

MY LIFE IN EM BY ANN DEWAR 1

Path Soc Meeting.....4

AFC & Physical Skills.....4

Publications.....5

The Myofibroblast.....6





ISSUE 5 | SUMMER 2008

ACEM focus

THE NEWSLETTER OF THE ASSOCIATION OF ELECTRON MICROSCOPISTS.

Last December, Alan Curry asked me if I would write an article for this ACEM newsletter. I retired last September after nearly 45 years working with electron microscopes, the last 30 years as EM Unit Manager at the National Heart and Lung Institute (NHLI), part of Imperial College since 1997.

My Life in EM

Ann Dewar, 2008

I hope my recollections below will inform, entertain and bring back some of your own happy memories.

Over the years at NHLI, I worked mainly with mammalian tissue, primarily human respiratory samples. My first electron microscope job, however, was quite different and involved the isolation, growth and characterisation of bacteriophages. This really was back in the 'good old days', in the mid-60s, when I joined the Department of Zoology, Edinburgh University, working for Dr D. E. Bradley. At the time, I didn't realise how eminent he was in the field (good pun here!) of electron microscopy. His publications included the first paper on platinum/carbon rods for replicas, articles investigating negative staining techniques and several chapters in the then EM 'bible', 'Techniques for Electron Microscopy', edited by D. H. Kay, 1961 and 2nd edition 1965. During my time there, I learned most of the basic TEM techniques and part of the training was a short film made by AEI, in which Dr Bradley played a role. I often wonder if that film is still hidden away somewhere. Shortly after I started, a junior technician, who was assisting with the first year biology practical classes, joined us in the EM unit. Thus started a long and special friendship with David Robertson who, as many of you will know, continued his career in electron microscopy in London at the Institute of Cancer Research.

The main equipment comprised a Siemens Elmiskop 1A (Figure 1), (serviced by the wonderful Houstin Martin, who later in life joined Jeol), the classic Huxley microtome and an Edwards coating unit (I remember the large size of the bell-jar being particularly useful for aluminium 're-silvering' the inside of vintage-car headlamps). I laugh, however, when I think back at some of the other equipment. Our embedding ovens were home-made from biscuit tins, with a hole in the side for the electric fitting and the use of 40 or 60 watt bulbs as required. Diamond knives were available but rare since they were really expensive. Making glass knives, on the other hand, could take all afternoon for only a few good ones. We got through a lot of, recycled broken plate glass from the



Figure 1

A very young Ann Dewar at the controls of a Siemens Elmiskop 1A



Figure 2

glazier, trying to get a good length of straight 'free-break'. Then we would position the special jig and scratch the lines that would form the knife. One day we found out that another department, way over on the other side of the King's Buildings campus, had a special machine to make glass knives. Yes, the exceptionally good and reliable LKB knifemaker had arrived, and we all learned how to beg, with charm!

Lecture slides were prepared from 2x2 inch glass photographic plates. There was no Letraset, and magnification lines and µm marks were manually drawn in ink straight on to the emulsion. A second, clear, glass protected the emulsion and the two were bound round with a special U-form 'lick and stick' black passe-partout tape. Our darkroom was above the boiler room and next door to a laboratory with cages of cockroaches that eventually escaped and infested the darkroom, so that when you went in and put the light on, the whole floor seemed to move as they scurried out of sight. Even with safelights on, I did a lot of dancing in that room to keep them at bay. We couldn't put poison down in case it was transferred to the insect department.

We had no freezer and our bacteriophage stocks were stored in the fridge but kept contaminant free by adding one crystal of thymol, which stopped any bacterial or fungal growth. (These days, if I ever need to go into hospital, I plan to take a sachet of this magic thymol with me.)

Disposable plastic Petri dishes became available and we quickly discovered the wonderful sculptures that formed when a stack was autoclaved, especially if colour was added. I don't remember how we ordered things, but I never forgot the wall cupboard full of small boxes of antibiotic because someone had failed to appreciate the purchase-unit, and so I never made such a mistake.

> "...it had become legal to brew your own beer and our culture laboratory had the perfect-temperature hot-room.

Around this time, it had become legal to brew your own beer and our culture laboratory had the perfect-temperature hot-room plus a supply of 'non-toxic' Winchesters to wash out, so we soon had an efficient production line on the go. We hadn't realised how much stronger it was and I still get a sore head when I think of that first party. Outside in the real word, we were driving Minis and Bubble-cars (where did they go?) and dancing to the Who, Beatles, Stones, Dylan etc.

(The editor used to drive a mini until a few years ago!)

That was my 'apprentice' training if you like and now I was ready for the 'journeyman' part of my life, so in 1969 I arrived in Vancouver, at the University of British Columbia, Faculty of Dentistry. While the work was experimental rather than clinical, it did require sectioning tissue, rather than just pellets of bacteria and this is where I learned to cut assuredly, even ribbons of sections transferred to large single slot grids. This was with glass knives using a Sorvall MT2 ultramicrotome. A Philips 300 (Figure 2) had very recently been installed and this seemed so modern after the old Siemens. Changing the filaments and aligning the column stood me in good stead for 20 years later when we had a second-hand Philips 301. Also, I was lucky to learn a variety of other, non-EM, techniques at this time. Most importantly, I am still in touch with some really good friends from that time, including my boss Dr P Osmanski.

In the mid 70s, my next EM job was back in the UK, in London at the National Heart and Lung Institute, then called the Cardiothoracic Institute, which has always had close links with the Royal Brompton Hospital. I had only been there two or three months when the head of Experimental Pathology, Prof. Lynne Reid, decided to leave the country (not my fault, honest!). The real reason was that even way back then, funding was increasingly difficult to get and so the UK's loss was Boston's gain, when most of this world-renowned team crossed the Atlantic a few years later. It was for me, however, a wonderful couple of years of a fast and steep learning curve in lung structure, while I helped with projects that needed to be finished. Particularly rewarding and edifying was the reinforcement of ultrastrucural features and terminology, as I worked my way through stacks of electron micrographs needing Letraset annotation.

Prof. Reid had established the EM facilities at Brompton in 1965, with MRC funding, and had bought that reliable old work-horse, the AEI EM6B. Younger readers should note that AEI at this time was a world leader in electron microscope manufacture and development. The service engineers were also rather special and I'm sure many of you will remember Roland Roberts and Colin Simmonds (Figure 3) as being quite a 'double act'. One of their stories told of being stuck inside a skip, and how I wish I could remember the details! The layout of the EM6B had a number of user-friendly features as compared with the Philips. First, the specimen-holder held 3 grids, which allowed one to choose the best section and also to choose the best position of a feature of interest with respect to grid bars etc. Second, the beam shift controls were both on the right hand side, which meant that it was easy to centre the beam with the right hand as you increased the magnification with the left. Third, one could go all the way to 30,000 times before there was any 'flip-around' of the image. On the other hand, the camera was definitely not user-friendly! It was quite quirky, and if you didn't turn the knob smoothly, with a special little halt at that special little point, then it just jammed on you. Also, it did not expose negative numbers onto the film, and so they were written on the emulsion side in pencil, in the darkroom while counting backwards from e.g. 36294.

Still, the excitement of the academic side of the work fully recompensed for any technical difficulties. I was hungry for all ultrastructural knowledge and would always have an issue of e.g. Am. J Pathology to read on the tube. At this time in the late 70's, electron microscopy at Brompton was becoming established as a key tool in three major, clinically linked, lines of investigations, both for diagnosis and pathogenesis. 1) Nasal and bronchial biopsies/brushings for the genetic disorder Primary Ciliary Dyskinesia (PCD) with Prof. P. Cole (1),
2) Bronchoalveolar lavage samples from various lung diseases with Dr P. Haslam (2) and
3) Miscellaneous lung tumour and non-tumour biopsies with Prof. B. Corrin (3). As found elsewhere, however, in the early 90s, as the use of light microscopy diagnostic immunostaining increased in use, diagnostic electron microscopy decreased. Nevertheless, difficult cases may still need every tool in the box. Also, one cannot truly understand the pathogenesis of a disease process without establishing the ultrastructural changes and I am very proud how EM has contributed to the understanding of lung disease.

An exception to this reduction in the diagnostic use of EM was for PCD. The first Brompton EM case was in 1978 and ultrastructural examination is still of crucial importance today in the confirmation of this disorder. The most common defects found concern the dynein arms, radial spokes and the

central pair plus outer doublets, the latter known as a transposition defect. It should be realised, however, that a number of cases seem to have normal ultrastructure and when these also have normal ciliary beat frequency (CBF) they are referred to as PCD-like. Thus, functional tests are very important although, unfortunately, not all can be performed on young children. Measurements for nasal clearance and CBF were established early on at Brompton and more recently, the test showing low nasal nitric oxide level in PCD cases is now used routinely. At the ACEM meeting in July 2000, we presented a workshop for ciliary studies. This was very early days for ACEM and I am pleased to say that the workshop-report is now available in the archive section of the web site. The message hasn't changed, but the referral opportunities have. In 2006, I was absolutely delighted to hear of the successful submission to NSCAG (National Specialist Commissioning Advisory Group) from the Royal Brompton Hospital, Leicester Royal Infirmary and Southampton University Hospital, thus enabling them to become established as national referral centres for the diagnosis of PCD. Returning to my 'time-line', the multi-user NHLI/Brompton EM facilities took a major leap forward in 1991 when we moved to a new building, with lots of new equipment including a Hitachi H7000 TEM (Figure 4) and S4000 field emission SEM. This was the first SEM I had ever used, never mind now being in charge of one and having to advise on appropriate techniques; another steep and fast learning curve! The clinical samples studied with the SEM consisted mainly of lung, de-waxed histology sections, mounted on Perspex not glass slides, to identify the inhaled particles. In a correlative manner, the areas of interest seen in the H&E stained section by light microscopy could easily be found on the neighbouring unstained section in the SEM, then the particles revealed via the backscatter detector were identified by energy-dispersive X-ray analysis.
Over the subsequent years, the TEM and SEM research projects have been numerous and various, originating from many different departments. Because the EM unit never had a multi-user junior technician, everybody had to be 'hands-on' with the techniques that were needed to process and view their samples. As you can imagine, a lot of really terrible sections have been photographed but all have given project information and thereby the encouragement to improve.
With about 100 peer review articles to choose from, it has been difficult to select favourites, but I have added a few references, which arise from the major investigations listed above 1,2,3,. That reminds me though; in the 'not so good old days' we didn't have computers or the wonders of Google and Pub. Med, both of which make finding information so easy these days. However, even with all the wonderful new technology, it is good to remember that 'it's what is behind the eyes, not what is in front of them that is important'.

I would like to take this opportunity to thank all of you I have known and worked with, throughout these 45 wonderful years in electron microscopy.

Ann selected the following references as key milestones in her career in EM.

1 Greenstone M, Rutman A, Dewar A, Mackay I, Cole PJ. Primary ciliary dyskinesia: cytological and clinical features. Q J Med. 1988 May; 67(253):405-23
O'Callaghan C, Chilvers M, Hogg C, Bush A, Lucas J. Diagnosing primary ciliary dyskinesia. Editorial. Thorax 2007Aug; 62(8):656-657

2 Haslam PL, Dewar A, Butchers P, Primett ZS, Newman-Taylor A, Turner-Warwick M. Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis. Comparison with other interstitial lung diseases. Am Rev Respir Dis. 1987Jan; 132(1):35-47

3 Dewar A, Valente M, Ring NP, Corrin B. Pleural mesothelioma of epithelial type and pulmonary adenocarcinoma: an ultrastructural and cytochemical comparison. J Pathol. 1987Aug; 152(4):309-16
Corrin B, Dewar A, Rodriguez-Roisin R, Turner-Warwick M. Fine structural changes in cryptogenic fibrosing alveolitis and asbestosis. J Pathol. 1985Oct;147(2):107-19
Corrin B, Dewar A. Pathogenesis of idiopathic interstitial pulmonary fibrosis. Ultrastruct Pathol. 1996Jul-Aug;20(4):369-71



Figure 3

Figure 1. The Siemens Elmiscop 1A at the Deparment of Zoology, University of Edinburgh; date mid 1960's

Figure 2. The Philips 300 at the Faculty of Dentistry, University of British Columbia; date probably 1970

Figure 3. The AEI EM6B at the National Heart and Lung Institute, Royal Brompton Hospital with service engineer Colin Simmonds repairing one of the panels; date late 1980's

Figure 4. The Hitachi 7000 which replaced the EM6B in 1991. A unusual occurrence with both the TEM (Martin Lyon) and SEM (Chris Watkins) service engineers on site at the same time; date probably early 2000's.



Figure 4

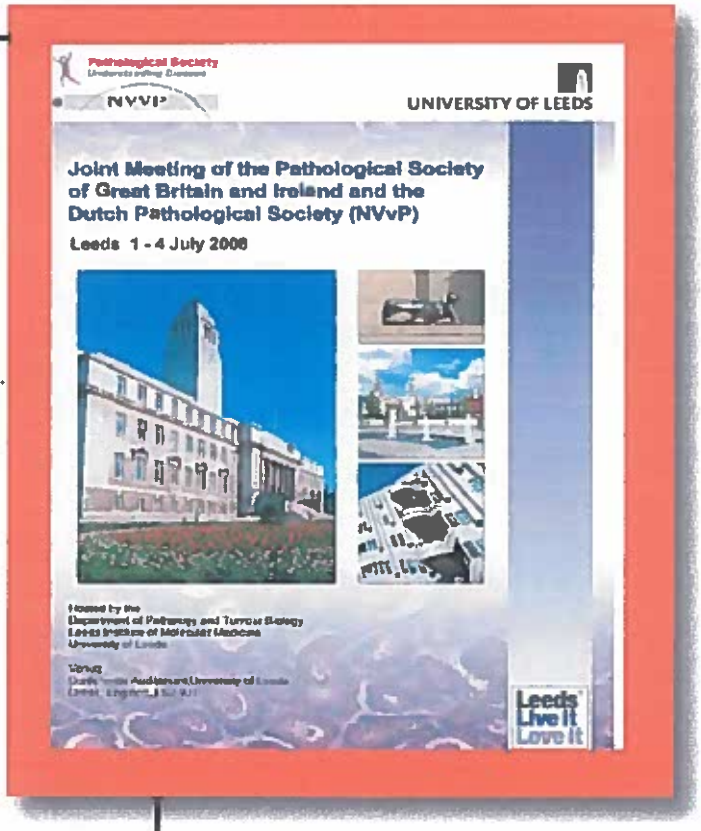
ENJOY YOUR RETIREMENT ANN!

PATH SOC MEETING

Leeds University -Thursday 3rd July 2008.
Association of Clinical Electron Microscopists. 11th annual scientific meeting
Satellite meeting to The Pathological Society of Great Britain and Ireland.

www.pathsoc.org.uk
www.acem.org.uk

9.00am:	Registration
9.30am – 10.20am:	The Liver Institute – What it does, & where EM fits in. Dr Alex Knisely, Consultant Histopathologist King's College Hospital, London Chair: Bart Wagner
10.20am – 10.30am:	Group photo
10.30am – 11.00am:	Coffee break
11.10am – 11.50am: immunohistochemical	CADASIL diagnosis – Ultrastructural and Mr Ray Moss, St George's Hospital, London Chair: Ian Shore
12.00 – 1.00pm:	Technical EM - EQA feed back. Mrs Tracey de Haro, Leicester Royal Infirmary Chair: Bart Wagner
1.00 - 2.00pm:	Lunch break.
2.05 – 2.50pm:	Gut dysmotility – Histopathology and ultrastructure Professor Jo Martin, Consultant Neuropathologist The Royal London Hospital Chair: Glenn Anderson
2.55 – 3.30pm:	Renal biopsy case presentation with review of literature Mr Bart Wagner, Northern General Hospital, Sheffield Chair: Trish Dopping-Hepenstal
3.30 – 4.00pm:	Tea break
4.00 – 5.00pm:	AGM – open to ACEM members and non-members. Chaired by: Dr Tim Ryder,



AFC and Physical Skills in EM

Many ACEM members will have been assessed under agenda for change (AFC) – one of the factors, 'Physical Skills' has almost universally been allocated a Level 3 (in a scale of 1 to 5). Traditionally EM has considered itself to need highly developed physical skills because of the small size of specimens we deal with and the precise hand to eye coordination required to do our job (particularly when using an ultramicrotome or operating an electron microscope). To be allocated a Level 3 in Physical Skills is insulting to most of us – does this mean that all laboratory procedures require the same skill level or, if we require above average skill in order to undertake EM, does this mean that other lab procedures require less skill to perform (is there a health and safety issue here!). Clearly this is nonsense! It seems that HR personnel are making judgements about skills required by laboratory staff and, in particular, seem to be basing their allocation on a literal interpretation of the examples given in the evaluation handbook i.e. if the procedure is clinical it is skilled (Level 4 and 5) and if its not clinical, then it requires less skill! Alternatively are all electron microscopists deluding themselves about the skill level required is that individuals with little or no experience of laboratory procedures are incorrectly interpreting the skill required to undertake EM. We live in a mad world, but we need to ensure that EM skills are not down graded by those without knowledge of EM.