Reflections

Reflections on an unconventional neuropathology career

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The author's CV has been attached as electronic supplementary material: CV supplementary material

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I have no idea if this short account will be of any interest to others but in writing it I have a strong sense of good fortune for having had the career I have had as a neuropathologist.

My interest in joining the medical profession emerged in my teens and I found myself in 1960, at the age of 18, commencing a degree in Physiology at St Hugh's College, Oxford University, as the first stage towards attaining this ambition. This 3-year course was to be followed by three years of clinical training and a supervised year of hospital practice before full registration as a medical doctor. Women made up about 10% of the medical students at Oxford at that time and, in due course, we were distributed in a wide range of medical specialties.

The early stages

My professional development was complicated by meeting and marrying a fellow medical student from Nigeria and embarking on bringing up a family while still a clinical student. This influenced my choice of specialty because it was almost impossible to specialise part-time and front line clinical specialties demanded very long hours of training which would have been incompatible with bringing up a family. Therefore, after completing my clinical studentship, I took up a scholarship from the UK Medical Research Council to have training in research methods as the next stage of my education. The training was undertaken under the supervision of Professor Graham Weddell in the Department of Anatomy. My brief was to spend a year learning the relatively new technique of electron microscopy and applying it to a study of diseased muscle in mice which had been infected with the leprosy bacillus, *Mycobacterium leprae*, after being immunosuppressed by whole body irradiation. Graham Weddell was an elderly, avuncular character with a kind but rather vague air. He was an expert in the structure of peripheral nerves, a site that the leprosy bacillus was known to damage. By reducing the immune response to the bacillus by subjecting the experimentally infected mice to irradiation, it was thought that light might be cast on how nerves were damaged in the naturally occurring human disease.

This was a completely new field of endeavour for me. I knew nothing about electron microscopy, nor leprosy, nor muscle. My first task was to learn how to prepare the tissues, sent to us from a collaborating laboratory in London, for light and electron microscopy. There were other people in the laboratory who were using the electron microscope for other purposes and they could share their expertise with a newcomer such as myself. It took

some time to become familiar with the hugely magnified features of the cells being examined with the electron microscope – it was another world that was being displayed before one's eyes. I went to the books and scientific papers to get help in deciphering the images I was seeing. I had never felt so much need to follow every letter of the message in papers and books about these ultrastructural features. My attention was much more riveted on what I was reading than it had been when I was reading papers for my tutorials as an undergraduate. Now the details were needed to help me make sense of what I could see.

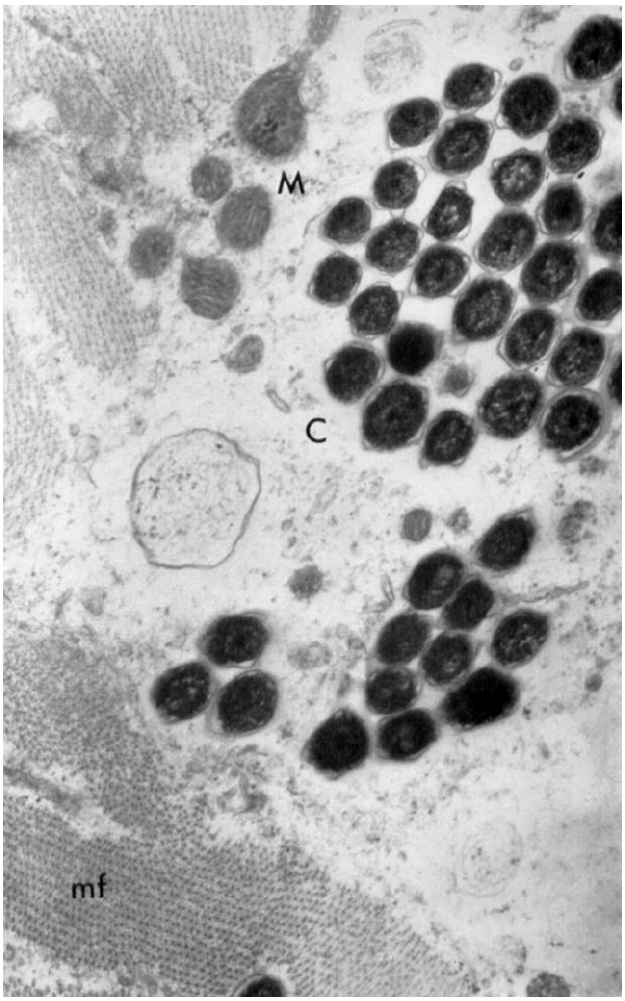

Fig. 1. *Mycobacterium leprae* in muscle

Colony (C) of Mycobacterium leprae in a foot-pad striated-muscle fibre. The bacillary membranes can be clearly distinguishedinthose sectioned transversely. The organisms are lying freely in a sarcoplasmic matrix. Myofibrils (mf) and mitochondria (M) are also present. Thymectomised-irradiated mouse given 10^7 *Myco. leprae* i.v. 11 mth previously. UA, LC, EM x36,000.

published with permission from Esiri MM et al. 1972; 106: 73-80.

Some of the muscle samples that I studied had been prepared for examination using a light microscope. This was to enable me to get more of a 'bird's eye' view of what I was studying under the electron microscope. I was frustrated to find that for quite a long time I could not find any leprosy bacilli in my electron microscope preparations despite the fact that I could clearly see clusters of them with the light microscope. This happened because the tissue samples used for electron microscopy were tiny compared with those viewed with the light microscope and it was easy to miss such minute structures as bacilli. I began to wonder if I would ever find any bacilli at the ultrastructural level, but then came a red letter day when a whole cluster of them, appearing large and black, were suddenly there in front of me in a new sample of tissue (Fig. 1). I fell on them, photographing them with abandon to preserve a record of their structure and their localization in the muscle fibres.

For the electron microscopy work I clearly needed to be present in the Anatomy Department but for the light microscopy I was given the loan of a microscope to take home so that some of my work could be carried on there at times that were the most convenient to me. It was typical of Graham Weddell that he went out of his way to enable my work to fit in with my home life. From time to time we would have a discussion about the work so that he could satisfy himself that it was progressing satisfactorily but most of the time he just left me to get on with it. In due course I wrote a short thesis and we prepared a paper describing our findings which was submitted to a scientific journal.

I was aware as I carried out my practical work in the Anatomy Department that Professor Weddell had regular visits from someone from the local teaching hospital, the Radcliffe Infirmary, who wished to familiarise himself with the electron microscopy of muscle and nerve. He wished to be able to extend to the ultrastructural level the examination he made of muscle and nerve biopsies from patients that were taken for diagnostic purposes. His name was Dr Trevor Hughes (Fig. 2) and he worked at the Neuropathology Department of the Radcliffe Infirmary. Trevor told me that he was hoping to obtain funds to support a Junior Re-

search Officer post in his department that would be devoted to muscle disease research. Would I be interested in applying? I explained that I needed to obtain my full General Medical Council registration by occupying a year of preregistration House Officer posts, but we agreed to keep in touch.

Fig. 2. Dr J Trevor Hughes

While I was carrying out this year of leprosy research, I had kept my hand in at dealing with patients by helping out once a week in an endocrinology clinic run by one of the consultants, Dr Derek Hockaday, whose clinical firm I attended for my second medical attachment as a student. Derek was aware of my difficulty in obtaining pre-registration house jobs while responsible for a young family. It was necessary to obtain the full GMC registration to enable me to progress to any subsequent clinical specialty. Derek was sympathetic to my plight. He had a wife who worked part-time as a paediatric neurologist and they had 3 young children. He eventually offered me a supernumerary pre-registration house job that would not pay me but would give me the experience necessary to secure full registration. This dealt with the 6 months' medical experience. To complete the additional 6 months' surgical experience that I needed, I managed to gain approval to work as a House Officer in Obstetrics, a specialty that did not have routine pre-registration jobs. The advantage of taking this route was that it entailed a 1-in-3 on call rota at night, whereas in surgery this rota would have been 1-in-2. It was also critical that I had the offer of such an obstetric post from the head of the Obstetric service, Professor John Stallworthy, whereas none of the surgeons was willing to offer me a post. Thus, I had secured the possibility of getting my full medical registration, albeit with an unconventional set of posts. I also needed to secure the home situation and here we were very lucky to find a very good young nanny, Jenny, who was willing to live in so that I could do my nights on call. I enjoyed this year of clinical work but I knew it would be impossible for me to carry on with this type of work in the future.

At the end of the year, I therefore applied for, and obtained, the Junior Research Officer post that Trevor Hughes had secured. The post was for 3 years and it would enable me to work for a DM research degree while allowing me at the same time to see what I thought about a career in Neuropathology. I recognised that this was a very small specialty with only about 20 departments in the UK and those confined to major medical centres. I had always kept at the back of my mind the possibility that I might end up working in Nigeria. If I trained in Neuropathology I would need to re-train if I were going to Nigeria. But since our young family had appeared, Nigeria had been experiencing a bloody civil war, ruling it out for settling in for the time being. The prospect of three further years living and working in Oxford helped us to decide that it would be best if the children were to be brought up with their roots in the UK. We did not think it would be a good idea to move the children to and fro between Nigeria and the UK even if the civil war had ended.

So, in 1970, I started as a Junior Research Officer in Neuropathology. I needed to decide on a topic to research on muscle disease. Perusal of the textbooks about human muscle disease seemed to

suggest that although there was a great variety of rare diseases, the two categories of disease that were more common were inflammatory myopathies and muscular dystrophies. I chose to study inflammatory myopathies because I had become interested in the role of the immune system in disease during my experience of studying leprosy. There were also not then the tools readily available to carry out genetic studies of muscular dystrophy that are now very effectively in play.

Inflammatory myopathies can occur at any age but are most common in late middle age. To make a definitive diagnosis, a small sample of muscle – a biopsy – needs to be removed and studied under the microscope to see if inflammation is present. The cause of the inflammation is not certain but it was thought likely that it was due to an immune reaction to some component of muscle, triggered by division and activation of subsets of lymphocytes. I decided to investigate blood lymphocytes taken from people with recently diagnosed inflammatory myopathies and compare their reactions in culture to a small concentration of homogenised muscle with the reactions of healthy people (myself and my colleagues). To do this I needed to find an immunology laboratory that could accommodate me and my lymphocyte cultures. There was such a laboratory in the Nuffield Department of Medicine. It was headed by Dr Ian McLellan. Although the laboratory was already full of researchers, he very generously allowed me to join them. I carried out my human lymphocyte studies there and also studied rats in which I attempted to produce a similar condition of inflammatory myopathy by injecting them with muscle homogenate combined with an adjuvant. While the lymphocytes were being cultured, I also examined the muscle biopsies from the same patients with light and electron microscopy. Although there would be no foreign organisms expected to be present in the muscle if this inflammatory myopathy is an autoimmune disease, it still seemed worthwhile to check for any invading organisms, such as viruses, which might be detectable with an electron microscope. To start with, I used the electron microscope in the Anatomy Department that I was already familiar with but the Radcliffe Infirmary soon acquired one of its own in the Pathology Department where it was particularly useful in diagnosing the nature of kidney disease from studying renal biopsies. I was able to use this electron microscope soon after it was installed and it turned out to be very useful for diagnosing some of the rare muscle diseases.

Just as I had initiated these studies and satisfied myself they were running well, I was overtaken by an unexpected event – a massive fire in March 1971 that destroyed the whole of the Neuropathology Department and the adjacent hospital library at the Radcliffe Infirmary. Luckily no one was injured. The fire occurred at night and I came in the next morning to find the department, including all my records, razed to the ground. The hospital authorities had to scurry round searching for somewhere to put us. The Professor of Surgery generously offered an office and there was some recently vacated animal house accommodation, rather dark and damp, into which we could move. Having scavenged a few chairs and a desk or two from sympathetic colleagues, we sat ourselves in the animal house to draw breath when who should appear but a former neuropathologist who had moved a few years earlier to London, Dr Sabina Strich. She had heard the news of the fire on the radio and promptly come to visit and commiserate and to provide us with a new electric kettle, mugs, coffee and chocolate wholemeal biscuits. It was such a welcome gesture! It raised all our spirits and made us determined to make the best of the situation. Fortunately, my lymphocyte studies could continue in the Immunology Laboratory which was in a different part of the hospital, even though the records of the first experiments were lost. There were also negatives from the electron microscope studies that could be retrieved and reprinted. Some of the brain specimens from earlier years were still retained in a hut in the grounds of another hospital and those that were most instructive for teaching purposes were retrieved and sections re-cut for microscopy. Some second hand equipment such as microscopes and microtomes, needed to cut thin sections, were rapidly acquired and the elements of a re-formed department thus assembled. Eventually the whole destroyed floor of the hospital was rebuilt, though this was not to happen for several years.

The three years of the junior research officer post passed quickly with my energies divided be-

tween the laboratories and the home. We now had a new nanny, Jane, who stayed with us until all the children were at full time school. The combination of research and pathology was much more compatible with family commitments. My husband was pursuing a surgical training and holding a succession of short term posts all over the country as was the usual practice in those days. He returned home whenever he was able to.

The lymphocyte studies yielded modest evidence of lymphocyte sensitivity to muscle in inflammatory myopathies. It was not a dramatic effect and could have been the result, rather than the cause, of the condition. It was difficult to provoke any more than a slight inflammatory reaction in the muscle of the sensitised rats. In the electron microscopic studies, there was only one biopsy that contained what might have been virus-like particles. Thus, the fruits of my studies were far from conclusive. I believe it remains the case today, more than 40 years after these studies were performed, that inflammatory myopathies are really little better understood. However, the thesis I wrote describing these studies earned me a DM degree and I published a couple of papers summarising the findings. I had found satisfaction from this work but now felt increasingly curious about the central nervous system and its diseases that I had started to become familiar with. However, before I was certain that neuropathology should become my chosen specialty, I needed to become familiar with other branches of pathology as part of my training.

What did these two short periods of research teach me? First they reinforced my admiration for science. I particularly admired the way science was prepared to embrace the contributions of people wherever they came from and whatever their background provided they worked with integrity and had innovative ideas. I admired the way science relied on reproducibility of findings to give them credence and not on the advertising ability of their perpetrators to give validity to their findings. I came to appreciate the importance of Karl Popper's dictum that scientists should seek to refute their ideas, not confirm them. Scientific 'facts' are always provisional and can be revised if subsequent experiments are incompatible with them. I learnt to recognise the importance of quantitation in recording results of experiments and the need, in studying disease, to include control samples as well as samples from the disease under investigation. It was important for the investigator, whenever possible, to be unaware of which samples were from controls and which from disease so that there was no chance of bias creeping into the observations being made. It seemed to me that if careful quantitative studies were performed they could almost always yield useful information even if they showed that the ideas that prompted the experiments turned out to be incorrect. In short, science satisfied my urge to ask questions and seek answers to them and having commenced my efforts in that direction I was going to continue if I could.

The next stage

To become a neuropathologist in the 1970s I needed first to gain experience of other specialties of clinical pathology. I became a trainee registrar with a post that rotated through haematology, bacteriology, virology and histopathology. The longest time (2 years) was spent in histopathology (also known as cellular pathology). There were exams to take for membership of the Royal College of Pathologists once the practical experience had been obtained. It was essential to gain this qualification before applying for a post as consultant in the NHS. The exam had a heavy component of histopathology, hence the longer time spent in that specialty.

I didn't find the haematology or bacteriology very interesting. The numbers of samples to be analysed in each of these departments were so overwhelming that much of the work had been automated. Many samples would typically be sent to each of these departments during the course of each patient's hospital admission and, in addition, many samples were sent in each day from local general practitioners. The departments were primarily concerned with delivering a diagnostic service and there was relatively little that I, as a newcomer, could contribute. They did not undertake active research. Histopathology was of greater interest, being more directly relevant to neuropathology. I learnt how to perform post mortem examinations (in the process giving myself the only nightmares I've ever had, in which the corpse came to life while I was busy and I attempted to replace

the larynx so that it could speak!) and to report on surgical specimens of tissue. Because of the large variety of specimens and of the conditions they exhibited, it took a fair time to be able to make correct judgements on the nature of the disease, often cancer. Samples were examined under the microscope, first by myself and then by a consultant who was able to share his or her experience and pass on to me the critical visual clues that yielded the essential evidence about the nature of the disease process.

The head of the Histopathology Department was Dr Robb-Smith, a slightly shy and enigmatic character whom I had encountered as a student at post mortem demonstrations. He was interested in attracting women to his specialty and had secured a trainee post that could be held part-time. This enabled me to have two free afternoons each week. I found the children were all occupied at these times so I used this time to write up my DM thesis, a task I had been putting off while I dealt with more immediate concerns at home and at work.

One of the great advantages for me of working in the Histopathology Department was that I not only gained essential diagnostic experience but I was also able to participate in research. There was a research officer there, Dr Ian Burns, who was experimenting with the use of antibodies to detect specific proteins in tissue sections. If antibodies could be produced towards a purified protein of interest and tagged with a marker substance such as a coloured dye or a fluorescent dye, this could be used to detect specific proteins in tissue sections with much greater specificity than could be provided with the traditional tinctorial stains. This was the principle behind the work that Ian Burns was carrying out and which has now become the hugely successful branch of pathology, immunohistology or immunocytochemistry. When Ian was working in the early 1970s, he was concentrating on the use of antibodies to detect immunoglobulins, themselves antibody proteins produced by plasma cells. I was able to make use of Ian's reagents to study plasma cells containing different types of immunoglobulins in sections of central nervous system tissue affected by different diseases.

This was research I undertook when I had moved back from the Histopathology Department to be a registrar in the Neuropathology Department. My experience in more general pathology had not seduced me into getting more interested in that field. Rather, I thought there were important things I had learnt there, particularly this new subject of immunohistology, that I could usefully apply in neuropathology. Back in the Neuropathology Department, I was also gaining experience of diagnostic aspects of the specialty by examining biopsies removed from brain tumours by the neurosurgeons as well as other biopsies such as those from muscle and peripheral nerves. I was also performing post mortem examinations on cases of neurological disease and brain trauma. This diagnostic work I found still left time for a limited amount of research in which I applied the antibody techniques I had been lucky enough to learn about from Ian Burns to nervous system diseases. This was an example of the wonderful way that collaboration can be developed in science and I was to become a great advocate of such collaboration, prizing it far above competition which, in some fields or departments of medical science sadly seemed to predominate.

I was lucky enough to obtain the modest funds needed to enable me to employ a technician to assist with the application of immunohistology in neuropathological material. I started by studying the lesions or areas damaged in the central nervous system in multiple sclerosis, most generally considered as an autoimmune disease[1]. This was a condition that was of interest to the other consultant in the Neuropathology Department, Dr David Oppenheimer (Fig. 3), who had written a DM thesis on it. He had seen many examples of this condition in post mortem studies and the tissues from some of these cases were still available in the department despite the fire that had destroyed much material in 1971. One of the beauties of the immunohistological technique was that it could be used on tissue that had been preserved in the department after being fixed in formalin and then embedded in paraffin wax to enable thin sections to be cut. Although some proteins that may be of interest in tissues are destroyed by such treatment, meaning that fresh frozen sections are needed to study them, there are many others that survive this

treatment. The advantage of being able to use formalin-fixed, paraffin-embedded sections is that this tissue shows better preservation of its structure than can usually be seen in frozen sections.

Fig. 3. Dr David Oppenheimer

I followed up the work on multiple sclerosis with studies of diseases that did have a foreign organism in the damaged tissues, starting with poliomyelitis of which we had cases dating from an epidemic of polio in the UK in the late 1950s. Later I extended the work to a study of cases of the rare complication of measles, subacute sclerosing panencephalitis. I wanted to know if these diseases showed a similar distribution of plasma cells containing immunoglobulins as I saw in multiple sclerosis. Although this turned out to be the case, the difficulty in interpreting the significance of the finding was that the arrangement of plasma cells might be just the same in autoimmune disease as in one with a foreign organism. While these studies were proceeding, more antibodies for use in immunohistology were being produced, including some directed to viruses. I was to make use of these for a later study when I was a Research Fellow.

The Neuropathology Department when I joined it was a small one. There were 2 consultants, Drs Hughes and Oppenheimer, a registrar (myself), 3 technicians and a laboratory assistant. Trevor Hughes had a particular interest in muscle disease and spinal cord disease. He had moved into neuropathology from histopathology. David Oppenheimer had a less conventional background. He had started his academic career with degrees in Philosophy, Politics and Economics and also Music. During the Second World War, he had been a conscientious objector and worked with an ambulance team in London rescuing injured people in the blitz. This experience awakened his interest in medicine and he read for his medical degree immediately after the war. Initially he worked on neuroanatomy and it was from there that he moved to neuropathology. He was initiated into diagnostic work by his predecessor, Sabina Strich, over a matter of a few months. He recognised that his lack of experience in histopathology and other pathology specialties was a handicap but his knowledge of neuroanatomy was most valuable. It made him particularly interested in the many important diseases of the nervous system in which there is a neuroanatomical basis for the way the disease is expressed or, in some cases, seems to spread. I had been introduced to the Neuropathology Department by Trevor Hughes but as time went on I became more interested in the conditions that interested David Oppenheimer, starting with multiple sclerosis.

One of the first opportunities that arose from the diagnostic work that I carried out was a chance to study a rare case of herpes zoster (shingles) in an elderly woman who happened to die just after the eruption developed. It wasn't the herpes zoster that killed her but an advanced state of multiple myeloma which had rendered her susceptible to developing herpes zoster. My patient had herpes zoster affecting the region supplied by part of the trigeminal nerve. Herpes zoster is caused by the same virus that causes chickenpox, varicella-zoster virus. The theory had been developed that in the natural history of infection with this virus, chickenpox is the first manifestation of infection. As the virus is overcome by the immune response that enables the person with chickenpox to recover, some virus survives and lies latent in the nerve cells of one or more sensory nerves. Circulating antibody which persists after recovery from chickenpox makes any reactivation of the virus uncommon until later in life when the level of antibody wanes or when the person has their immunity compro-

mised by disease. When the virus reactivates in the sensory nerve cells of a particular nerve, it travels along the nerve to the skin where it causes the eruption of herpes zoster. Although this theory was widely accepted, the reactivation of the virus in nerve cells had never been demonstrated. It was this reactivation that I was able to show by electron microscopy which revealed the virus particles in the nerve cells of the trigeminal ganglion (Fig. 4). A colleague in the Virology Department, Dr Albert Tomlinson, carried out immunofluorescence using an antibody to the varicella-zoster virus on frozen sections of the ganglion and the nerve and was able to show the virus at both sites.

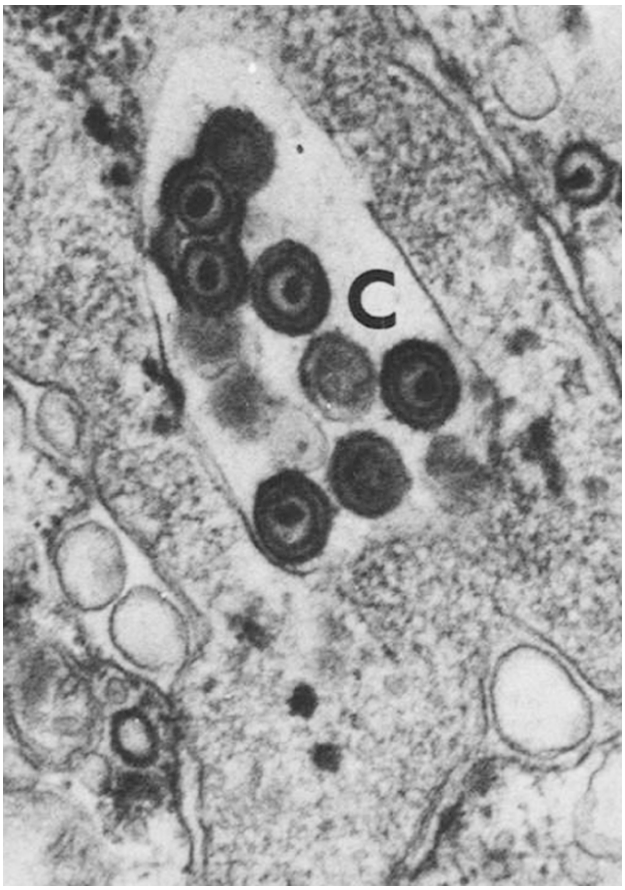

Fig. 4. Herpes zoster viral particles in the cytoplasm (C) of a trigeminal ganglion neuron.

From Esiri MM, Tomlinson AH J Neurol Sci 1982; 15(1); 35—48.

The varicella-zoster virus that Dr Tomlinson was able to show in frozen sections of the nerve and ganglion of our case could not be seen when we tried to detect the virus in formalin fixed tissue – the viral protein was an example of an antigen that could not survive formalin fixation. This observation made me think that there could be more we could learn from our post mortem and surgical specimens if we thought carefully about whether we should preserve a portion of tissue by snap freezing it in liquid nitrogen and then storing it in a -70◦C freezer. Snap freezing in liquid nitrogen preserved the tissue structure better than slowly freezing tissue in a freezer but only if the tissue sample was small. The opening of our minds to the potential importance of deep freezing some tissue was to prove important in years to come, not only to enable immunohistology to be performed for detection of antigens that were sensitive to formalin but also to enable biochemical and genetic studies to be carried out. It led to Oxford being early in the field of brain banking for research (for a history of brain banking in the UK see [2]).

The highly trained technicians that we worked with in neuropathology played an absolutely essential role in all that we did. Unlike in histopathology, where most tissue sample sections were subjected to a single stain, in neuropathology it was common to have consecutive thin sections from the same block of tissue stained with several different stains, some of which were quite difficult to perform. Each technician had their special expertise in carrying out a selection of these stains. Some were histochemical stains that enabled enzymes to be displayed in frozen sections. These histochemical stains were mainly used on muscle biopsies. Each of the tinctorial stains picked out a particular feature of the nervous system to highlight. Nowadays the need for these special tinctorial stains is much reduced because immunohistology can convey the same information using antibodies that are more specific and more straightforward to apply. Some of the reagents used in the tinctorial reactions are quite toxic and it is far preferable that technicians can now avoid them.

My closest colleagues were those in the Neuropathology Department. Trevor and David took it in turns, several months at a time, to supervise my diagnostic work and I had the close assistance of the technicians in the preparation of the sections I needed to do this work. I liaised closely with the neurosurgeons and neurologists over the results. At times there was another trainee doing a project in the department or a visitor making use of the ma-

terial we had available for them to study. I remember particularly a very pleasant, recently retired, Canadian professor of anatomy, Richard Saunders, who came to spend a few winters in Oxford with his wife to escape the worst of the winter in Nova Scotia. They had a son who was an Oxford Rhodes Scholar. Richard had an intimate knowledge of the structure of the hippocampus which he did his best to impart to me. It turned out to be useful knowledge when I became interested in Alzheimer's disease.

I made regular visits to local district general hospitals where I provided a diagnostic service on neurological post mortem cases. I discovered that histopathologists in these hospitals had to deal with quite a lot of neurological cases as the hospitals had consultant neurologists working in them. This visiting service yielded many interesting cases from which I brought back to our department selected samples for expert processing. I would then send reports on these cases back to the histopathologists.

Once or twice a year there were meetings of the UK neuropathology community. Trainee and consultant neuropathologists could apply to join the British Neuropathological Society, which organised these meetings, after they had delivered one sponsored paper or poster at one of the meetings. Some overseas neuropathologists also joined the Society. My first paper to the Society, sponsored by Trevor, described the work I had done on experimental leprosy infection in the Anatomy Department. It was interesting to get to know neuropathologists working in other centres and to compare notes on our respective research projects. I found them to be a very congenial group. Collaboration with some of them became an important asset in devising some research projects for which more cases of a particular disease might be needed than were available in one centre. It was also helpful at times to be able to send a puzzling diagnostic specimen to someone in another centre to obtain a second opinion on the diagnosis.

Towards the end of my time as a registrar I made the acquaintance of a recently appointed consultant in geriatric medicine, Gordon Wilcock. Gordon had come to enquire if there might be someone interested in collaborating with him to study the neuropathology of dementia, particularly Alzheimer's disease. He was himself interested in learning some neuropathology and he had many patients under his care in the geriatric medicine wards on whom, if they agreed, he had carried out a simple cognitive test to determine whether or not they had dementia. A considerable number of them had dementia but there were others who did not, including quite a number who had had strokes. If any of these patients subsequently died, and their next-of-kin was willing for a post mortem to be performed, we were able to see what abnormalities there were in the brain that contributed to making them demented. It was known that in Alzheimer's disease there were two distinct microscopic abnormalities: the presence of plaques and tangles. Gordon and I wanted to see if we could relate either plaques or tangles (or both) to the presence of dementia in his cognitively tested patients.

We had the impression that the distribution of tangles in Alzheimer's disease affected parts of the brain that were becoming known to be anatomically linked together. We took to discussing the neuroanatomy of Alzheimer's disease on Friday afternoons with experts on these cortico-cortical and cortico-hippocampal connections, Drs Tom Powell and his former DPhil student, Carl Pearson. Tom had spent his career on meticulous studies of the cerebral cortex and was greatly pleased to find that some of his work might have relevance to an understanding of such an important disease as Alzheimer's disease. From these discussions came a study of the detailed distribution of plaques and tangles in the different layers of the cerebral cortex[3]. The findings fitted well with what was known of the manner in which one part of the cortex was connected to others and to the hippocampus. Those regions most closely linked to the hippocampus had many tangles while those parts of the cortex which were most remote from the hippocampus had the fewest tangles. This work was my introduction to trying to understand Alzheimer's disease from a neuropathological perspective.

Another influence in fuelling my new interest in dementia was a publication by a neurochemist at London's Institute of Neurology, Dr David Bowen. In the mid-1970s, he published a paper showing

that in post mortem brain specimens that had been frozen at -70◦C immediately after removal from the body there were many enzymes whose activity could be reliably measured. He furthermore showed that in Alzheimer's disease the enzyme, choline acetyl transferase, required for the synthesis of acetyl choline, was deficient. A similar finding was reported from two other laboratories at about the same time. This paper was a real eye-opener for me. I had previously assumed that after death all enzyme activity would cease. Gordon and I had discussions with David about providing him with frozen brain samples from the patients Gordon had tested for cognition on the wards and we were soon adopting the policy of deep freezing one side of the brain for David's studies and carrying out our microscopic studies on the other side. This led to a realisation that it is the burden of neurofibrillary tangles that is held within the brain that determines the extent of dementia in Alzheimer's disease, much more so than the amyloid load[4]. I have watched with dismay the dominance of amyloid in thinking about Alzheimer's disease and how to prevent it that has lasted for decades. Hopefully, now that may change[5].

Not long after we had set up this programme to study dementia, Gordon was offered a professorship in care of the elderly at Bristol University and left Oxford but there was an interested consultant in geriatric psychiatry at the large Littlemore mental hospital near Oxford, Dr John Robinson, who was able to play a similar role in supplying cases of dementia, though he was not in a position, as Gordon had been, to supply cases that did not have dementia.

This collaboration with clinicians in our research on dementia was the first time I had had the opportunity to work directly with doctors looking after patients to devise a research strategy. Neuropathologists are too remote from direct patient care to be in a position to recruit patients to a study themselves. Some neuropathologists developed interests in brain tumours that they could follow using the biopsy specimens they received. Others developed experimental models of diseases that could be studied alongside human post mortem material. But I think the most valuable contribution that clinical neuropathologists can make is to work closely with their clinical colleagues to recruit patients into clinic-pathological studies of human disease.

By the mid-1970s, I had acquired enough experience to take the Royal College of Pathologists examination in Histopathology slanted to Neuropathology. The written examinations were taken in London and for the practical exam I was instructed to go to Glasgow. I remember casting my eye hastily over my notes on the long train journey to Glasgow and thinking that to be taking examinations at the age of 35 years was too old! Fortunately, I passed this last one and was then in a position to think about the next step in my career. For the sake of our children who were happily settled in schools in Oxford, I wanted to remain there but there was only one academic post in Neuropathology and that would be occupied by David Oppenheimer for another few years. One of my colleagues in the Histopathology Department made a most helpful move at this point. He wrote to the Medical Research Council on my behalf and asked if there might be a prospect of getting support from this source to fund research for a few years in Oxford. An encouraging reply to this enquiry led to me applying for a Senior Clinical Fellowship from the Medical Research Council.

Becoming a Senior Clinical Fellow

I put together an application for this Senior Clinical Fellowship to study a disease that had long been of interest to me – herpes simplex encephalitis. This is a rare condition but one that had a considerable mortality at that time and which I had the chance to study post mortem. There were several enigmatic aspects to this disease which intrigued me. It seemed to affect people of a wide range of ages in whom it developed quite unexpectedly. Yet it was caused by a virus that infected some 90% of people in some of whom it caused recurrent cold sores. Like its cousin, varicella-zoster virus, which I had already encountered, it had the capacity to lie latent in sensory ganglia (for this virus, usually the trigeminal ganglion, because it usually causes a facial infection) from which it could reactivate under a range of conditions such as fever, exposure to strong sunlight or the common cold. What was the process by which it could occasionally cause a devastating encephalitis? I knew I would be able to

study the distribution of the virus in the brain using immunohistology and an anti-herpes simplex antibody, and I had tissue from enough cases, which had survived for varying periods of time after disease onset, to enable me to try to piece together the pathological process. Armed with this intention I was lucky enough to get the Fellowship for 5 years at consultant level salary plus some part-time technical help and costs of consumables.

This period of full-time research reinforced my passion for investigating brain diseases of which there seemed so many without an effective treatment, in part a consequence of inadequate understanding of the disease process – hardly surprising given the complexity of the nervous system and the difficulty of accessing brain tissue during the course of many of these diseases. At the time, brain imaging was still rudimentary compared with how it is today and the post mortem examination, for those diseases that were fatal, gave the best lead to increase understanding.

Herpes simplex encephalitis has a very intriguing distribution of damage in the brain with an emphasis on the temporal lobes but with the damage usually asymmetrically distributed[6]. The focus of damage overlaps with that in Alzheimer's disease with the hippocampus and sites connected to it badly affected. From my visits to the Anatomy Department to discuss Alzheimer's disease with Tom Powell and Carl Pearson, I knew these areas had close connections with the olfactory system so I studied the olfactory bulbs in these cases of herpes simplex encephalitis because it seemed possible that the virus, with its propensity to travel along axons, might reach the brain along this route. I could indeed detect the virus there but the olfactory bulbs were not as damaged as sites in the amygdala and piriform cortex to which the olfactory tracts projected. Thus it seemed possible that the virus reached the olfactory bulbs by moving centrifugally, not centripetally, perhaps from a site of latency within the brain, perhaps the hippocampus. The asymmetry of damage to the temporal lobes, which was more marked in those dying early in the course of the disease, might be explicable if it reactivated from inside one hippocampus and then travelled to the contralateral one via anatomical connections between the two.

To take this work further I teamed up again with Albert Tomlinson, the virologist who had helped me examine the case of herpes zoster. We developed a mouse model of herpes simplex encephalitis and showed that inoculation of the face led to the virus ascending to the brain via both the trigeminal and olfactory routes and the development of both brain stem and temporal lobe inflammation. Others have pursued the possibility that latent infection of the brain with herpes simplex virus may predispose to the development of Alzheimer's disease but the opportunity to further investigate the human acute encephalitis was lost on account of the fortunate development of an effective treatment which reduced the fatality of the disease and resulted in many people affected recovering at least partially.

A change of direction?

While I was intent on this research, my children had grown into their teens and my husband was exploring the possibility of returning to Nigeria to provide medical care there. This posed a dilemma for me. Should I abandon my neuropathological career and re-train in tropical medicine or could I carry on with my neuropathology in Oxford part-time while taking periods of unpaid leave to join my husband in Nigeria during school terms when our two younger children were at boarding school? The other alternative - to pursue neuropathology in Nigeria, in the very remote region in which my husband decided to set up a medical practice - would have been impossible. I was able to negotiate to carry on part-time in Oxford for the last part of my research project, working full-time while in Oxford and visiting Nigeria between times. Strange though such an arrangement might seem, it actually worked well and, by the time the research was completed, David Oppenheimer had retired, giving me the expectation that I might apply for his (now vacant) post.

That expectation was thrown into confusion by the government of the day deciding to impose drastic cuts to university financial support. As a result, all re-filling of posts was frozen and many posts were lost. I remained in a form of professional limbo with short-term Medical Research Council support lasting 3 months at a time until a new pro-

fessor, John Newsom-Davis, was appointed in the Clinical Neurology Department on the retirement of the previous postholder. John offered to attempt to reinstate the lectureship in Neuropathology which had previously been held in the Cellular Pathology Department, but only if it could be held in the Clinical Neurology Department. The post was rescued in this way and I was appointed to it on a part-time basis that allowed me to continue with my visits to Nigeria.

My husband's choice of where to establish his clinic was partly determined by his wish to live in a part of the country that is decidedly cooler than the rest. He hated the tropical heat of most of the country and he knew I would too. His clinic was established in a town, Gembu, on the Mambilla Plateau, some 1,500 metres high and not far from the Cameroon border which lies to the east of Nigeria. The countryside is made up of grass-covered hills, interspersed with deep, fertile, valleys and the climate is really perfect, with temperate, warm, sunny weather and abundant rainfall. The roads were rudimentary in the extreme and getting to the place took some 12-15 hours of uncomfortable land rover motoring after a domestic air flight to the nearest place with an airport, Yola. Over the years, a tarred road of sorts was created that mounted the steep escarpment to the plateau but it was soon full of potholes that were hardly ever repaired.

Spending 2 months at a time in Gembu in the spring and autumn of each year became my routine for about 20 years from the early 1980s. Because I couldn't continue with neuropathological research while I was away, I decided to turn my attention to writing about the diseases I was interested in. My aim was to inform those coming after me about what I had learnt so far. My first book was written with John Booss, a neurologist from Yale, who came to Oxford on a sabbatical to study multiple sclerosis. Together we wrote a book on viral encephalitis[7]. To write my parts, I took piles of references with me to read and digest in Gembu, mainly in the afternoons and evenings after I had helped in the mornings at my husband's clinic. My offerings there were decidedly amateurish but one felt that in such a place, where medical care barely existed, one was justified in doing one's best. Once I had gained a little confidence from seeing people recover or, at worst, return the next day for a rethink about the diagnosis, I thoroughly enjoyed this experience. With no diversions such as newspapers or television to occupy the time later in the day I was able to think and write without interruption, although, in the evenings, any activity had to be carried out by the dim illumination offered by a 'tilly' light, fuelled by kerosene.

To follow up after publication of Viral Encephalitis, I approached David Oppenheimer, who had recently retired, to see if he would be willing to write a book on diagnostic neuropathology with me. I felt greatly honoured to have had much tuition from David during my training. He was a truly inspiring person and I felt it would be good if other people could benefit from his teaching even if it had to be in the form of a textbook. I knew he wrote superbly so could convey his knowledge in that way. He thought about it and agreed to write a 'pamphlet' in which we would divide up the topics evenly between us. He had kept the photographic slides that he had collected over his career so was able to make use of these and entered into the writing of the 'pamphlet' with enthusiasm. I continued to learn from him as we wrote and it was a complete pleasure, at the end of a working day when I was in Oxford, to visit him and his wife on my way home and to share the new text he had written during the day. I think he also enjoyed being able to extend his teaching of neuropathology in this way. The result was a textbook of nearly 400 pages that was published as Diagnostic Neuropathology[8].

The third book that I initiated was one on the neuropathology of dementia[9], which was co-edited with my colleague James Morris, who had stepped into the post that Trevor Hughes had vacated when he retired. That all these three books went in to 2nd editions pleased me as it suggested they had found useful homes among those in the neuroscience community.

By this time, a major longitudinal study of ageing and dementia, the Oxford Project to Investigate Memory and Dementia (OPTIMA,) had been started by David Smith, Professor of Pharmacology in Oxford, and we were benefitting from the opportunity to study the brains of dementia sufferers and healthy elderly controls who had been followed up

in a detailed way during life and had consented to donating their brain for research after their death. This study grew slowly, as such longitudinal studies do, but we eventually collected over 500 brains that helped to increase understanding of dementia, and still continue to do so.

Forging ahead

Once I had been appointed to an academic position in neuropathology in the mid-1980s, I started to supervise graduate students who wished to study the brain and its diseases. I always insisted that students should have two co-supervisors because of the periods I spent in Nigeria and out of contact with the department. Two months was the maximum that I considered possible to remove myself from all the activities that go on in a department that has diagnostic, teaching and research interests. Sometimes I would return to find a pile of problems to solve but usually everything had continued smoothly and I had the pleasure of hearing about the new findings that had emerged while I was away. Because of these periods of leave I kept my other absences from the department to a minimum which meant attending very few conferences and meetings – a sacrifice that I was willing to make in return for the privilege of being able to continue with my professional work in my rather unusual personal circumstances.

The department was expanding at this time (the late 1980s) with the arrival in Oxford of Professor Tim Crow, a psychiatrist with a deep interest in biological aspects of psychiatric disease, particularly schizophrenia. We were able to expand into a new small laboratory and recruit an experienced technical manager and a small group of research students to work on psychiatric disease. We were also joined by a research group created by a local academic psychiatrist, Dr Paul Harrison. I was never in any doubt that study of the brain was needed as much to understand these diseases as it was for dementia and ageing and for neurological diseases. In the neuropathology department, I had wonderful collaborations with psychiatrists as well as neurologists and neurosurgeons. It seemed very odd to me that there was little direct communication between clinical neurologists and psychiatrists and little shared training for the two disciplines but this may be changing now. There were also superb opportunities in Oxford to collaborate and learn from neuroscientists in the basic science departments from which we benefitted enormously.

A great boost to our research on schizophrenia came from the generous provision of a substantial group of brains donated for research in Belfast, Northern Ireland. Professor Ingrid Allen, the senior neuropathologist in Belfast, and her colleague in psychiatry, Dr Steven Cooper, were instrumental in creating this initiative with Tim Crow. Tim had a fascinating hypothesis about schizophrenia: that it was somehow related to subtle differences in cerebral asymmetry, that differs normally between men and women, but which he thought might be disturbed in those with schizophrenia. This led to our studies of the brain in schizophrenia being carried out having regard to sex and cerebral hemisphere side. We also studied the corpus callosum and other tracts that connect the two hemispheres. We found tantalising findings on this basis that seemed to indicate, among other things, that schizophrenic males have cerebral hemispheres more akin to normal females than occurs normally in males and vice versa for female schizophrenics. We should not have found these differences if we had studied schizophrenia without distinguishing between the sex of subjects whose brains we studied. Although it has become fashionable in psychology to downplay differences in brain structure between males and females I think these differences are important and may have significant roles in psychiatric disease that we are not yet able to understand.

While these attempts to understand psychiatric disease were launched, we were also continuing to investigate dementia and ageing with the OPTIMA resource. A graduate student, Zsuzsa Nagy, who remained for a while in the department as a postgraduate research scientist, discovered that neurons expressed antigens indicating they had re-entered the cell division cycle in elderly subjects. This discovery, which has been repeatedly confirmed by others, led to studies of lymphocytes and the suggestion that properties of these cells in culture might have diagnostic value for Alzheimer's disease. Although this work has been followed up, we are still without a simple method of diagnosing this disease, particularly in its earliest phase, when symptoms are absent or minimal, but at the best

time for any intervention to be made to slow or stop its development.

My interest in multiple sclerosis was re-ignited at about this time by two developments. The first was provoked by a paper that came out in The Lancet linking episodes of clinical expression at the start and in relapse in multiple sclerosis to episodes of previous sinusitis. The author, Frederick Gay, was a general practitioner and he made his observations using general practitioner records. He thought it possible that bacterial antigens derived from microorganisms in the paranasal sinuses might reach the CNS compartment in some individuals and lead to an immune response that created the demyelinating, inflammatory lesions typical of this condition. We worked together on this idea but, given its unfashionable nature at a time when most interest lay in viruses having a role in the disease (if, indeed, any foreign antigens were involved), funding to make progress was hard to acquire. The idea, however still has great attractions, in my opinion, and it is still being actively pursued.

The second development that brought back my active interest in multiple sclerosis was applying immunohistology yet again but this time using an antibody to amyloid precursor protein as a marker of damaged axons. Although axons have long been known to be damaged in MS, the extent of that damage, its timing and its role in progression of the disease were little appreciated largely because there had been no sensitive method of assessing its extent. Being able to detect damaged axons with great sensitivity, which detection of amyloid precursor protein provided, changed this situation and when we applied the technique to multiple sclerosis lesions of differing age I was greatly surprised by the result. I had expected that we should see damaged axons in chronic lesions which are common in progressive disease but it was actually in the most recent lesions that axon damage was most prolific[10] (Fig. 5). This was despite the fact that recent lesions are more common at earlier phases of the disease when recovery from relapses is common. Initially it seemed hard to understand how damage to axons, which we assumed was irreversible, was compatible with a history of relapse followed often by remission. This pattern of illness seemed more compatible with pathology that was reversible, likely demyelination and the inflammation that accompanied it. But we soon realised that because there is much redundancy in the CNS, progression of damage would only occur when a threshold of axon loss and other forms of neurodegeneration was reached, leaving room for recovery between episodes of inflammation before that point was arrived at.

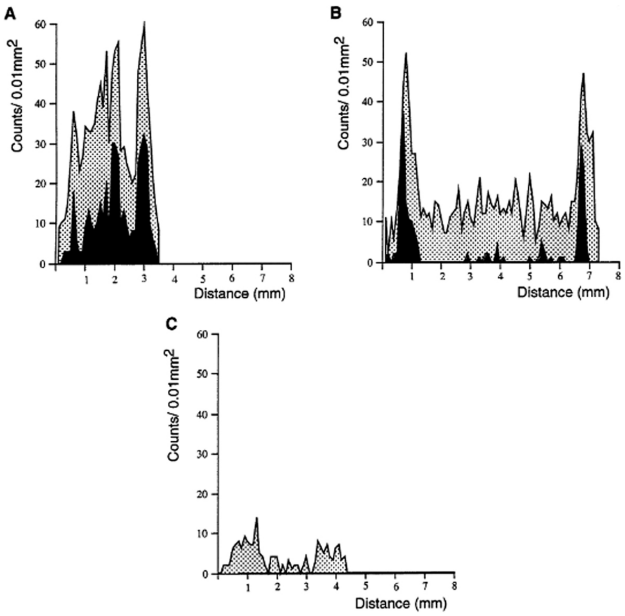

Fig. 5. Graphs to illustrate the profile of axonal injury (APP stippled areas) and the number of macrophages (black areas) in a typical acute multiple sclerosis lesion (A), active chronic multiple sclerosis lesion (B) and chronic multiple sclerosis lesion (C).

From ref. 10

This reassessment of multiple sclerosis and, in particular, its neurodegenerative aspects, made me acutely aware of how progress in understanding disease depends on new techniques being applied to enable new knowledge to be acquired. The spectacular developments in neuroimaging over the past few decades have enabled much progress to be made in understanding CNS diseases and this will continue. Nevertheless, there is still a place for pathological studies because these are still needed to penetrate to the cellular and molecular levels of change that accompany disease. Without knowledge at these levels rational treatment or prevention cannot be achieved. The new frontier relating to knowledge that has grown up in the last two decades is about genetics. Here there is such a wealth of new information that new ways of man-

aging data are needed but I remain convinced that it is the protein and other structural aspects of cells that will provide the key answers to what happens to the state of the body in disease and, therefore, what can be done to remedy it.

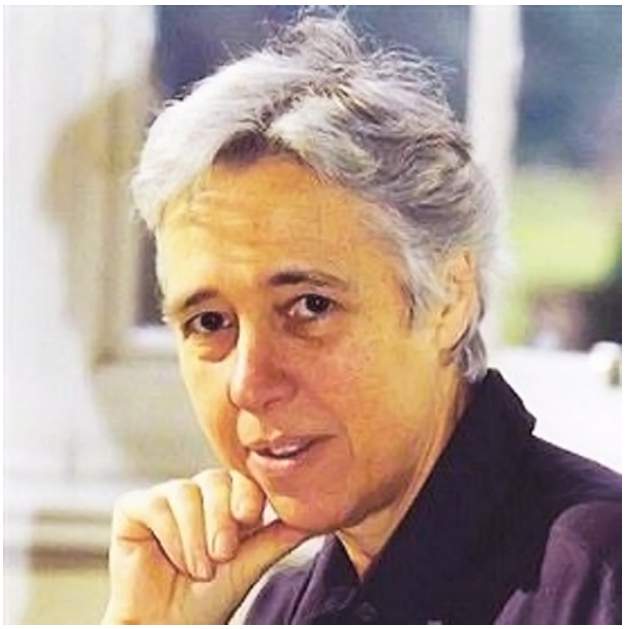

Fig. 6. Margaret M Esiri (2000)

The future

Along with new technical developments enabling fresh knowledge to be acquired about disease, it is vital that there are new ideas. These arise from human imagination and ingenuity. Ideas can come to a researcher from all sorts of experiences and at any stage of their career but, in the long run, a steady supply of new research students with novel ideas is essential. That is why I have been so fortunate in having a career in an outstanding university in which tutors take extraordinary efforts to select the best students some of whom can then become the generators, along with newcomers from elsewhere, of the next wave of insights. They start off without the baggage of long indoctrination with the previous and current dogmas and bring creativity to a research team. Students that I have supervised have repaid my confidence in them in spades and I feel their hands are fully capable of taking forward the fields that came to fascinate me. The person I have kept in closest touch with is Gabriele DeLuca, now a consultant neurologist at Oxford with a lively research group, who brings energy, creativity and flare to his research and teaching, making him an inspiration for yet another generation of young medical students and scientists.

One thing writing a memoir brings home is the manner in which attitudes and knowledge reach you and which you, in turn, pass on to others. There is a social angle to all this, with an amplifying effect of teaching and example, that, at best, is inspiring and uplifting.

At the age of 78, I still find it impossible to abandon my interests in neuropathology and I am incredibly lucky to have a department that still welcomes me into its fold.

Fig. 7. Left: Margaret M Esiri, **Right:** A composite painting depicting the three buildings in which I worked as a neuropathologist: Radcliffe Infirmary (left), St Hugh's College (centre), John Radcliffe Hospital, West Wing (right).

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