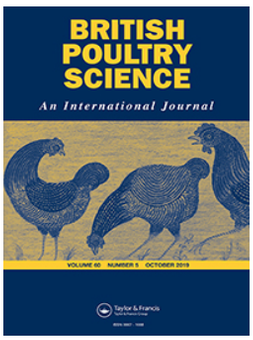

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The Gordon Memorial Lecture: novel approaches to controlling bacterial infections

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ABSTRACT

1. There is huge emphasis in veterinary and agricultural science in understanding the basics of processes and exploiting them for benefits to the economy and human and animal welfare. It is always valuable to be able to step back from existing or favourite hypotheses and paradigms to look at an area of work or problem and see whether a different approach might be productive particularly by drawing parallels with other sometimes unrelated problems.
2. This approach has been used to explore (i) the use of live, attenuated Salmonella vaccines to generate a new form of competitive exclusion, (ii) gene expression technology for the design of improved inactivated vaccines (iii) use of cytokine therapy to reduce persistent carriage by Salmonella, (iv) using bacteriophages to reduce carcass contamination by food-borne pathogens and reduce carriage of antibiotic resistance plasmids.
3. The potential for extending virus therapy to parasite infections is also discussed.

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Introduction

The aim of this lecture is to emphasise the importance of imagination and looking at things differently in the research that I have carried out mainly on *Salmonella* in poultry over more than 3 decades. I worked much of my professional life in the scientific civil service which was set up in the immediate post-war period to improve agriculture such that the country would no longer be so reliant on foreign food should we be once again cut off by naval blockade during war. At that time scientists were provided with sufficient money to be comfortable and with the freedom and time to sit back and think in addition to carrying out basic and applied science. There is huge merit in occasionally sitting back and looking at your work from a distance, working out why you are doing it, whether you can do things differently and whether there are any analogous situations from which you can learn and which may be indirectly relevant to your work. There is also sometimes merit in not following the latest fashion in science, whether this (in my area of work) is virulence plasmids, pathogenicity islands, Toll-like receptors or the microbiome. Francis Bacon (not the artist) said ' He who will not accept new remedies must accept new evils' Not a bad epigram for us.

This lecture covers my personal history followed by some of the work that I have done over more than 35 years grouped into three areas with much of it centred on *Salmonella* in poultry: (i) intestinal colonisation, (ii) vaccination and immunity, and (iii) bacteriophage use. I will also touch on speculation in the related area of using parasite viruses and on the current research funding climate.

Personal history

My generation is sometimes unaware of how lucky we are both in terms of the post-world war II geopolitical landscape, in addition to the national political scene which facilitated social mobility enabling 'poor boys' to reach the heights previously only attained by those families who could afford a first class education. I, like others, have to thank the Beveridge Report and a series of post-war governments of both colours for providing the environment enabling less well-off families to leave their social class previously limited to working in trade and the services. I have also to thank the Grammar School system which gave me a good academic education.

I discussed these benefits with Prof. Trevor Bagust, when we met in Bangkok, two years before he died, and we both agreed that to some extent this had been a golden era for us with (i) better health and diet in comparison with that of our parents and grandparents, (ii) participation in no major wars, (iii) free high-quality school and virtually free university education (so much has changed for the worse in this area, which is important to the success of any small nation with few natural resources other than cerebral resources. Education creates informed and imaginative minds which generate income for the country which leads to higher taxes which support shibboleths such as health services, in addition to other 'essentials'). (iv) We have also enjoyed contact with the rest of the world and European academics and companies following our joining the European Economic Community, now the European Union. (v) There was also post-war government support for agricultural research to avoid dependence on foreign countries for food which was an Achilles heel during WWII.

My education started at Eglwys Wen junior school in Cardiff, Wales with me as an erratic student but who, when I got through the 11+ examination went up to Whitchurch Grammar School and discovered the joy of 'learning' about science, geography and languages. On joining the 6th form I chafed at the inability to take German in addition to the three sciences, such was the restricted curriculum in the mid-1960s.

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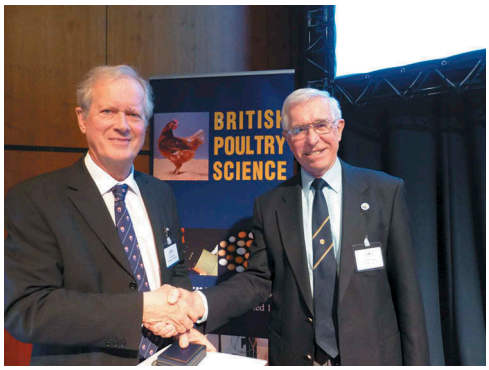

The Gordon Memorial Lecture given at the Annual Meeting of the WPSA UK Branch in Edinburgh on 10th April 2019. The Lecturer, Professor Paul Barrow (left), is seen receiving the commemoration medal from Professor Colin Whitehead (right) of the Gordon Memorial Trust at the conclusion of the lecture.

I went to Bristol University and joined the Microbiology course under Prof. Mark Richmond who stretched our minds and got us to look at things in a slightly different way. Developing an interest in the gut flora in the third year I went to the National Institute for Research in Dairying, Shinfield, Reading to work towards a PhD (Reading) on the role of the attached lactobacillus flora in the pig stomach in regulating gut health with Dr. Roy Fuller, who has been central to the current probiotic agenda. I met my future wife there so there were additional benefits other than just getting a PhD. While searching for a suitable post-doc position I did casual labour followed by a 4-month stint in Black and Decker in Didcot, Oxfordshire where I was promoted to fork-lift truck driver and gang leader, more promotions in 4 months than I had in the following 10 years! I also managed during this time to develop and teach a HND Medical Microbiology part-time course at Oxford Polytechnic College.

A post-doctoral position eventually turned up at the London School of Hygiene and Tropical Medicine and London, working on the transmission of bovine tuberculosis from badgers to cattle. There I was mentored by Prof. Bo Drasar and met Sir. Graham Wilson who was still there producing the 8th edition of Topley and Wilson's Principles of Bacteriology. I was also involved in teaching on the MSc course in Medical Microbiology.

An unhappy year was spent at Unilever Research, Colworth House, Bedford working on coliform mastitis, for which nevertheless I have maintained an interest.

I then had the huge opportunity of working with H. Williams (Willie) Smith, probably the best post-war veterinary microbiology in the world, at Houghton Poultry Research Station (HPRS), Huntingdon. I had admired Smith's work since I was an undergraduate, and it was a dream come true to work with him. Through him and his PhD supervisor, Sir Graham Wilson, I also inherited a line of descent going back to Pasteur through W.W.C. Topley, Sheridan Delepine and Emile Roux. I got to know Prof. Peter Biggs and the enormity of working under two Royal Society Fellows was difficult to appreciate at first. This was a formative time, and I learnt so much from Willie, who was a hard-working, brilliant but prickly and sometimes difficult scientist but who was the closest to genius I have known. He was also Welsh so we shared many jokes around this peppered with his very dry and sarcastic humour. I had 6 years with him before he died and on the closure of HPRS in 1991 moved with my whole group to the Institute for Animal Health, Compton, Berkshire where the facilities also allowed me to work on pigs and calves in addition to poultry. In both Institutes I collaborated with Nat Bumstead (geneticist) on genetic resistance to salmonellosis and who became a good friend, and Jim Kaufmann and Adrian Smith on avian immune response to *Salmonella* infection. I also initiated a European network supported by FP6 and FP7 funding in addition to the core MAFF/Defra funding that sustained us for many years.

The early 2000s were increasingly difficult with shrinking funding and by 2006 it had reached the stage where loss of permanent technical staff was inevitable. The opening of the new vet school in Nottingham (Sutton Bonington) gave us a new opportunity, and I was pleased that the whole group (including Mike Jones) decided to move there with me, in addition to two other postdocs who had worked with me and were then at Oxford (Neil Foster) and Bristol (Rob Atterbury) who joined us. In the first few years of the school, it was 'all shoulders to the wheel', and I was made Research Director a position which I held for 9 years raising the school's research profile in the university, establishing internal collaborations between different groups and helping to establish systems to allow research to function effectively. I then did two years as Global Engagement Lead, largely as a result of my extensive links in Brazil and China. I stepped down in 2018 to concentrate on my research in *Salmonella* in poultry, exploiting bacteriophages, and also exploring the potential of using parasite viruses for disease therapy.

Intestinal colonisation – scientific discoveries and applications

Basic science

When I started work at HPRS the understanding of intestinal colonisation by *Salmonella* of the chicken intestine was largely informed by Willie Smith's seminal work on the mechanisms of colonisation of the pig intestine by enterotoxigenic *Escherichia coli*, namely that they avoided being flushed out by adhering to the small intestinal mucosa. This became the accepted paradigm for intestinal colonisation, but it seemed an unlikely mechanism for caecal colonisation where the flow rate of contents was so slow. We showed that association with the mucosa did occur but that it was related to tissue invasion rather than adhesion (Barrow et al. 1988).

We started to explore the mechanism by assessing colonisation ability of randomly generated transposon mutants of *S.* Typhimurium which indicated that colonisation was more of a metabolic/physiological phenotype with bacteria showing stress responses (Turner et al. 1998). A further signature-tagged mutagenesis study expanded on this but interestingly did indicate that adhesion might also be involved (Morgan et al. 2004). Taking this one step further, gene expression studies on *S.* Typhimurium harvested from the caeca of very young chicks by microarray showed that bacterial growth took place closest to the mucosa where oxygen and nutrient levels are highest (Harvey et al. 2011). The bacteria used carbon sources such as 1,2-propanediol, propionate and ethanolamine with tetrathionate as electron acceptor and using cobalamin. Bacteria were less motile but again indicated an association with the mucosa through up-regulation of SPI-3 and SPI-5. The exact nature

of this association and its role in colonisation and immunity remain to be determined, but a close association is likely since, with Adrian Smith, we showed that bursectomised chickens eliminated *Salmonella* from the intestine at exactly the same rate as entire birds suggesting a role for T cells in gut clearance, again indicating a likely close association with the mucosa during colonisation and immunity, perhaps at the caecal tonsil (Beal et al. 2006).

The role of these carbon sources and electron acceptor in colonisation by serovars such as *S.* Typhimurium and *S.* Enteritidis was highlighted by genome sequencing of *S.* Gallinarum and *S.* Enteritidis where pseudogenes were seen to have accumulated in the relevant operons in *S.* Gallinarum, which does not colonise the intestine extensively (Thomson et al. 2008).

We also found that the virulence plasmids of *Salmonella* did not play a role in colonisation but were essential for the virulence of *S.* Gallinarum and Pullorum. Exchange of these plasmids between these two serovars and *S.* Typhimurium suggested that the plasmid genes were a switch rather than defining specific essential virulence functions per se (see Barrow & Lovell, 1989).

Application in competitive exclusion

A more applied area of work initiated by Willie Smith when I started was to find a bacterial strain which was *Salmonella*-like in its colonisation characteristics but *E. coli*-like in its virulence. We used an *in vivo* system in one-day-old chicks with environmental or faeces samples inoculated orally followed 24 h later by a *S.* Typhimurium strain to find samples and ultimately isolated bacteria from the samples that could inhibit the *Salmonella* colonisation. We eventually found three strains of *E. coli* which when given together produced moderate inhibition of *Salmonella* colonisation. The control for these experiments was birds inoculated first with a rough attenuated *S.* Typhimurium 24 h prior to challenge with the virulent strain. This choice of control arose from our early colonisation studies where we found that a group of young chickens were resistant to colonisation by a *S.* Typhimurium strain because it had become infected via the feed with a *S.* Montevideo strain. We isolated the *S.* Montevideo strain and found that it did inhibit colonisation by *S.* Typhimurium as did a rough Typhimurium strain in the baby chick colonisation-inhibition assay. We decided to study this inhibition between related strains in more detail and found that the inhibitory effect was related to the presence of live bacteria and was not an immunological effect or related to bacteriophage activity. It was genus-specific and some strains were more inhibitory than others (Barrow et al. 1987). The inhibition was profound and was also able to prevent disease if the challenge strains were virulent. When modelled *in vitro* we thought it related to the competition for nutrients under the prevailing redox conditions. We found a similar effect between isogenic Salmonella strains in germ-free pigs and between strains of *E. coli, Citrobacter* (Barrow et al. 1987) and *Campylobacter* (Barrow and Page 2000) in chickens. This approach was novel and was introduced for orally administered live *Salmonella* vaccines by Lohmann Animal Health and now by Elanco. It meant that live *Salmonella* vaccines administered in this way will generate a rapid exclusion effect against colonisation, but the vaccine strain will persist and induce protective adaptive immunity in the normal way.

Immunity and vaccination

We started exploring the nature of immunity to *Salmonella* colonisation in chickens together with colleagues in Cambridge because virtually nothing was known in detail in comparison with murine salmonellosis. Basic studies on the serological response led to the development of a commercial ELISA for use with poultry which has been commercialised by Guildhay and Bommeli and enables differentiation between some invasive serotypes including flagellate and non-flagellate serotypes (Barrow 1992). More basic studies explored the Th1/Th2 bias during *S.* Typhimurium intestinal colonisation and persistent *S.* Pullorum infection and the role of B cells in intestinal clearance (Beal et al. 2006).

An interesting application

We, like others, have shown early on that infection with either *S.* Typhimurium of *S.* Enteritidis generates protective immunity to re-infection by the same serotype. We knew that (i) the *S.* Gallinarum 9R vaccine, developed by Willie Smith in the 1950s (Smith 1956), was highly protective against fowl typhoid (*S.* Gallinarum), (ii) LPS is a major protective antigen and (iii) *S.* Gallinarum and *S.* Enteritidis share many antigens including LPS. We therefore hypothesised that the 9R vaccine might protect chickens against *S.* Enteritidis. We assessed this in laying hens and obtained a high degree of protection against localisation in the liver, spleen and ovaries (Barrow et al. 1991). This was also applied extensively in the field by Dutch scientists and commercially by Intervet/MSD.

Improved inactivated vaccines

Live vaccines are much more protective than inactivated because they stimulate both humoral and cell-mediated arms of the immune system and because they persist longer in the tissues. Inactivated bacterial vaccines were produced historically by culturing bacteria in broth followed by inactivation resulting in a vaccine which is rich in antigens appropriate for *in vitro* growth in broth! Intervet addressed this problem by culturing *Salmonella* in synthetic broth containing dipyridyl, an iron chelator, with the rationale that Fe^{3+} sequestration is important for bacterial virulence *in vivo*. However, studies on patterns of gene expression of *S.* Typhimurium in mouse macrophages indicated many other physiological changes. So, we cultured an Avian Pathogenic *E. coli* (APEC) strain in an avian macrophage cell line and looked at bacterial gene expression by microarray. We found that restriction of iron, magnesium and manganese were important and different carbon sources were used in comparison with growth in nutrient broth. What we did then was to reproduce these conditions in a synthetic broth medium and culture the APEC strain followed by inactivation. When tested as a vaccine against a second APEC strain we found much improved protection over the vaccine produced by culture in nutrient broth (unpublished results). This again indicated that a novel application could result from normal investigative science.

This approach may have scope for improved inactivated vaccines against bacterial infections where septicaemia and/or pneumonia might be significant aspects of the disease, such as caused by APEC, but also by *Pasteurella multocida*

in chickens, turkeys and pigs, *Riemerella anatipestifer* in ducks, *Mannheimia haemolytica* in calves and *Ornithobacterium rhinotraccheale* in poultry.

Cytokine and vaccine therapy

We have had a long interest in the persistent infection that follows convalescence from S. Pullorum infection that still occurs frequently following hatchery infection in many countries. Following experimental oral infection of commercial layers at 4 days of age the bacteria multiply in the liver and spleen and are then controlled but are never completely eliminated, and the bacteria persist in these organs and ovary until, at sexual maturity, a surge in sex hormones reduces T cell responsiveness (Wigley et al. 2005), and the bacteria again multiply in these organs and the oviduct transferring infection to the developing egg.

It has always been a puzzle why the bacteria are not completely eliminated despite the presence of high titre specific antibody. We knew that they persisted within splenic macrophages (Wigley et al. 2001). We established cultures of blood-derived macrophages co-cultured with T cells and found, in contrast to *S.* Enteritidis after macrophage infection, *S.* Pullorum induces much lower levels of IFNγ, IL-12, IL-17F and IL-18 and higher levels of IL-4 and IL-13, classic signs of a Th2-type response, with weaker cell-mediated immunity, as opposed to a Th1/17 response induced by *S.* Enteritidis. This was reflected in similar result from using *ex vivo* splenocytes or *in vivo* infections (Tang et al. 2018).

We know that some pathogens can differentially modulate the host immune response with a contribution from the host genetic background. This can be seen in leprosy and also in some parasite infections. Lepromatous leprosy shows extensive tissue damage and is characterised by a Th2 response which can be reversed to some extent by injections of human IFNγ into the lesions. We therefore argued that a similar form of immune modulation could reduce persistent *S.* Pullorum infection. We injected recombinant chicken IFNγ intravenously into persistently infected birds and observed a significant reduction in *S.* Pullorum numbers in liver and spleen. Clearly further work is need on this approach but although this seems an unrealistic scenario for controlling *S.* Pullorum infection it might be of practical and economic value for other *Salmonella* infections where persistence is a key feature as in *S.* Dublin in cattle, *S.* Abortusovis in sheep and also *S.* Typhi in man.

Competitive exclusion by live vaccines – something new!?

In the section above on colonisation – competitive exclusion, I talked about the protective effect of oral administration of live, attenuated *Salmonella* vaccines which generates profound resistance to oral challenge by virulent *Salmonella* strains, through, we think, a mainly physiological effect. As part of these studies, we sought a *Salmonella* strain which had a protective effect against a wide range of challenge strains. We found a strain of *S.* Infantis which had this characteristic. When we had access to young germ-free pigs at IAH, Compton we tested this strain against a virulent *S.* Typhimurium strain. When inoculated alone *S.* Typhimurium produces fulminating enteritis within 12 h. The *S.* Infantis strain administered on its own is avirulent and when germ-free pigs infected with this strain were challenged with the virulent *S.* Typhimurium strain just 12-24 h later there was complete protection against the enteritis, despite the fact that there was no prevention of colonisation by the challenge strain! The Typhimurium strain reached densities in the intestine reached by this strain when administered alone but in this case without disease. We found that the avirulent *S.* Infantis was mildly invasive and stimulated the production of IL-8 in the intestine which induced diapedesis of activated neutrophils which generated a protection in the intestine (Foster et al. 2003).

This seemed completely novel to us but as usual, there is nothing, or very rarely anything, new under the sun! Willie Smith showed with his studies on the 9R vaccine in 1956 (Smith 1956) that parenteral vaccination with that vaccine could induce protection against the virulent *S.* Gallinarum strain when vaccination took place just a few days before or even simultaneously with challenge – or even one day after! Well, therapeutic vaccination is not new and Pasteur showed this with his first rabies vaccine – it is a race between the induction of non-specific or specific protection and the development of the disease. Frank Collins and his group in Canada in the 1960s also showed that non-specific protection could be demonstrated in mice and which he regarded as being mediated largely by macrophages, rather than neutrophils/heterophils, He also demonstrated cross protection within short periods of time between unrelated bacteria indicating the non-specific nature off the protection (Blanden et al. 1966; Collins et al. 1966). However, what we showed for the first time was that this effect could be expressed in the intestine, and there is clearly considerable potential in exploiting this to protect rapidly against intestinal salmonellosis.

Bacteriophages – a new take on an old idea

Soon after they were discovered independently in 1915 and 1917 by Twort and d'Herelle, the latter realised that there was scope for bacteriophage (phage) application in disease therapy. At that time nothing was known of their nature or of their interaction with bacteria. It is perhaps not surprising that some of the work carried out at that time was poorly conceived, studied and controlled. With the advent of antibiotics in the 1940s, phage fell out of favour except in the Soviet bloc. The idea was resurrected in the 1980s by Willie Smith, with some very elegant experimental work showing how effective they could be in treating *E. coli* enteritis and septicaemia (see Barrow & Soothill, 1997 for review). I followed his work with interest and tested some phages specific for certain serotypes of *E. coli* that produce systemic disease in man and poultry. The phages were highly effective in preventing and treating coli septicaemia in chickens and colostrum-deprived calves. In chickens, we were able to delay phage administration until birds became sick and still rescued 90% of them (Barrow et al. 1998).

One of the perceived problems with using phage is the development of resistance during therapy and the issue of restriction of phage DNA so that not all potentially susceptible bacterial strains are able to sustain phage growth because the phage nucleic acid is destroyed. A standard way of avoiding this is by using a combination of phages, which Smith used in his studies so that the second phage attacks the resistant mutants that arise from the action of

the first phage. A second is to apply phages in an environment where there is little chance of recycling of phage and bacterial culture which normally results in resistance development. The issue of nucleic acid restriction can be tackled by using phages at such a high multiplicity of infection (moi) that non-specific lysis occurs where so many phages attach to and penetrate the bacterial cell wall that the cell dies from loss of membrane potential. We used this approach to test the ability of phage administration at high moi to reduce surface contamination of carcasses with food-poisoning organisms and found that this was highly effective in reducing *Salmonella* and *Campylobacter* numbers on poultry skin (Goode et al. 2003) and *Salmonella* on pigskin. This was a novel application of phage. At relatively low levels of bacterial contamination, the skin was sterilised of the pathogen. Avoiding recycling is also an issue for phage treatment of enteric infections, either in livestock or for humans, where, after treatment, the bacteria and phage are excreted in the environment with the capacity for new infections or re-infections. In the case of human cholera, there is the opportunity for breaking this cycle through treatment in hospitals or clinics where the faeces of the infected and treated individuals can be collected and composted. We and others have shown that lytic phage can be highly effective and reducing *V. cholerae* numbers in the gut and associated clinical signs using experimental animal infections (Bhandare et al. 2018).

A third approach to countering the problem of development of phage resistance during treatment is to use phages for which the receptors are surface virulence determinants. In this case, most phage-resistant mutants that develop will have lost their phage receptors and thereby show reduced virulence. This was done by Smith and by our group (Barrow et al. 1998) using phages which target the K1 capsule in *E. coli* strains that produce systemic infections.

An extension of this approach has been used by us and others to tackle the problem of plasmid-mediated antibiotic resistance. In most of the Enterobacteriaceae plasmid self-transmissibility involves the production by the transfer region of the plasmid of hair-like sex pili which attach to recipient bacterial cells. Many types of phage attach to these pili and which are able to reduce bacterial numbers but also reduce plasmid transmission. In addition, the phages select for the small number of spontaneous mutants within the culture which have lost their plasmid and have thus become phage-resistant but also antibiotic sensitive from loss of the plasmid. In addition, in many cases, the plasmid-free cells multiply faster than the parent cells. We have shown this *in vitro* but also in groups of chickens infected with an AMR F plasmid-containing *S*. Enteritidis strain where the AMR strain was replaced almost completely after a few days of phage treatment by an antibiotic-sensitive mutant (Barrow et al., unpublished results). There is thus huge potential in applying this approach to reduce AMR in *E. coli* (which is thought to be a major driver of AMR) and related bacteria both in groups of livestock or humans, the latter, for example, in hospital wards, or during cases of septicaemia. Practical limitations include the fact that many plasmids show repression of transmissibility so that, although all bacterial cells possess the plasmid, they may not all express pili and therefore show partial phage-resistance. There are many different plasmid (Incompatibility) groups which possess different types of pili and are targets for different phages so some knowledge of the predominant plasmid types would be required. This novel idea is being explored in more detail by our group and by Matti Jalasvuori in Finland.

Exploring virus therapy one step further – viruses for parasite control

If we can use bacterial viruses to treat bacterial infections and oncolytic viruses to treat cancers in a limited way why should we also not consider looking at the potential of using parasite viruses for treating some parasite infections? Many parasite infections are difficult to treat by chemotherapy and vaccines are unavailable or poorly protective. The idea of using parasite viruses in this way is new (Hyman et al. 2013) but was something that I thought about 20 years ago before many parasite viruses had been discovered.

Two questions need to be posed to explore this: (i) what do we know about parasite viruses and (ii) have eukaryotic viruses been used in disease treatment?

(i) Parasite viruses. An increasing number of nematodes, cestodes, trematodes and protozoans have been shown to be infected with viruses. These include
- free-living (e.g., *Acanthamoeba*) and parasitic amoebae including *E. histolytica*,
- the flagellates *Trichomonas vaginalis*, a cause of vaginitis; *Giardia duodenalis*, a cause of intestinal disease; *Naegleria fowleri*, a rare cause of brain cysts; *Leishmania guyanensis, L. braziliensis* and the sheep parasite *Trypanosoma melophagium*.
- the apicomplexans *Plasmodium cynomolgi* and *P. gallinaceum* (virus-like particles); *Babesia*, the cause of cattle tick fever and human infection; *Eimeria* spp. including *E. acervulina, E. tenella* and *E. necatrix* the cause of avian coccidiosis; and *Cryptosporidium spp*, a cause of diarrhoea,
- platyhelminths.
- *Caenorhabditis elegans*

Fungi and algae also have viruses associated with the cells.

(ii) Evidence of effective virus therapy. The American Chestnut tree (*Castanea dentate*) is attacked by a fungal blight (*Chryphonectria parasiticum*) which causes extensive damage. Some strains of the fungus may be infected with a dsRNA mycovirus (CHV-1) which causes hypovirulence in the fungus. Superinfection of a diseased tree with a hypovirulent fungus strain results in transmission of the virus which attenuates the virulent strain resulting in reduced tree damage and healing of lesions.

It is known that some parasite viruses (for example those affecting *Entamoeba histolytica* and *Trichomonas vaginalis*) result in parasite pathology and death so there may be scope for exploiting their use directly for disease control as has been done with phage.

One can start to speculate on the nature of the likely epidemiology of the virus infections in the relevant hosts. Viruses are likely to multiply maximally where the parasite also shows maximum multiplication. In *Eimeria,* this is where vegetative replication takes place in the intestine.

If *Plasmodium* viruses are found these are more likely to replicate in places such as the mosquito salivary gland rather than in the mammalian host. In *Fasciola, hepatica* viruses might be found in the free living larval forms rather than in the mammal. In both these latter cases parasites viruses might nevertheless be used to control infection in the mammalian host.

There thus remains huge scope for exploring the potential of these viruses for infection and disease control. However, they have not been found for some major parasites including the malarial parasites and liver flukes, human trypanosomids and *Toxoplasma*. Viruses must be sought in these major pathogens. The infection biology and the role of the virus in regulating parasite multiplication and infection must be studied before we evaluate the potential for their use in human and veterinary medicine either by virtue of their inherent lytic activity or in manipulating their genomes to enhance virulence.

Other outputs

In addition to the above themes involving looking at infection control in novel ways, we have produced other practical and scientific outputs which perhaps don't fall within these topics. These include

(1) The demonstration that growth-promoting antibiotics exacerbate *Salmonella* excretion by poultry, which contributed to their withdrawal by the EU in 2006 (Barrow et al. 1984).
(2) A study of Mendelian inheritance of resistance to systemic *Salmonella* infections (led by Nat Bumstead) and colonisation by *Salmonella* and *Campylobacter*. The genes are being identified by the Roslin Institute (Bumstead & Barrow, 1988; Barrow et al. 2003).
(3) The development of ELISAs for *S.* Typhimurium, *S.* Enteritidis and *S.* Gallinarum/Pullorum for chickens, commercialised by Guildhay and Bommeli (Barrow 1992).

Future fears and SWOT analysis

Much of the above work has been done with commissioned Defra and Research Institute support which has allowed a degree of freedom not easily available these days with competitive peer-reviewed research funding in Universities which is the worst of all systems except for all the others – rather like democracy. But it is a crude and insensitive method to review research proposals in that it follows trends too readily, and there is always too much emphasis on the major centres of research ignoring outlying institutions where good original work takes place. This can result in haphazard funding streams creating problems for research idea development and also for careers. There is also the concern for loss of expertise through ageing and retirement of older, more experienced staff leading to skills not being handed on to younger staff if funding is not available or if there is too much regulation. This can result in research effectively being transferred to countries in the Far East where funds are more easily accessible and where the research active population is clearly larger.

Passing on skills, advice and information to the next generation, particularly those from countries where the standard of education is less well developed is so important. It is incumbent on us to raise international standards in scientific research and to be inclusive in doing this.

I feel that I have been a lucky member of a lucky generation, to come back to this theme, by being influenced by some major scientists (Willie Smith, Graham Wilson, Bruce Stocker, Martin Raff, Stan Falkow, Peter Biggs, Roy Curtiss III) while developing an extensive network of contacts, too large to list, both in the UK, EU, Brazil, China and elsewhere) and I have been lucky enough to have a number of PhD students (26) and post-docs (20) from many countries which have become good colleagues and friends.

The final parting piece of advice for any younger scientist reading this is to remember the power of the imagination and to occasionally think differently. Dare to be different!

Conclusions

The research environment is changing with less funding available and more of it concentrated in fewer centres which reduces originality in approaching problems. For these reasons, there is much merit in more international collaboration so that more effort can be directed with more outcomes for less individual financial input. However, it is important to remember that ideas cost little and original ideas take some time to come to fruition and persistence is as important as funding.

Disclosure statement

No potential conflict of interest was reported by the author.

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