fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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UNIVERSITY OF OXFORD

We are very grateful to Judie Waldmann for photographic images of the Dunn School

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Editorial

It is some 18 years since I joined the Dunn school. A lot has happened in that time, but it all seems to have rushed by so very quickly. Needless to say, however, this has been a very enriching and happy period. There are many reasons for this, but the main one which I can only call 'the perpetuation of its soul', is hard to nail down. It is some composite of the Dunn School's history, architecture, and the people who have chosen people. The result is that each working day can be happy and productive in a buzzing community where everyone plays their part.

In being given the role of 'temporary guardian' I have been very fortunate to have had guidance, support and major contributions from many colleagues-and I hope they forgive me for not creating lists of names, but they all know who they are, and I daren't offend anyone but not mentioning them in any select list!

There is moreover, a special advantage to a department whose mission is to conduct strong basic science in the context of disease. The incentive to place one's research and teaching in the context of the alleviation of suffering is part and parcel of our heritage, as so succinctly summarised in Eric Sidebottom's historical webpage. We are privileged to be able to provide Oxford medical students with their first taste of disease mechanisms, and I know that our courses have been treasured as particularly formative for generations of qualified physicians and biomedical scientists. Our mission has provided a framework for the future which gives us a distinct role in Oxford biomedicine, one which I am confident will continue to determine the composition of the scientific projects we undertake.

I would now like to say a little about the science that we have been able to undertake in our time here. It has been directed to the immune system, and specifically how the immune system normally avoids attacking components of our own bodies. Our goal has been to understand all the routes that ensure 'self-tolerance' and to find which can be manipulated therapeutically. The clinical payoffs are potentially huge, all based on the notion that, through a short course of treatment, we might reprogram a faulty immune system and switch off unwanted immune responses in autoimmune diseases such as multiple sclerosis and diabetes and following the transplantation of organs and bone marrow. Current treatments involve unpleasant immunosuppressive drugs, which have a range of unwanted side-effects, and often need to be given life-long.

One of our most encouraging discoveries has been the finding that we can identify short term treatments that empower the immune system to police itself indefinitely. The cells that do the policing appear to be converts to the job, and this has opened up a whole new area of research focussed on how to selectively vaccinate the cells that police the immune system rather than the cells that do damage.

In clinical trials, one of our therapeutic antibody drugs (trade name Lemtrada) has achieved its desired endpoints in relapsing remitting Multiple Sclerosis, in Phase 3 trials conducted by Sanofi–Genzyme. This drug, administered over just a few days, has an impact on prevention of relapse and accumulated disability that appears to last for some years. On a more disappointing note, another drug which can arrest damage to insulin-producing cells in juvenile diabetes, did not succeed in Phæse 3 trials, where the Pharmaceutical partners used a therapeutic dose which was some 16 times less than we had found effective. Clearly, there remains much to be done to bring immune reprogramming into normal clinical practice, but hopefully we can still play our part in encouraging big-Pharma to accept that such treatments have a future.

I wish to finish this short article by thanking everyone who has been part of Dunn school life in the past 18 years for helping create such a vibrant and collegial environment, and in particular, for making my job such a pleasant one to fulfil. I am sure the School will be an important part of UK science for a long-time to come, and I look forward to keeping up with developments through my personal copies of *Fusion!* Most important of all, though, I am very happy that the Dunn School will be developing under the guidance of Matthew Freeman. His enthusiasm and energy in attracting young outstanding scientists is already apparent and I really look forward to the new directions that will come with his leadership.

Herman Waldmann

Editorial

I am thrilled to be taking the helm at the Dunn School, and although the MRC Laboratory of Molecular Biology (LMB) in Cambridge, where I have been for the last 20 years, is a difficult place to leave, I am convinced I have made a great decision.

Oxford biomedical science is thriving and I am looking forward to helping with the further development of South Parks Road, as well as strengthening links with the excellent science done 'up the hill' at the various hospital sites. More specifically, though, all the work that has been done over the last 15-or-so years to modernise and expand the facilities makes this a great time to come to the Dunn School. I am probably not in the best position to write in detail about all the changes made by Herman Waldmann, but the huge debt that the Department owes him is clear. His long-term vision makes my new job perhaps the most attractive in UK biomedical science. I arrive at a time when there is fantastic scope for taking advantage of the excellent new lab space available in the Oxford Molecular Pathology Institute (OMPI). Combined with the outstanding recruitments that Herman has overseen, I am convinced that we can take the Dunn School to even greater levels of success.

A brief word about me. Since returning in 1992 from a postdoc in Berkeley, I have been a group leader in, and latterly Head of, the Division of Cell Biology at the LMB. My background is in *Drosophila* genetics, specifically of intercellular signalling, but in the last ten years my own science has evolved towards more obviously medically-relevant cell biology of signalling. My group is currently most excited about our work on the intersection between cellular quality control mechanisms and the regulation of signalling by growth factors and cytokines. One of our most recent pieces of work exemplifies our approaches nicely: it started as a *Drosophila* genetics project, moved towards mice and human cells, and has now led to a collaboration with Novartis to develop anti-inflammatory therapeutic strategies.

I don't formally take over until January 2013 but I am already quite immersed in planning future developments. Mostly, however, I am talking to people and learning. One aim that I am already clear about is to use some of the space in OMPI as a magnet for outstanding young group leaders with external career development fellowship funding. For example, the newly established Henry Dale Fellowships, which merge the former Wellcome Trust and Royal Society Schemes, are competitive and prestigious and therefore attract the best. We can provide space, mentorship, excellent facilities and a thriving research environment; they will bring with them their flair and scientific potential. Not only will this have obvious research benefits, but such people are often enthusiastic contributors to teaching, providing students with direct contact with excellent young scientists.

Let me finish by reiterating what I have already said: my main current activity is to learn the ropes of a system that is quite new to me. If you have thoughts about the Dunn School and its future, please don't hesitate to get in touch. I am easily findable and try hard to be responsive. I have met many members of the Department already: I look forward to meeting the others soon and to becoming acquainted with the extended Dunn School 'family' that has an interest in our future developments.

Matthew Freeman

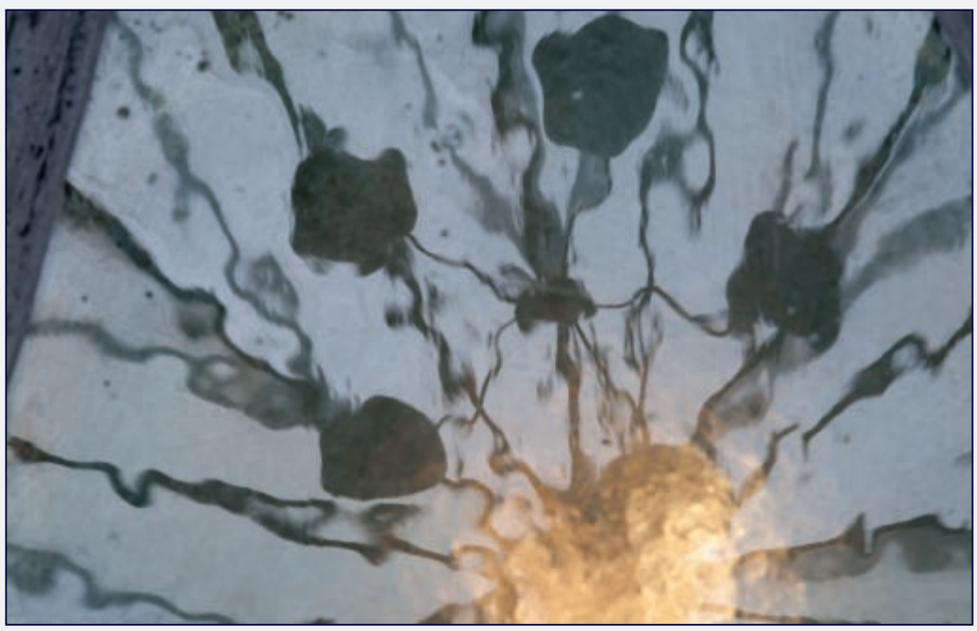


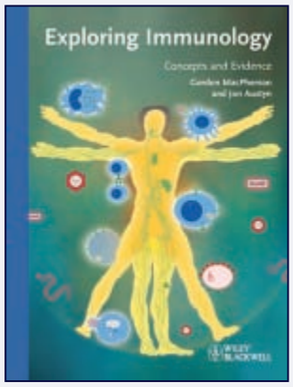
Photo by: Judie Waldmann

Book Review: *Exploring Immunology* by MacPherson and Austyn

A new Immunology textbook, written by two former members of the Dunn School, was published earlier this year by Wiley-Blackwell. Here, Marion Brown reviews their efforts to capture the essence of this rapidly-evolving field and present it in an accessible manner.

In *Exploring Immunology,* we benefit from the combined wealth of experience of Gordon MacPherson and Jon Austyn in teaching at the University of Oxford. The result is a textbook of 'joined up thinking' about immunology, including cells, anatomy, molecules, disease and therapy. Gordon and Jon have produced a tome of manageable size which encourages — as I did — reading the whole volume right through, even if such a strategy does preclude pausing to contemplate all the challenging questions posed throughout to stimulate thought and the manipulation of knowledge. Thankfully, the answers to the questions are provided at the end. The style of the book is didactic but conversational while the intent to explain is serious. Phenomena are explained in relatively plain English without unnecessary jargon, although it suffers from the inevitable overload of acrostics. Analogies are sensible, for example apoptosis is described as 'enforced suicide rather than murder'.

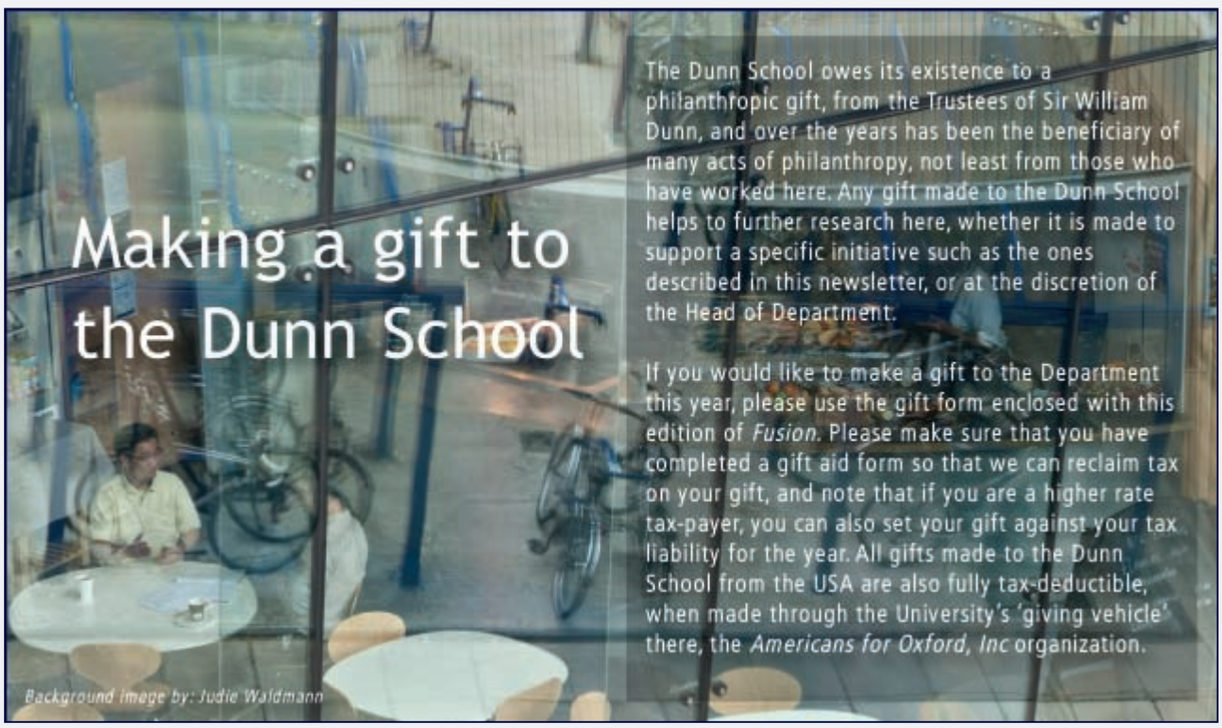
With their long experience of educating, Gordon and Jon know how to regulate the rate at which knowledge is disseminated. A classic approach is adopted of telling us what they are going to tell us, telling us and then telling us what they have just told us. There is also sufficient repetition in each chapter to withstand the time and the memory lapse between reading each one. Summaries at the end of each chapter are helpful for teacher and pupil alike. These will be of great benefit for setting tutorial and exam questions. For those who have not been educated in this country, infiltrating the Oxford way of teaching may seem daunting: *Exploring Immunology* will, therefore, be a great comfort to those wanting to develop their teaching skills.



I have the benefit of knowing both the authors personally and so can vouch for the authenticity of their style. The information provided is comprehensive and includes explanation of techniques and experimental approaches. The diagrams are clear without the unnecessary use of multiple, garish colours. Important discoveries which won Nobel prizes are described. Case studies are included, encouraging theoretical knowledge to be put into practise. Particularly in the last chapter, ethical dilemmas are raised, avenues of research suggested and opinions offered. For example, by way of a conclusion to a discussion on why allergies and autoimmune disorders are increasing in the developing world and the associated "hygiene hypothesis", "the authors will continue to encourage their respective grandchildren and step-grandchildren to play in the dirt". Besides being a valuable educational tool, *Exploring Immunology* is fun to read.

Marion H. Brown

Exploring Immunology: Concepts and Evidence is published by Wiley-Blackwell (ISBN: 978-3-527-32430-9). An accompanying website will be launched in Michaelmas Term 2012 and will contain all 250 colour figures and legends, with a little introductory text.



Introducing Chris Tang

October 2011 was one of those months...

The group had been preparing to move from Imperial College to the Sir William Dunn School of Pathology for several months. But, much like moving house, no amount of planning could have prepared us for the chaos that descended when the removal vans arrived at the labs at Imperial on the first Monday of that month. To make matters worse, the vans were delayed by the inevitable London traffic, and the special truck that was to transport our precious bacterial strains to Oxford had broken down the week before and the company had not found a replacement. Despite these setbacks, by lunchtime we were heading up the M40 to our new home on the second floor of the Oxford Molecular Pathology Institute. This is an outstanding research facility which had been opened earlier that year and houses state-of-the-art laboratory space and facilities; one of the rooms on the floor had been refurbished by Roger Payne and the estates team to accommodate our work with human bacterial pathogens. The rest of that week was taken up with unpacking boxes, except for a welcome break for the Departmental seminar day which provided a whistle stop introduction of the interesting and diverse research that makes the Dunn School such a stimulating place to work.

Rather than having any time to settle in, the remainder of the month was spent giving lectures and practical classes to the second year undergraduate medical students studying the Microbiology component of the *Principles of Pathology* course. This was a daunting task because of the unique history and contributions of the Dunn School to Microbiology and Infectious Diseases. Indeed, I was casually informed that the practical classes were originally devised by Lord Florey and his team, so no pressure there then! Nevertheless, I was fortunate to receive help and support from David Greaves, who is a devoted and respected teacher, as well as the demonstrators, drawn from DPhil students and post-doctoral scientists at the Dunn School, who make the practical classes so popular with the undergraduates.

Even from before we arrived, the group (Figure 1) has been made to feel very welcome by everyone including the Head of Department, fellow academics, and other members of the Dunn School. And almost a year later, I am happy to report that we have settled in well and are all glad not only to have the move behind us, but to be moving forward with our research again.

Figure 1.

The group primarily studies two bacterial pathogens, *Shigella flexneri* and *Neisseria meningitidis* (Figures 2 and 3). These microbes cause medically-important but quite distinct conditions. *N. meningitidis* lives in the upper respiratory tract of humans, and is a leading cause of bacterial meningitis and septicaemia in children. It has also been responsible for outbreaks among school children and University students. In contrast, infection with *Shigella* results in diarrhoea and dysentery, and while this bacterium is not common in the UK, it results in around a million deaths each year in other parts of the world: shigellosis is truly a disease of the poor, and affects refugees and impoverished children living in less wealthy countries.

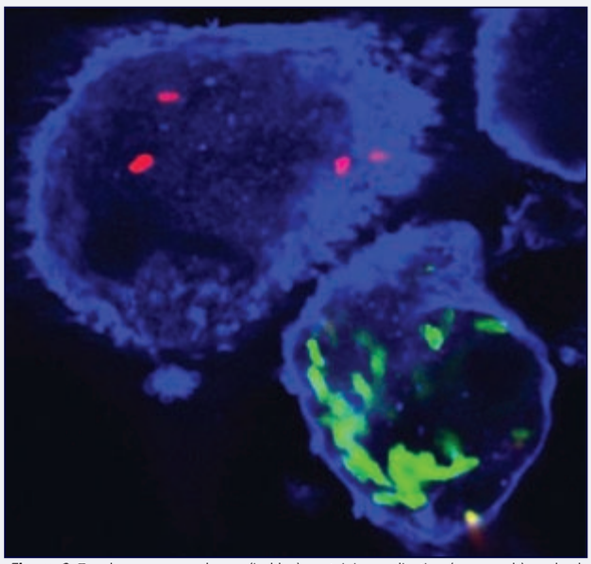


Figure 2. Two human macrophages (in blue) containing replicating (green rods) or dead *Shigella* bacteria (red rods).

Although the meningococcus and *Shigella* occupy different niches in the body and cause totally distinct forms of disease, they have two common features which are the principal reasons why we study them in the laboratory. First both species are human-specific pathogens, and have no known reservoir outside the body. As a consequence, they are exquisitely adapted to life in and around humans, and have evolved over hundreds of thousands of years (and millions and millions of bacterial generations) to avoid our immune system and to occupy specific microenvironments. This makes them fascinating to study and understanding the mechanisms by which they manipulate the body's defences can reveal insights into human physiology and immunity. Second, both bacteria are important causes of human disease, and there is an urgent need for vaccines against these pathogens. A major goal of work in the group is to develop approaches to protect individuals from the devastating effects of meningococcal disease and shigellosis through understanding the strategies these bacteria employ to cause harm to their hosts.

It is being increasingly recognised that we are confronted by a vast array of microbes as part of our normal flora. At any one time, we harmlessly carry several thousand bacterial species. Most of these are kept in check by barriers provided by the skin and mucosal surfaces and by the activity of the immune system. Indeed, these 'commensal' bacteria are vital to health by stimulating our immune systems to prepare for attack by pathogens, and by providing us with essential nutrients. Only a few, highly-specialised bacteria are capable of causing disease, by producing

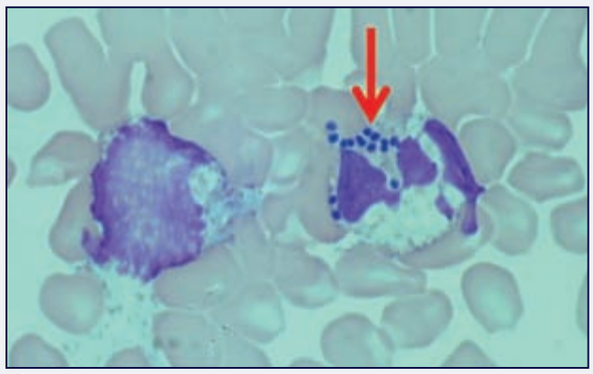


Figure 3. The meningococcus (small blue dots, arrowed) with two human phagocytes in a blood smear from a patient with meningococcal disease.

virulence factors which often set them apart from harmless microbes. Our research aims to identify bacterial virulence factors and define their mechanism of action.

The meningococus remains the leading cause of death from infection in children in the UK. The bacterium strikes rapidly and indiscriminately, with disease developing over the course of just a few hours. The bacterium is a master of disguise, and has evolved multiple ways of by-passing recognition by our immune system. It expresses several molecules on its surface which are direct copies of human structures and do not, therefore, stimulate immune responses. Furthermore, it has evolved a dedicated machinery to change a series of proteins on its surface through a process known as antigenic variation. These characteristics make the development of vaccines extremely challenging and, although there have been some notable successes, there is still no effective vaccine against the most common strains which circulate in Europe and North America.

We discovered that the bacterium takes advantage of human proteins that regulate a key part of our immune system, called complement. The activation of complement is carefully controlled in the body, as inappropriate deposition of complement factors would harm our own cells. This is achieved by a protein called factor H, which switches off complement activity in the bloodstream and on endothelial cells that line the blood vessels. The meningococcus can scavenge factor H from our bloodstream and cells and attach it to its surface: this allows the bacterium to disarm immune responses and promote its own survival. It achieves this feat by expressing a molecule, known as factor H binding protein (fHbp), which can efficiently scavenge factor H from the body. This is a key interaction between the host and the pathogen. Recent studies have shown that human genetic polymorphisms close to the factor H gene determine susceptibility to infection, while fHbp is a leading antigen in vaccines that are currently being assessed in clinical trials.

We work on fHbp with Susan Lea here at the Dunn School, so our move to Oxford has helped to cement this collaboration, and will enable us to make rapid progress. Our studies are focused on modifying fHbp to make it into an ideal vaccine candidate. We have engineered modified versions of this molecule to inactivate its function (in a way that has been done more crudely with de-toxified vaccines in the past) and will test them in clinical trials in the future to see if they are effective in preventing meningococcal disease.

Our group is also studying why certain strains of the meningococcus do not vary their surface structures to any great extent, yet, paradoxically, can still cause outbreaks of disease. We have found that a protein called pilin, which enables the bacterium to adhere to human cells, has not changed appreciably for up to 30 years in some strains, while others alter their pilin every few days in the body. This has obvious implications for vaccine development.

Shigella has a very different lifestyle from the meningococcus, and is one of a select group of pathogens that can actually invade human cells, and gain access to, and thrive within, the cytosolic compartment. Hidden within human cells, *Shigella* avoids recognition by the immune system. We study the invasion process which is mediated by a specialised secretion system that acts like a microscopic needle and syringe. This pumps toxins from the bacterium directly into human cells, where they re-shape the scaffold of the cell, driving bacterial entry. We recently found that the secretion system is triggered by low concentrations of oxygen found within the intestinal tract. This means that the toxins are delivered precisely where they are needed, at the mucosal surface in the gut. We are now using this information to find what other molecules the bacterium expresses at this site in the body. This should reveal novel information about the behaviour of *Shigella* during the invasion process and might provide novel vaccine targets which are only switched on in the human body. We are part of a large network of European researchers involving over 12 groups trying to exploit this information for the prevention of disease.

So for both the meningococcus and *Shigella*, we are attempting to derive clinical benefits from fundamental research which we hope will move some of our work from the bench to the bedside over the coming years. We are also aiming to integrate the teaching of microbiology with other disciplines in the *Principles of Pathology* course. Pathogens do not respect either anatomic or system boundaries and elaborate complex mechanisms to bypass cellular barriers, escape immune detection and trigger multi-organ disease. Therefore, they are not only important causes of human suffering worldwide, but can be used to illustrate the role of inflammation and immunity in health and disease.

Naren Srinivasan is a DPhil student in Kevin Maloy's laboratory and was the worthy recipient of last year's Peter Beaconsfield Prize in Physiological Sciences. The prize is awarded to young researchers who are 'capable of escaping from the stereotype of narrow specialization and who display a wider grasp of the significance and potential applicability of their research'. Here, we are delighted to reproduce his winning essay.

Getting by with a little help from our friends: commensals and disease

Naren Srinivasan

Apart from infectious diseases, which are triggered by clearly identifiable causative agents such as bacteria and viruses, the causes of many non-infectious chronic inflammatory diseases are multifactorial and complex, often involving interactions between genetic mutations and environmental triggers. One such disease, that is the focus of my research, is inflammatory bowel disease (IBD) which involves chronic inflammation of the intestine. Present within the intestine is the greatest density of microbes in the body comprising bacteria, viruses, fungi and parasites. Most people are astounded to know that about 100 trillion bacteria dwell within the gut in a largely peaceful co-existence with their human hosts. Compare this to the mere 10 trillion cells that make up our own body and you soon realize that we are 90% microbial, suggesting that humans are 'super-organisms' and their gut-microbes constitute *bona fide*, but forgotten, organs.

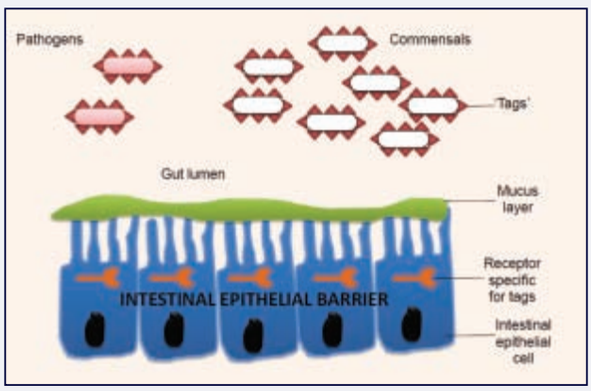


Figure 1. *An immunological paradox: how are pathogens and commensals discriminated in the gut?*

However, this symbiotic relationship with our microbes is paradoxical given how mammals have evolved to sense and kill bacteria. To defend ourselves against dangerous pathogens, we must first detect and identify pathogens, whilst distinguishing them from our own

tissues to avoid autoimmune responses. To do this, we possess a class of receptors that sense 'tags' found solely on microbes, for example sugar studded cell walls that encapsulate fungi. Since these 'tags' are restricted to microbes and are not found on mammalian cells, we can distinguish between self and non-self and thus focus our inflammatory responses accordingly.

One unsolved mystery however is how we discriminate between beneficial commensal bacteria and harmful pathogenic bacteria, given that they express the same, evolutionarily conserved, 'tags'. Nowhere is this question more relevant than in the intestine where commensal bacteria normally outnumber pathogenic intruders by many orders of magnitude (Figure 1). Commensal bacteria play crucial roles in human health: they prevent pathogenic bacterial colonisation, aid human metabolism by digesting complex plant sugars, and play roles in priming the immune system. Thus, it is in our interest to suppress killing of commensals, yet not let our immunological guard down against pathogenic bacteria.

IBD may be due to a breakdown in the ability of the host to tell apart commensals from pathogens. Two lines of evidence support this idea; administering antibiotics to IBD patients partially relieves intestinal inflammation, suggesting that bacteria present in the gut drive the inflammation. Secondly, mice that are genetically altered to develop spontaneous intestinal inflammation show no symptoms when reared in germ-free conditions (these mice are completely devoid of microbes). However when these germ-free genetically-susceptible mice are fed with a single strain of commensal bacteria, they develop intestinal inflammation. IBD may hence arise when the line between commensals and pathogens in the gut becomes blurred.

During my DPhil, I have had time to put this theory to the test. To do this, I have used mouse models of IBD and compared the severity of disease between normal mice (wild type) and genetically modified mice that lack particular genes. In my case, I used mice deficient in genes that I reasoned might be responsible for mediating the discrimination between commensal bacteria and pathogenic bacteria. These genes encode a particular receptor (NOD-like receptor) which senses damage or stress caused by infection. Since pathogens, but not commensal bacteria, cause cell stress and damage, these receptors ensure that inflammatory immune responses are restricted towards pathogens. Interestingly, some of these genes were associated with IBD by other researchers in the field, using novel bioinformatics approaches known as Genome Wide Association Studies (GWAS). GWAS works by scanning and comparing markers across DNA between healthy subjects and patients with the disease of interest. Mutations in particular genes that occur more frequently in sick versus healthy patients are identified as being associated with that disease. I have found that mice lacking genes that potentially mediate the discrimination between commensals and pathogens have a higher susceptibility to IBD and develop exacerbated intestinal inflammation (Figure 2), findings that are currently being prepared for publication.

Having found a genetic association that increases the severity of IBD, my future work will examine how the environment, particularly the intestinal bacteria, influences this phenotype. I will use novel bacterial sequencing techniques which amplify highly conserved parts of bacterial DNA to identify and classify which bacterial strains are associated with the increased severity. By understanding how

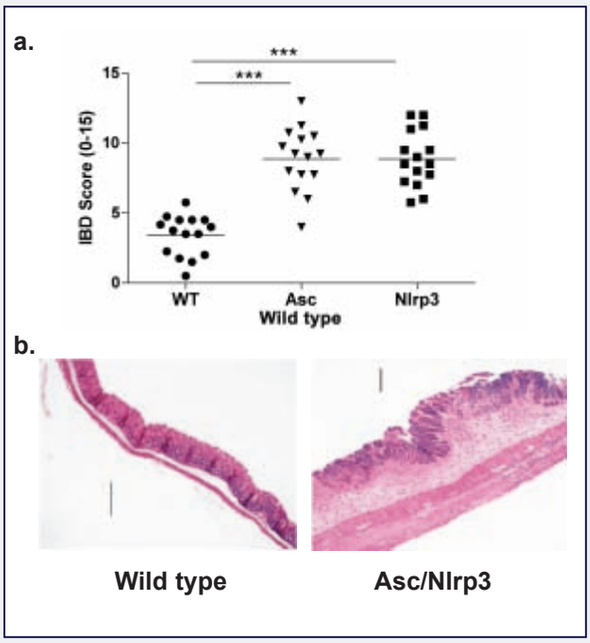


Figure 2. *Mice deficient in genes (Asc and Nlrp3) which sense damage or stress upon infection have significantly greater intestinal inflammation than wild type mice (a). This was measured by examining the intestinal tissues stained with Haematoxylin and Eosin (H&E) (b) and assigning scores to parameters such as oedema, damage, fibrosis and hyperplasia.*

changes in the intestinal bacteria predispose genetically susceptible people to IBD, it may be possible to reverse the microbial shifts by targeting pathogenic bacteria or supplementing the growth or activities of protective commensal bacteria (probiotics).

Embedded in our discussion of IBD is a more fundamental question: what causes non-infectious chronic inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and Type 1 diabetes? Scientists have defined many of the genetic associations associated with these disorders, but the environmental contributions remain poorly characterized. A clue to the answer may lie in a well known observation: over the past decades the incidence of allergic, autoimmune and chronic inflammatory diseases has risen sharply in industrialized, predominantly westernized societies, in comparison to developing countries. The argument known as the 'hygiene hypothesis' claims that urbanization and modernization leads to increased standards of sanitization and consequently a reduction in exposure to commensal bacteria. This leads to impaired maturation of the immune system which predisposes hosts to aberrant, excessive immune responses. In light of this, the gut intestinal bacteria are emerging as important cofactors in a variety of inflammatory disorders. New advances in genetic sequencing technologies are equipping scientists to probe the makeup of intestinal bacterial communities and follow how their structures change with disease. Such technology has already implicated changes in the intestinal bacteria as being associated with obesity and IBD in humans. Recent studies in mice have also shown that certain intestinal bacterial species can drive arthritis whilst others can protect against mouse models of IBD and Type 1 diabetes. Ultimately, the methodology used to identify the cause of IBD could be useful in finding factors that influence the incidence of a host of other diseases and eventually direct the design of novel therapies.

"Are we nearly there yet?" — How macrophages make the long journey to sites of inflammation

David R. Greaves

Cells of the innate immune system need to be rapidly recruited to sites of infection and injury to defend the body against microbes. Chemokines are key players in this process. First identified in the early 1990s, chemokines are a family of secreted signalling proteins that are made in large amounts at sites of tissue damage. These proteins act as powerful chemoattractants for different sets of host immune cells by interacting with a series of specific G protein coupled receptors on the cell surface. There are 48 chemokine genes in the human genome that act through a series of 20 different chemokine receptors.

Tissue resident macrophages, which are derived from blood monocytes, play an important role as sentinel cells in vascularized tissues. When macrophages sense the presence of microbes or pathogen-associated molecular patterns (PAMPs) they amplify this signal and recruit more immune cells by secreting a broad range of chemokines and other chemoattractant signalling molecules including prostaglandins and leukotrienes. A particular group of chemokines, known as the CC chemokines, which include MCP-1 (CCL2) and RANTES (CCL5), are important components of this potent signalling cocktail.

Acting via their cognate chemokine receptors (CCR2 and CCR1/CCR5 respectively) these CC chemokines activate monocytes loosely bound to nearby inflamed endothelium and direct their migration through tissues to the site of tissue damage. This leads to a build up of more macrophages, which help in the clearance of dead microbes, apoptotic neutrophils and initiate tissue repair. It is now appreciated that chemokines can act systemically as well as locally and direct the increased production of innate immune cells in the bone marrow and hasten their release into the bloodstream.

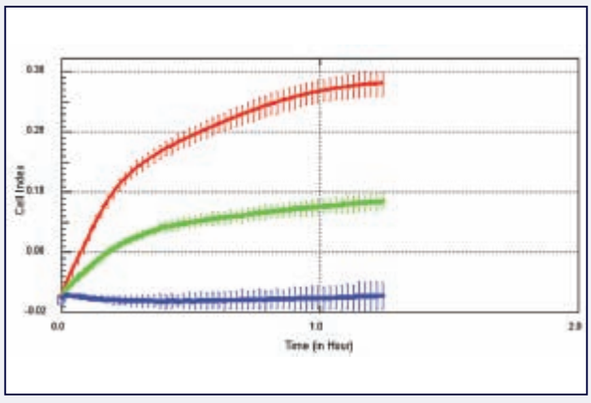


Figure 1: *ECIS Chemotaxis experiment using primary murine macrophages and the CC chemokine CCL5 (Asif Iqbal, unpublished data).*

While macrophages play a key role in the initiation and resolution of acute inflammation they can also orchestrate many of the harmful effects of chronic inflammation. Failure to turn off the powerful CC chemokine signalling system in diseases such as arthritis results in a build up of activated macrophages and the development of chronic inflammation. For this reason, many groups have been developing methods to block CC chemokine activity *in vivo* to reduce monocyte recruitment and macrophage accumulation at sites of chronic inflammation.

Chemotaxis has a long and distinguished history in the Dunn School. When Henry Harris first arrived in the Department, Howard Florey was keen for him to develop methods to study leukocyte chemotaxis (1). Until recently, the modified Boyden chamber used in the 1960s for these experiments was the standard method to measure chemokine bioactivity. Over the past year, my group has adapted Electrical Cell-substrate Impedance Sensing (ECIS) technology for real time analysis of leukocyte chemotaxis and adhesion. An example of such an ECIS chemotaxis experiment performed by Asif Iqbal is shown in Figure 1. Murine macrophages are placed in an upper chamber separated from the chemokine CCL5 in the lower chamber by a filter containing 8-micron pores. A chemokine gradient is developed over a period of 30 minutes and macrophage migration through the pores is measured by following changes in electrical signalling on a gold grid printed on the underside of the filter.

In the absence of both cells and the chemokine CCL5 there is no signal, in the presence of cells but no CCL5 there is a low background level of migration, but when there is a concentration gradient of CCL5 from the bottom well to the top well, it is possible to follow

macrophage chemotaxis and adhesion to the lower filter. This signal is dose dependent and we have shown that the electrical signal corresponds to adherent macrophages using scanning electron microscopy (Figure 2). Asif Iqbal has demonstrated the utility of this ECIS chemotaxis system to follow migration of human neutrophils and monocytes, as well as mouse macrophages, to a wide range of chemoattractants.

Now that we have a sophisticated system to follow chemotaxis of primary myeloid cells in real time, what biological questions can we study using this technique?

Two questions we have started to address are, firstly, which signalling pathways are important in mediating macrophage migration to chemokines and, secondly, are they different from the pathways that mediate chemokine-directed adhesion? We have shown that both chemotaxis and adhesion are dependent on signalling via the Gαi component of the chemokine G protein coupled receptor (GPCR). This result is not unexpected but in early experiments we have revealed a role for ROCK signalling in macrophage chemotaxis that is not required for subsequent adhesion. Work is ongoing to define the signalling cascades initiated by chemokines that lead to leukocyte migration.

Macrophage recruitment, retention and activation are known to be important in atherosclerosis, the disease process that leads to heart attacks and strokes. An important risk factor for accelerated atherosclerosis is diabetes. Both Type I and Type II diabetes are associated with elevated plasma glucose levels which lead to a wide range of clinical conditions, including accelerated atherosclerosis and problems with wound repair. However, the exact cellular mechanisms that account for enhanced atherosclerosis in the arteries of diabetic patients are not known.

Ed Fisher of New York University, a recent sabbatical visitor to the Dunn School as the Eastman Professor, has developed animal models of atherosclerotic plaque regression which shows that macrophages can leave the atherosclerotic plaque when hyperlipidemia is normalised. Ed Fisher's lab showed that macrophages in the aortic lesions of Type I diabetic mice were retained in the lesion when low density lipoprotein (LDL) levels were reduced. We reasoned that this observation could have been due to increased recruitment of monocytes into the lesion, decreased macrophage emigration from the lesion, or a combination of the two. Using our ECIS chemotaxis technique, we have shown that primary murine macrophage chemotaxis to physiological concentrations of CC chemokines *in vitro* is

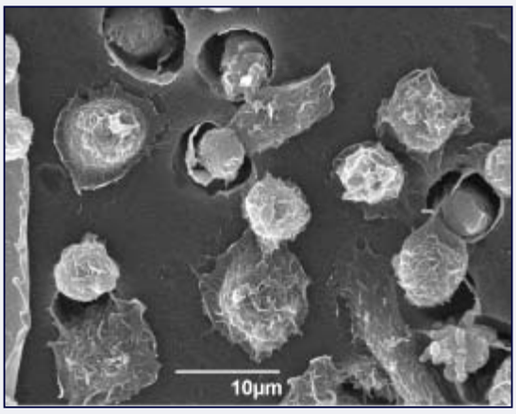


Figure 2. *Scanning EM image of the underside of a filter in an ECIS Chemotaxis experiment using murine macrophages and the CC chemokine CCL5 (Asif Iqbal and Mike Shaw).*

significantly enhanced by acute treatment of macrophages with hyperglycemic levels of D-glucose (15mM) when compared to incubation under normoglycemic conditions (5.5mM D-glucose). We are exploring the specificity of this effect by looking at the effect of glucose concentration on macrophage migration to other chemoattractants and we are exploring the cellular signalling mechanisms that underlie glucose-enhanced chemotaxis.

Finally we want to use our ECIS chemotaxis assay to test new approaches to block macrophage responses to CC chemokine signalling. Because of the central role chemokines play in animal models of inflammatory cell recruitment, chemokine receptors have been widely seen as an excellent target for the development of novel anti-inflammatory drugs (2). Our own approach has been to engineer a poxvirus CC chemokine binding protein for *in vivo* delivery as an Fc fusion protein - 35K-Fc (3). This 35K-Fc fusion protein blocks primary macrophage chemotaxis in a dose-dependent fashion in ECIS chemotaxis assays.

In answer to the question in the title, when it comes to understanding the biology of chemotaxis we are not quite there yet, but we have made significant progress on our journey over the past 10 years. I believe that we are now very well placed to understand what regulates macrophage recruitment both *in vitro* and *in vivo,* and how this process is dysregulated in disease states. My goal is to use this knowledge to develop better treatments for a wide range of human diseases characterized by chronic inflammation.

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3) White GE *et al.* (2011) *Mol Pharm.* 80: 328

Self-determination in a hostile world

Frank Vreede

Frank Vreede was recently awarded an MRC New Investigator Grant to study the regulation of influenza A virus replication and transcription. Here, he gives a flavour of the research he intends to pursue.

Negative stranded RNA viruses, like influenza A, find themselves in a bit of a pickle — their genomes are the opposite of sense! In order to make sense, they need to be replicated first for infection to proceed, and herein lies the devil — replication requires an RNA-dependent RNA polymerase and their cellular hosts are not kind enough to provide adequate facilities for such exotic activities. So RNA viruses are forced to carry this fairly bulky enzyme around and to encode it in their genomes, no mean feat given the structural and genetic constraints to which they are subjected. The inclusion of this functionality alone into the viral genome can double its size, risking the potential for higher genome error rates per replication cycle, even without any consideration for the regulation of the polymerase activity.

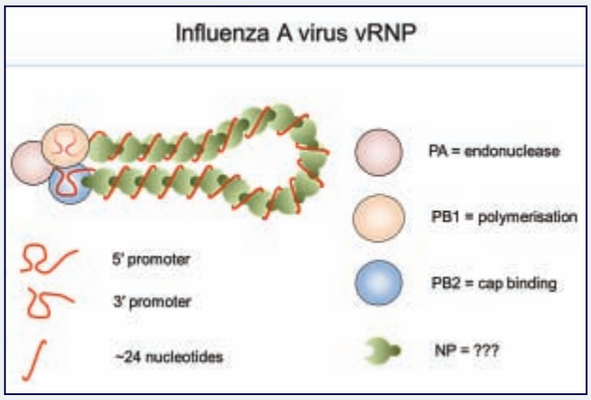


Figure 1.

The trimeric influenza A virus polymerase forms a complex with the viral genomic RNA (vRNA) and oligomers of nucleoprotein (NP) to form the ribonucleoprotein (vRNP) complex (Figure 1) that is responsible for the transcription of positive sense mRNA and replication of the viral genome via an anti-genomic RNP (cRNP) intermediate. Thus the viral polymerase, together with NP, utilizes two complementary templates to produce three different viral RNAs accumulating at varying rates and at different times during the viral infection cycle (Figure 2). The vRNP complex has emerged as a major determinant of host range and pathogenicity which, together with its critical role in the viral life cycle and high level of overall conservation, makes it a potentially important drug target. However, the underlying molecular mechanisms are still poorly understood due, at least in part, to a lack of knowledge about the regulatory mechanisms controlling polymerase activity, both host- and virus-mediated. The objective of my research is, therefore, to elucidate the molecular mechanisms by which the activity of the influenza virus RNA-dependent RNA polymerase is regulated.

As a post-doc with George Brownlee, I proposed a new model that the transcription and replication by the virion-associated RNP is not intrinsically regulated. Instead, I proposed that regulation is mediated by competition between host cell factors, specifically cellular nucleases, and the expression of viral polymerase and NP which would stabilise the RNA by assembly of an RNP complex. This model challenged the established wisdom at the time and renewed interest in the field. Although this model has been widely recognised and cited, it remains a model requiring evidence for the proposed role of nucleases. Thus, one of the projects that the MRC New Investigator Award will enable me to pursue, is to investigate the role of host cellular nucleases in influenza A virus infection.

NP is an essential and major component of the RNP complex yet surprisingly its function in RNP activity and the mechanism by which RNA-dependent RNA polymerase is able to read an RNA template that is associated with NP are not understood. I have recently sought further insight into the regulation of RNP activity by investigating the role of NP. Together with Lauren Turrell, then a Part II student but now a DPhil student in the lab, I firstly demonstrated that NP is, in fact, entirely dispensable for efficient replication and transcription of short virus-like RNA templates *in vivo* and established that NP represents an elongation factor for the viral polymerase. However, we do not yet understand the molecular mechanism of action. I then also determined the elements of NP essential for the stabilisation of nascent RNPs which, surprisingly, did not include homo-oligomerisation. This feature, enabling neighbouring NP molecules to interact, is absolutely essential for RNP activity, and the finding that it is not required for stabilisation of viral RNPs hinted at intriguing alternative roles. It turns out from my recent work, that homo-oligomerisation of NP plays a role in recruitment of NP to the nascent RNP. Through competition experiments, I was able to identify a chain terminating dominant negative mutant of NP that suggests that NP assembly onto the RNP occurs in a unidirectional manner and independent of RNA-binding. Thus the award from the MRC is already allowing me the opportunity to examine the role and assembly mechanisms of NP in greater detail. In future, I hope to study the mechanism by which the viral polymerase traverses the RNP,

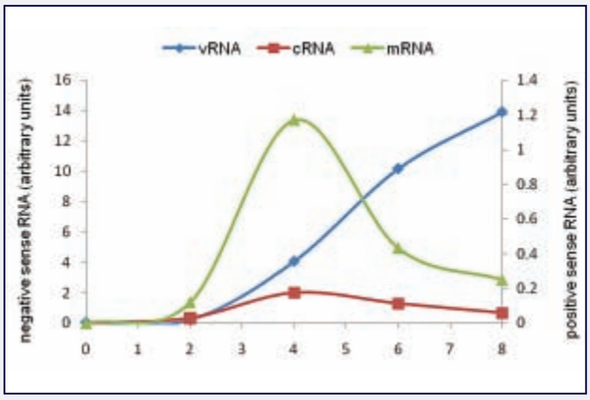


Figure 2.

specifically examining whether homo-oligomerisation of NP may play an additional role to enable transient displacement and repositioning of the NP on the template during the passage of the polymerase.

Much of my research described above focuses on *in vivo* studies. Although broadly relevant to viral infections, elucidating fine molecular detail with this approach is complicated by the scope of cellular factors which have been shown to play both positive and negative roles in viral RNP regulation. Therefore, I have finally also proposed to develop a reductionist *in vitro* system using single plasmon resonance. The successful implementation of this approach using highly-purified viral polymerase and NP will provide a powerful platform to study RNP assembly and activity.

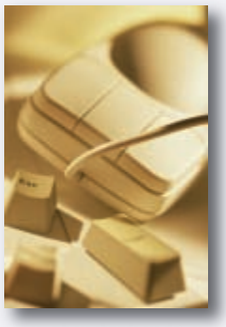
Altogether, it is hoped that understanding the mechanisms by which RNPs are assembled and replicated may suggest possible novel antiviral approaches for targeting these highly-conserved components of influenza A virus.

Interview with John Marriott

John Marriott joined the Dunn School as Computing Officer in 1994. Since that time he has had to navigate rapidly-evolving technology to keep pace with the changing requirements of the Department.* Fusion *caught up with him to find out more about the demands this role has placed on him over the years.

How have the IT needs of the Dunn School changed during the 18 years you have spent here?

In the mid 90s, the department was originally quite Mac orientated with solid machines like SE/30s and IICXs connected to the data network (you can still see a few of these in the background if you look closely at some of the photographs of the students displayed on the walls). Since there were few networked PCs at that time, some of my first jobs were to fit ethernet cards to 286 PCs so that they could be connected to the data network via DEC Pathworks. Macs were heavily used in the former Cellular Immunology Unit and Chemical Pathology Unit and I think PCs were generally used elsewhere, Wordperfect 5 for DOS being very popular on the PC front for Word Processing purposes. Pathology originally shared a VAX cluster with the then Molbiol service which provided the convenience of a single username to access both. We outgrew that system as sharing files between Mac and PC users was becoming difficult and then moved to Novell networking for file storage which we continue to run now, although connected to a larger storage system elsewhere. In 18 years the Department has moved from about 70 networked objects to just under 700. As time has progressed we have seen instrumentation become much more PC based, since many instruments now require PCs to operate, collect and analyse the data. We now have around 1500 active data sockets around the site and have had to have some large holes drilled in the Dunn School to accommodate the amount of cabling required. I've now seen four new buildings go up on the site and been fortunate to be involved in the provision of cabling and data networks in each.



Email used to be quite a simple communication method (we did run this locally at one time) but has now evolved into the Nexus service run by Oxford University Computing Services (OUCS) which includes calendaring to link to mobile devices, a service on which people are now highly dependent. We also keep an eye on the telephones here and liaise with Telecommunications on any necessary changes. The anitcipated change of the University Telephone system to VoIP (Voice over IP) by 2017, currently under discussion, should be an interesting development.

We also maintain the audiovisual systems of the seminar rooms: facilities like video-conferencing are now taken as standard requirements. There are also large central software systems for Finance, Personnel and Student records which are relatively new and therefore bring their own challenges, as most people only used their own databases.

How have hardware and software evolved over the years to meet the demands of modern scientific research?

One of the best things I have seen introduced into the University is the way it handles wireless networking using OWL (Oxford Wireless LAN) which enables a department to deliver the same wireless services that are delivered elsewhere in the University, if it wishes to do so. This makes it easy for people to move with their laptops, tablets and smart phones between the colleges and departments. Back in the 90s, people could leave work with all their collected data on a single 128MB Magneto-optical drive, which for most applications today, would be totally inadequate. The challenge of where to store all the data is a growing challenge, since there are lots of potentially expensive solutions to choose from. The website, which was created around 1997, has undergone two rewrites over the years and has now become an important point of information for people who want to find out more about the Dunn School and research undertaken here.

What has been the greatest IT calamity that you have been aware of during the time you have spent at the Dunn School?

We had a serious server problem in December 2005. Although the hardware problem was eventually fixed, the server continued to suffer performance problems which took some weeks to resolve with the vendor. All the data had to be moved elsewhere, which caused us to change strategy completely, as a result of which we now rent space elsewhere.

What are the most rewarding aspects of the service you provide and what are some of the frustrations?

One of the most tricky areas to deal with, not surprisingly, are passwords. We can generally tell when someone is not using the appropriate password but have a way of resetting the system if trying to coax out the right password fails. We like things to run smoothly and, since some people are highly reliant on laptops to function, enjoy turning repairs around quickly to get equipment back in use. One of the good aspects of IT is getting your hands on new pieces of equipment and seeing how they work. We enjoyed the completion of the OMPI building in 2011 and the challenge of fitting out the data kit in a short period of time, from stacks of boxes to a functional data network. Furthermore, it is the interaction with people that makes the job more worthwhile. Indeed, on a personal note, I would like to thank Herman Waldmann, our outgoing Head of Department, who has always been highly supportive of IT initiatives and projects.

With no particular place to go...

Steve Clarke

Over the last four decades, Steve Clarke has become almost synonymous with the Dunn School and will be greatly missed following his retirement earlier this year. Here, he shares a few parting reflections on his rather unconventional career path...

It is time to leave, after more than 41 years of working in the Dunn School. So how did I get here?

My career could not by any means be described as the pursuance of established career goals, but rather one of responding to opportunities when encouraged to do so by others, who recognized more potential in me than I did myself. My arrival at the Dunn School was due to happenstance: I had completed A-Levels and had not achieved the required grades to study Geography at University, which was my ambition at the time. So, I was faced with re-takes of maths and physics, and the need to have a job in the meantime. Unlike today, there was plenty of work available in the 70s: one could pick and choose to a large extent. The first job suggested to me by the employment exchange was in the Dyson Perrins Chemistry Laboratory, but at the last moment the clerk decided I should apply, instead, for a technician post in the Dunn School (a friend of mine took the job in Chemistry but said it was rubbish – something of a close shave from my perspective!).

Mrs Peggy Turner, the Administrator at that time, managed to overlook my long-haired, hippy appearance, and thought I would probably do, although, as she interviewed me with straw in her hair from grooming her beloved horses that morning, I had a few reservations of my own. I received an immensely welcoming, if rather eccentric introduction to the Dunn School: there were still a number of people from the penicillin era, who had built up a camaraderie and informal management style, partly as a result of working in the Department throughout the 1939–45 war.

I worked first with Paul Dendy on diphtheria toxin studies, and it took only a short time to realize that I quite enjoyed the novel processes involved in cell biology. Apart from work encouragement, he initiated some cultural interests such as T.S. Eliot's *The Waste Land,* and Stravinksy's *Rite of Spring,* for which I have always been grateful.

During the last 40 years, concerns over Health and Safety have grown considerably, and it seems strange now to recall the more relaxed attitude to such matters, which would, quite rightly, never be permitted today: in the 70s, people would brew coffee on Bunsen burners and keep their packed lunches in lab fridges, alongside bacterial and viral samples. Each Christmas we enjoyed drinks in the workshop, as we danced amongst the lathes and band saws!

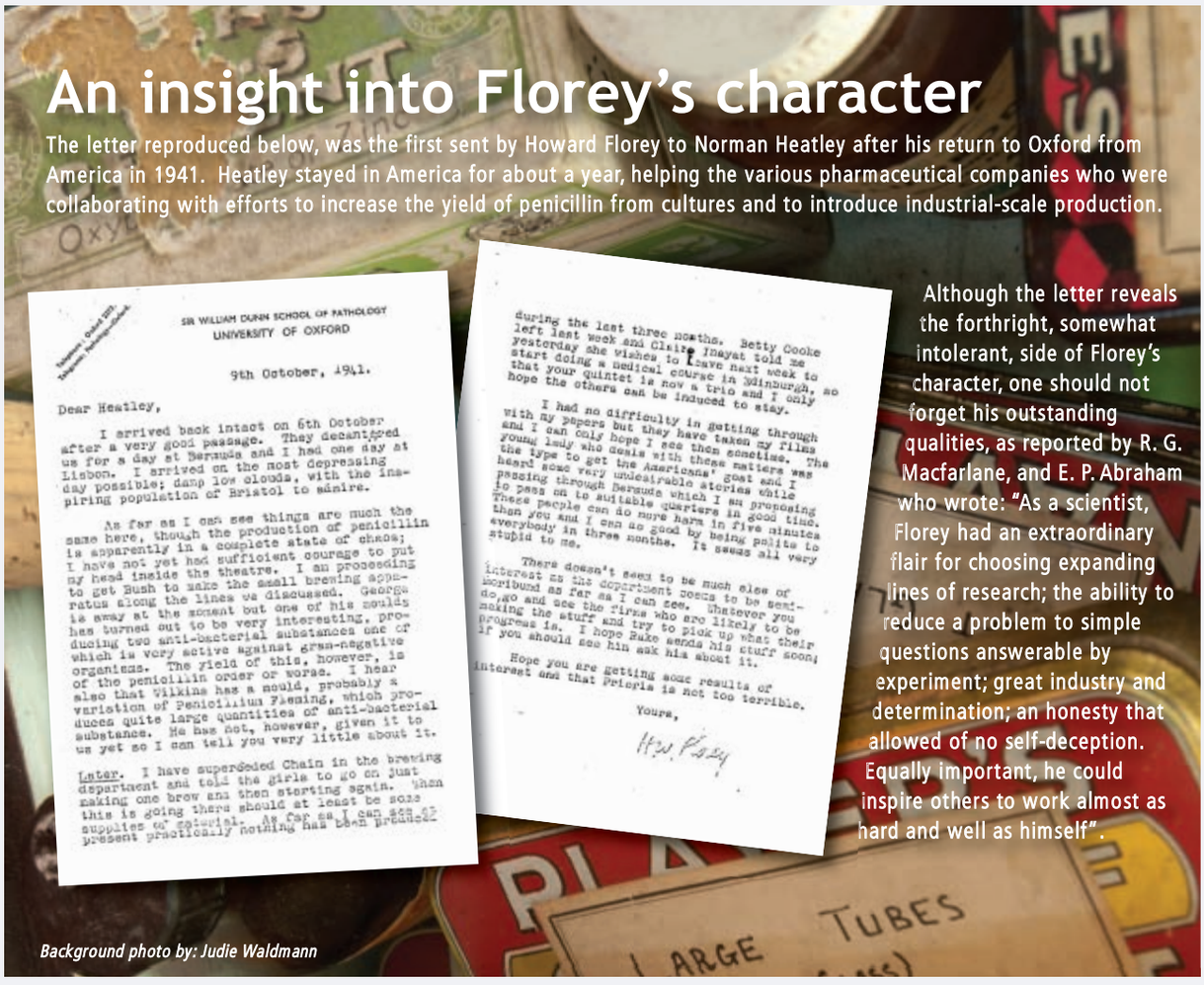
During this time, I was fortunate to develop skills and experience, particularly when working with Erich Jost on DNA binding proteins, and then with Eric Sidebottom on tumour metastasis. I owe them both a great debt, as they made me realize that I was in this for the long term. As a result, I needed to further my education. I embarked on an Open University degree course, and was greatly supported by the Department, both in terms of study time, and financial assistance. This took six rewarding years to complete, since it was all done in my spare time, most of it at weekends. After a brief respite from study, and at the suggestion of Eric Sidebottom, with strong support from the Head of Department, Sir Henry Harris, I started working towards a DPhil, which I successfully completed in 1986.

My first departmental role gave me the opportunity to participate in the development of the replacement scientific support facility. This was a time of steep learning curves! There were many different aspects of the role, and we had to respond to the rapidly-changing requirements and expectations at departmental, university and governmental level. There was much to be done to ensure that robust compliance measures were in place. With a new Head of Department from 1994, there was a bit of a roller-coaster of new expectations and challenges. The transfer from the old to the new facility (PSB), and the establishment and management of new standards and goals was simultaneously frustrating, rewarding, exhausting and stimulating. Thankfully, there was a lot of support (and some challenges) from Herman Waldmann, for which I am most grateful.

I feel I was extremely fortunate to have joined the Department in the 70s, when employment was easier to obtain than it is today. I certainly didn't arrive through the regular routes, and I hope the Dunn School will continue to provide an environment which enables people from less conventional backgrounds to benefit from similar educational and professional development. I am conscious that I owe a huge debt to many people in the Dunn School, some of whom I have mentioned (apologies to the many I have not!), who were willing to take a chance on me and to provide vital encouragement and support, which enabled me to respond to the opportunities as they arose.

An insight into Florey's character

The letter reproduced below, was the first sent by Howard Florey to Norman Heatley after his return to Oxford from America in 1941. Heatley stayed in America for about a year, helping the various pharmaceutical companies who were collaborating with efforts to increase the yield of penicillin from cultures and to introduce industrial-scale production.



SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
UNIVERSITY OF OXFORD

9th October, 1941.

Dear Heatley,

I arrived back intact on 6th October after a very good passage. They decantoped us for a day at Bermuda and I had one day at Lisbon. I arrived on the most depressing day possible; damp low clouds, with the inspiring population of Bristol to admire.

As far as I can see things are much the same here, though the production of penicillin is apparently in a complete state of chaos; I have not yet had sufficient courage to put my head inside the theatre. I am proceeding to get Bush to make the small brewing apparatus along the lines we discussed. George is away at the moment but one of his moulds has turned out to be very interesting, producing two anti-bacterial substances one of which is very active against gram-negative organisms. The yield of this, however, is of the penicillin order or worse. I hear also that Wilkins has a mould, probably a variation of Penicillium Flexing, which produces quite large quantities of anti-bacterial substance. He has not, however, given it to us yet so I can tell you very little about it.

Later. I have superseded Chain in the brewing department and told the girls to go on just making one brew and then starting again. When this is going there should at least be some supplies of material. As far as I can see at present practically nothing has been produced during the last three months. Betty Cooke left last week and Claire Inayat told me yesterday she wishes to leave next week to start doing a medical course in Edinburgh, so that your quintet is now a trio and I only hope the others can be induced to stay.

I had no difficulty in getting through with my papers but they have taken my films and I can only hope I see them sometime. The young lady who deals with these matters was the type to get the Americans' goat and I heard some very undesirable stories while passing through Bermuda which I am proposing to pass on to suitable quarters in good time. These people can do more harm in five minutes than you and I can do good by being polite to everybody in three months. It seems all very stupid to me.

There doesn't seem to be much else of interest as the department seems to be scurbund as far as I can see. Whatever you do go and see the firms who are likely to be making the stuff and try to pick up what their progress is. I hope Duke sends his stuff soon; if you should see him ask him about it.

Hope you are getting some results of interest and that Priorla is not too terrible.

Yours,

H.W. Florey

Although the letter reveals the forthright, somewhat intolerant, side of Florey's character, one should not forget his outstanding qualities, as reported by R. G. Macfarlane, and E. P. Abraham who wrote: "As a scientist, Florey had an extraordinary flair for choosing expanding lines of research; the ability to reduce a problem to simple questions answerable by experiment; great industry and determination; an honesty that allowed of no self-deception. Equally important, he could inspire others to work almost as hard and well as himself".

Background photo by: Judie Waldmann

Standing ovation for Gowans

A recent paper by Irving Weissman "Lymphocytes, Jim Gowans and *in vivo veritas*" published in *Nature Immunology* (1), reminds us just how important and unexpected Gowans discovery was of the recirculation of small lymphocytes in the early 1960s. Weissman was a medical student in the audience at the New York Academy of Sciences in 1962 when Gowans described his experiments and their interpretation. His talk was greeted with a standing ovation – "the only one I have ever heard for a scientific paper" says Weissman who, in 1964 while still a medical student, came to work with Gowans in the Dunn School. Weissman's contacts with Gowans set the stage for most of his career. He notes that when publishing the work he had done at the Dunn School,

HISTORICAL COMMENTARY

Lymphocytes, Jim Gowans and *in vivo veritas*

Irving Weissman

Jim Gowans opened a new era of immunology by combining physiological *in vivo* studies with prospective enrichment of small lymphocytes, validating the clonal selection theory, discovering lymphocyte homing and recirculation, and training others to follow.

Figure 1: *Weissman I (2010)* Nature Immunol. *11(12):1073–1075*

Gowans declined to be a co-author, saying that "he had not been there enough", "an uncharacteristic generosity then and now" as Weissman comments. Reading this historical commentary reinforces one's feeling that Gowan's work merited a Nobel prize which, though nominated, he has never received.

Here we reproduce an image of Weissman's Historical Commentary and photographs of Gowans and Weissman from the 1960's, when they worked together at the Dunn School.

Figure 2: *Irving Weissman (left) and Jim Gowans (right), c1964*

Bonanza to Brazil

Eric Sidebottom

The Dunn School has hosted many star graduates over the years. Here, Eric Sidebottom describes his recent reunion with Manuel Odorico Moraes who continues his work on cancer in the Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará, Brazil.

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I have just returned from one of the most extraordinary and satisfying journeys of my life. It was to Fortaleza in Brazil, at the invitation of the last graduate student I supervised before I left the Dunn School in 1989: Odorico Moraes (Figure 1).

Figure 1.

Odorico is now a shining star on the Brazilian medical research and teaching firmament. He recently received two honours; a professorship in the Brazilian Academy of Medicine and the *"Ordem Nacional do Mérito Científico"*, which he modestly explains would be somewhere between an FRS and a knighthood on the British scene. He is also in the middle of a huge project to build Brazil's first custom designed department for basic research in Clinical Pharmacology and Oncology combined with a 64 bed unit for Clinical Trials in these areas. The Clinical Trials are run by his wife Bete, who also completed a DPhil in Oxford in 1989, under the supervision of David Grahame-Smith and Jeff Aronson. The building is a massive 10,000m^2, the basic structure of which is now complete (Figure 2), and the detailed fitting out is about to start.

My invitation was to give 5 lectures to his graduate students and staff about the impact of disease on world history (my current hobby horse). About 40 people attended and seemed genuinely interested in what I had to say (although it is impossible for me to know what instructions they had received beforehand!). For this my wife and I received 'royal treatment'. We were accommodated and dined at some of the best hotels and restaurants in Fortaleza. We were taken in a friend's six-seater private plane along the spectacular coast of Ceara. The friend, a surgeon, also turned out to have done a DPhil in Oxford in the 1980s working with Mike Kettlewell. We were taken to a 'paradise beach resort', cavorting in the warm Atlantic ocean and driving miles along the almost white sands. We were then taken to Manaus, the crazy industrial metropolis with 2 million inhabitants in the middle of the Amazon jungle, 1000 miles inland, where the famous meeting of the Rio Negra and the Rio Solimaes takes place to form the 5 mile wide Amazon. Manaus has a world famous opera house and spectacular jungle hotels, one with an 8km treetop walkway.

Figure 2: *Brazil's first custom designed department for basic research in Clinical Pharmacology and Oncology*

Figure 3.

Odorico's Oxford thesis was on the behaviour of spontaneously transformed rat cell lines and he has continued this line of fundamental cancer cell biology in Brazil. He has, where possible, taken advantage of the huge range of natural products available in Brazil and, together with his wife, is pioneering developments from the laboratory to the bedside in cancer treatment. He has over 200 publications listed in *PubMed*, 10 of them in 2012. Typical titles are: 'Synthesis and cytotoxic activity of new acridine-thiazolidine derivatives', and '*In vitro* and *in vivo* antitumor effects of the essential oil from the leaves of *Guatteria friesiana*'.

When embarking on this visit, I had no real idea of the pleasure it would give me to see at first hand the success of one of my students and to learn how grateful such students are for the opportunities that Oxford provided them. It is very clear that an Oxford DPhil confers enormous status in Brazil. Long may that continue!

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