauline Barber	(Chairman) 01 837 6411 x2042
Mr Chris Walker	(Vice-Chairman) 01 889 7607
Dr Jill Lewis	(Secretary)01 982 6160
Ms Susan Barnes	(Treasurer)01 938 9348
Mr Clive Wells	01 601 8888 x8552
Mr David Gunner	01 928 9292 x3306

SEMT

Society of Electron Microscope Technology

Affiliated to the Royal Microscopical Society



PROGRAMME

9.30	Registration and coffee.
10.30	Chairman's Introduction.
10.40	Dr. Robin Harris (N.E. Thames Regional Transfusion Centre, Brentwood.) "Cell fusion and syncytium formation induced by HIV"
11.10	Professor Hellmuth Sitte (Univ. des Saarlandes) "News in Cryotechnology for Transmission E. M."
12.10-1.40	Lunch, Trade Exhibition and Poster Display.
1.40	Dr. Peter Szczesny (Institute of Ophthalmology) and Mr. Don Claugher. "Ultrastructural studies of the human photoreceptor cell"
2.10	Dr. Giselle Hodges (Imperial Cancer Research Fund) "Gold label imaging and Quantification; facts and factors."
2.50	Tea.
3.30	Ms. Aviva Burnstock (National Gallery, Trafalgar Square) "Microscopy in the examination and restoration of paintings".
4.00	Poster Presenters' Discussion period.
4.15	Chairman's summing-up.
4.20	Sherry reception followed by Annual General Meeting.

Cell fusion and syncytium formation induced by HIV.

Dr. Robin Harris (N.E. Thames Regional Transfusion Centre, Brentwood.)

HIV-1 is known to induce syncytium formation in vivo, particularly in brain, spleen and lymph nodes in AIDS patients. In cultures of CD4+ lymphoid cells, cell fusion and multinucleate giant cell or syncytium formation is a well-defined cytopathic phenomenon and is used light microscopically as a method of assaying the presence of HIV-1. When compared to the uninfected cell, distinct morphological changes can be defined within the infected cell and the syncytia, which become more pronounced with increasing syncytial size as they progress towards senescence and lysis.

From an electron microscopical study of thin sectioned samples taken from cultures of C8166 cells infected with HIV-1, it has been possible to define the following ultrastructural changes:

1) The varying stages of cell fusion, with breakdown of the plasma membrane and increase in syncytial size.

2) The progressive vacuolation of the syncytia, with extensive cytoplasmic ballooning of the larger syncytia.

3) Initially, viral release occurs only at the syncytial surface, but release into the internal vacuoles is more pronounced in the larger syncytia.

4) The nuclear envelopes of infected cells and small syncytia which are actively releasing virus show pronounced "plug-like" nuclear pore complexes.

5) Overlapping or multiple budding events at the vacuolar membranes result in the accumulation of numerous multicored aberrant viruses.

6) Pronounced margination occurs, with production of an organelle-free periphery and central clustering of nuclei and mitochondria. There is breakdown of the rER with proliferation of free ribosomes.

7) The nuclei become progressively shrunken and distorted, with nuclear envelope invagination and chromatin condensation; nucleoli become "spongy" or reticulated as the nuclei become metabolically inactive.

8) The surface of single cells and small syncytia is covered with microvilli; these tend to be lost with increasing syncytial size. At the stage of syncytial senescence, when viral production has ceased, there is nuclear pycnosis and the formation of a deviant surface microvillar coat. At the stage fo syncytial lysis, massive release of vacuolar enclosed HIV-1 occurs.

News in cryotechnology for transmission electron microscopy.

Professor Hellmuth Sitte (Univ. des Saarlandes, Homburg).

Since cryo ultramicrotomy for immuno cytochemistry, element analysis and morphological work in the frozen hydrated state is nowadays an established method, additional information can only be gained with new methods and new instrumentation. Recent work in our laboratory (L. Edelmann) was therefore mainly directed to the improvement of cryofixation followed by cryosubstitution and low temperature embedding. The state of preservation of cryofixed specimens depends mainly on the temperature-time-schedule during the substitution procedure: elongated substitution times up to 7 days at temperatures below 200 K result in considerably better structural preservation even without stabilizing additives as OsO_4, uranyl acetate or aldehydes if the subsequent embedding procedure is realized at approx. 210 K. Proper curing following this protocol is possible by UV light if the embedding moulds are properly sealed and if the specimen size is kept below 0.5 mm. The resulting blocks are immediately sectionable at ambient temperature without subsequent hardening after warming up. They often show a reproducible distribution of elements (ions) in the different cellular compartments, which corresponds the expectations and the results obtained with other methods. Further work (L. Edelmann) was directed to freeze drying of tissue blocks for subsequent resin embedding at ambient or low temperatures and for subsequent TEM observation of ultrathin sections after freeze drying. The use of an adapted conventional cryosubstitution apparatus allows efficient work which safely excludes any risk of ion redistribution due to the uptake of water traces during the different steps of the procedure. Low temperature acrylic resin infiltration and low temperature embedding as a follow up procedure is possible in the already described way. Further considerations are directed to the routine immersion cryofixation of thin suspension film ("ice embedding" or "bare grid method" according to Adrian at al.) and the improvement of cryosectioning procedures and instrumentation. The different methods and possibilities will be discussed in comparison.

Ultrastructural studies of the human photoreceptor cell.

Piotr J. Szczesny and Don Claugher* (Inst. of Ophthalmology, London
* British Museum (Natural History)

Generalised defects in the cilium linking the inner and outer segments of the human photoreceptor have been indicated as a possible factor leading to photoreceptor death and blindness in various retinal dystrophies.

Electron microscopy, both transmission and scanning is applied to investigate the ultrastructural details related to the photoreceptor cilium and ciliary plasma membrane morphology in normal and dystrophic retinas. HRSEM combined with freeze fracture and a modified technique of tissue preparation developed by Tanaka are used in this study.

Results revealing new details with regard to membrane organization during the formation of the outer segments in healthy and dystrophic retinae will be discussed.

Gold label imaging and quantitation by scanning EM; facts and factors.

Dr. Gisele Hodges (Imperial Cancer Research Fund, London)

An overview will be given of gold label imaging by scanning EM. The main discussion will centre-

a) on studies illustrating the effective contribution of the gold marker system coupled to different SEM analysis procedures using secondary electrons, backscattered electrons or X-rays to immuno- and lectin-cytochemical investigation; and

b) on factors likely to influence direct quantitation of the gold label with the SEM.

Microscopy in the examination and restoration of paintings.

Ms. Aviva Burnstock (The National Gallery, London)

Paintings in the National collection are examined before and during their restoration. In order to gain information about the materials and techniques used by the artist and to differentiate original paint from later restorer's additions, small samples are taken and examined using various microscopical techniques. Problems such as colour change and changes in the surface of paint and varnish will be discussed with reference to paintings in the National Gallery.

The structure of Iron Hexacyanoferrates; a crystallographic and spectroscopic study.

J.P.Cassella, J.Hay and D.Cairns (St. Thomas' Hosp. Med. Sch.)

Analysis of three Iron Hexacyanoferrate complexes by X-ray powder diffraction, Mossbauer spectroscopy and a novel colorimetric technique reveals differences between them although a common unit cell structure may exist, and a resonance structure is likely.

Ultrastructural immunocytochemistry of the Toxoplasma tissue cyst.

Dr. John Hay (Leicester Polytechnic)

Electron immunocytochemistry, using rabbit polyclonal anti-Toxoplasma IgG as the primary layer in immunogold staining, was used to assess the distribution of Toxoplasma antigen within, and in relation to intact tissue cysts in brain tissue from mice with congenital toxoplasmosis.

Blood nerve barrier: anionic site distribution in endothelial membranes and basal lamina.

Dr. M.S.Bush (UC and Middx. Med. Sch.)

The distribution of anionic sites on the cell membranes and basal laminae of vascular endothelial cells in the rat sciatic nerve was investigated using cationic ferritin and cationic colloidal gold. EM study revealed microdomains of various charge densities.

Fine structure of photoreceptors in normal and dystrophic retinae in man.

P.Szczesny, D.Claugher and J.Marshall (Inst. Ophthalmology, London)

Ultrastructual changes with regard to membrane organization during the formation of the outer segments of the photoreceptor are described in healthy and dystrophic human retinae.

S.E.M.T. One-Day Meeting 27 October 1989

Cell fusion & syncytium formation induced by HIV

Dr. Robin Harris
N.E.Thames Regional Transfusion Centre, Brentwood

Gelderblom is the authority on HIV structure.
Working inwards from the outside of the particle, the layers are:
glycoprotein 120
GP41 in a lipid membrane
P 18
P 24 is around the core of RNA + reverse transcriptase.
The morphology of the core is still unclear.

GP 120 connects to the CD4 on the cell. Infected cells fuse to form syncytia with uninfected cells.

The nucleopore plug complexes are more dense & more complex than normal; this suggests that the cell's metabolism has been turned over to viral production. The cell buds off immature virus without the core structure, and these stay clustered around the cell; the outer ones are more mature. There are some double- or triple-headed particles.

Transformed cell lines produce much more virus than do actual AIDS patients. The nucleopore plugs are very dense and complex, and extend into the cytoplasm.

Fusion between cells begins within 5 - 7 days of culture; we cannot keep the culture going longer than this because of the syncytia. Cytoplasm appears to be transferred between cells. Fusion is a dynamic metabolic effect following infection. If the plasma membrane is absent, the cells have fused. There are vaculoes in the syncytia, but not in Sinde infected cells. There may be more than 100 nuclei clustering in a syncytium, leaving patches of clear cytoplasm. There is patching of areas of release of virus, and it is possible to relate these to underlying nuclei.

The nuclei gradually disintegrate after incorporation into the syncytia. Degenerate nuclei become dense and pyknotic. The vacuoles fill with virus. It is not clear whether the vacuoles are surrounded by plasmalemma membrane. The mitochondrai also cluster a bit, leaving clear cytoplasm. Even within a vacuole, there is patching of the areas of virus release.
In highly degenerate nuclei, there is a spongy nucleous. Mutlinucleate cells are found in the brains and lymph nodes of AIDS patients. This suggests removal of T4 lymphocytes by fusion. T4s are essential for a full immunological response. In blood cells, they have <u>not</u> yet detected even binucleate lymphocotyes.
In clustering, there are certainly cellular antigens with immature virus; it is possible that the glycocalyx holds these in position.

News in Cryotechnology for TEM

Prof. Hellmuth Sitte
Univ. des Saarlandes

1940 Sjostrand & Gersh - freeze-dry
1960 Fernandez-Moran rapid freeze
1964 Van Harrevald - metal mirror cryofix & freeze-substitution
1964 Moor & Muhletahler - freeze-fracture
1965 Bernhard - cryoultramictotomy

1968 Moor & Riehle - freeze under high pressure
1969 Dollhopf et al - small cryochambers for sectioning
1973 Tokuyasu - sucrose cryoprotection for sectioning
1975 Edelmann & Pfaller - freeze-dry --> resin embed
1976 Taylor & Glaser - frozen hydrated films
1976 Heuser - release of synaptic vesicles by rapid freeae
1979 Boyne - bounce-free on metal mirror freeze
1980 Lichtenegger et al - side-entry cryo-transfer
1980 Carlemalm - acrylics for low temperature embedding
1980 Sitte - cryochamber
1983 Muller & Moor - high-pressure cryofixation
1986 Zeiss TEM top-entry cryo-transfer

We still have the old problems of artefacts from the freezing, substitution, embedding, freeze-drying, sectioning, EM, etc.

The metal mirror gives the best cryo-fixation. But if there is bouncing on the mirror plane, this slows the freezing.Even if a pad of foam is used, you may still get air bubbles. But if the specimen is fragile, it may squash & splatter.
Liquid nitrogen is as effectvie as liquid helium.
It is importnat to compare the results with light microscopy.

Cryosubstutution
Acetone is still the best substituting liquid, plus 1 - 2% osmium to stabilise the ultrastructure. Substitution within one day is too short, without additives; extend the substitution over several days, and the consumption of liquid nitrogen remains low. It is possible to flat-embed if there is a good seal above the specimen and contact below to the aluminium disk, at -65°C. K4M will cure in 24 hours with UV; it is necessary to lower the temperature before starting to cure, because there is a rise in temperature during curing.

Cryo-dehydration = freeze-drying
Hagler & Buja 1986 - lipid does not melt during cryo-TEM.
Frozen-dried specimens are very hydrophilic and take up water from the air.
Specimens shrink during freeze-drying. It is best to dry very slowly;
1 hour at -100°C, then 6 hours at -80°C; this will give only 5% linear shrinkage (Edelmann 1986)
Molecular sieve under liquid nitrogen removes gases.

Cryosectioning
Zierold 1988 picking up dry cryo-sections; better reproducibilty & preventing compression.
Jesior 1986 - use a low-angle Diatome diamond knife for cryosections - the small angle helps to reduce compression.
Tissue samples are more complicated than bacterial suspensions.
Pictures can be obtained without any heavy metal staining.

Cryo-chamber
This is a heat-exchange tank between the outer liquid-nitrogen tank and the specimen.

Henkelman & Ottensmeyer 1974, a modification of Castain & Henry 1962, used an electrostatic mirror for an energy filter for TEM.

Zeiss uses contrast enhancement by tuning - this is very important for cryosections; so you don't need to use underfocus.

Immuno-staining with monoclonal antibodies can be demonstrated more easily on cryosections than with cryo-substitution etc. It is best to use both methods and compare the results.

Ultrastructural studies of the human photoreceptor cell
Dr. Peter Szczesny & Mr. Don Claugher
Institute of Ophthalmology

Retinitis pigmentosa has characteristic pigmentary changes in the periphery of the retina; the cause is still unknown.
There are various types of inheritance for rod-cone degeneration, cone-rod, etc. Even at 10 years of age, ultrastructural abnormalities may be visible, although substantial impairment of vision may not occur until decades later.
Abnormal nasal cilia, sperm, and photoreceptors may be correlated.
For high-resolution scanning, the tissue is frozen, then macerated in 1% osmium. If the tissue is stored in fixative, it is still suitable for freeze-fracture studies.

Gold label imaging & quantification: facts & factors
Dr. Giselle Hodges
ICRF, London

Gold can be conjugated to a wide range of ligands - proteins, lectins, glycoproteins, polysaccharides; to primary & secondary antibodies; to the protein of interest.
Range of sizes of gold label: 40 nm for ease of detection; 15-20 nm for ease of EM detection; 1-5 nm if the pathway to the structure is obscured, there is high particle concentration, or targets are close together.
It is possible to sue multiple labelling for EM; silver amplification of small gold particles; coloured silver-intensified gold.

Gold gives strong emission of secondary & back-scattered electron.
Back-scattered electrons are used for atomic number contrast; secondary electrons for topography & topology. Topography = description & measurement; topology = quantitation.

For examining the urinary bladder, an area having scalloped edge with plaques, techniqwues using concanavalinA + gold label; followed by glut/osmium, then Pt layer to examine the cell surface.

Quantification of cell-bound markers needs:
- target accessibility
- target density
- size/configuration of marker complex
- steric hindrance

Wash; prefix; wash with buffer + 0.2M glycine to block free aldehyde groups; normal serum from species supplying secondary antibody, to block non-specific antibodies; label, etc.
Then: glut; wqsh; osmium; wash; dehydrate through alcohols; critical point dry; metal/carbon coating.
Try air-drying as well, in case there is loss of gold.
Microfuge the primary & secondary antibodies to rmeove micro-aggregations.
If the reagents contain azide, this may affect the architecture of the cell.

Optimum size of gold particles - because of steric hindrance, use the same size of particles to compare between experiments. Large particles may preclude 1:1 correspondance of the particle and the target.
With a thicker metal/carbon caoting, we tend to lose the gold, although it can still be imaged by back-scatter.

If smaller gold particles are used, they can be amplified; either with silvcer, or with the three-layer technique - gold-tagged second and third layers.
Depending on the instrumentation, it may be possible to mix back-scattered & secondary electron signals.
10 - 20 nm coating can obscure small structures and particles, and give "decoration". Small surface topography may mask gold particles or mimic them.
It is possible to reverse the polarity of the back-scattered electrons; this is especially useful for image analysis.
We should determine the labelling density and labelling index.

Microscopy in the examination & restoration of paintings
Ms. Aviva Burnstock
National Gallery, London

On a hand painted by Rembrandt: azurite blue can react with oil paint, and become yellower. Cleaning reagents amy fluoresce.

A Leonardo cartoon was shown to be on linen flax paper.
The green and brown pigments sued by Poussin fade.
Acrylic varnish peels, and si matt.

? from poster presentation;

With AIDS and immunosuppressed patients, Toxoplasma becomes very important; it can cross the placenta in an asymptomatic mother, to produce severe ocular disease in the offspring in adolescence. Most infection comes from undercooked meat.