HUMAN VACCINES & IMMUNOTHERAPEUTICS
2017, VOL. 13, NO. 1, 11–14
http://dx.doi.org/10.1080/21645515.2016.1207439

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PORTRAIT

A view from the virology research laboratory

John Oxford

Blizard Institute, Queen Mary College, London, UK

As a school boy I read *Microbe Hunters* by Paul de Kruit about the hunt for cholera, yellow fever, typhoid and botulinum and I volunteered immediately for a couple of weeks in the laboratory of the local hospital. It was not quite what I expected. I was sent off on the second day to take blood spots from patients using a triangular needle and then told by the Chief Technician to ask the sister of the obstetrics ward for a glass fallopian tube! So by the age of 14 I directed my thoughts more toward science and the research laboratory. I had my own small chemistry laboratory in a garden shed and we made rockets.

Professor BCJG Knight the microbiologist at the University of Reading astounded me. He was an awful lecturer but a profound character. He had worked on Gram positive clostridia and gas gangrene in the years between the wars. His laboratory was a temporary wooden structure off the London Road. My color vision was not perfect but he was reassuring. "Any fool can tell the difference between blue and red, "he said," so the Gram stain will not worry you! There was nothing more exciting than going into the laboratory each morning and peering down the microscope and searching for pink acid fast bacilli. Also I met a wonderful red haired student and later we married. It soon became clear though that I should study for a PhD and I went for an interview at Lodge Moor Infectious Diseases Hospital on the outskirts of Sheffield. The Professor of Medicine, Sir Charles Stuart-Harris was an influenza virologist. He was a member of the Laidlaw, Andrewes and Smith team in London who in 1933 first isolated influenza in a ferret. His clinical registrars used to complain that he spent too much time with his blue eyed scientist boys, namely G. C. Schild and C. W. Potter. I doubt whether they even knew of my existence. But I had a big asset - an ex post office van which I painted blue. When it was still green too many people would rush after me with their letters unposted. So I used to travel the Sheffield Hospitals collecting aborted fetuses and bring them back to Lodge Moor to dissect out the kidneys. Geoffrey Schild had taught me the basics of cell culture and I would chop up and then trypsinize the kidneys and he would grow common cold and polio viruses. Monkey kidney cells would arrive weekly from Porton Down near Salisbury in a baby's feeding bottle. We all used glass test tubes with rubber bungs to seed cells and viewed them every day. We worked in solid wood and glass cabinets with a Bunsen burner to flame all equipment. The rooms overlooked the moors and so we cultured our viruses with a view.

No one had a "game plan" for someone like me, a PhD student. It was a question of seeing what came up in those first months. As it happened K. McCarthy in Liverpool found that teratogenic rubella virus could grow on a rabbit kidney cell line called RK-13. So for the next 2 years I researched animal models in hamsters, ferrets, mice and even hedgehogs. Today PhD research is massively over planned and certainly is lacking in the fun side.

I loved the University library and would spend happy evenings there reading papers of virology. My own first paper was published in *Nature* and I established that the genome of rubella was RNA. But Sir Charles was none too pleased. "Take my advice, Oxford," he said, "publish in a journal that everyone reads like the *British Medical Journal*." Nowadays an early paper in *Nature* is viewed as gold dust.

As I was teaching medical students at that stage I thought that I might switch to clinical medicine but after successfully applying, 2 things happened to change my mind. I had my first paper in *Virology*, secondly there was the announcement at home of our first baby Catherina. It was a wise decision. I have observed how clinical virologists can be lured away from research. But as a lecturer in medical microbiology I learnt a lot teaching the students and also the art of sensible communication.

Essential for the new researcher, then and now, is the writing and publishing of quality scientific papers. Around 8 good papers a year was sound and still is. I found that presenting data at scientific conferences in England and abroad made me new friends and collaborators and gave me ideas. My wife Gillian, a scientist herself, invited my co-workers home and very often she accompanied me on scientific visits abroad and still does. I feel this family approach now with the input of 5 children is key to a solid scientific research career.

I was encouraged by Professor C. Beattie to apply for a Lectureship in Medical Microbiology of Sheffield. In fact I was the only applicant, although when I resigned 4 years later there were 100 candidates. So the job market had altered dramatically. Professor Beattie and Jack Beverly worked on pneumocystis carinii in cats and dogs and humans. At that time these microbes seemed very academic but with the advent of HIV 15 years later they took center stage as an important microbe in AIDS patients. I feel now that every bug needs someone to love and understand it and that such devotion, in the long term, bears fruit to the community. I was offered the job but I objected to the salary because it was less than my wife's who was working in a hospital laboratory as a researcher. The interview committee immediately recognized that a husband should not be paid less than his wife and so my salary was increased on the spot!

CONTACT John Oxford j.oxford@qmul.ac.uk 70 Holden Road, Woodside Park, London N12 7DY, UK.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/khvi.

About John Oxford

Dr. Oxford is Emeritus Professor of Virology at Queen Mary's School of Medicine and Dentistry. He is Founder of Retroscreen Virology Ltd and Oxford Media and Medicine Ltd. He has co-authored 2 standard texts: *Influenza, the Viruses and the Disease* with Sir Charles Stuart-Harris and G. C. Schild, and most recently *Human Virology, a Text for Students of Medicine, Dentistry and Microbiology* with L. Collier and P. Kellam, now in its fourth edition, published by Oxford University Press. Dr. Oxford has also published 300 scientific papers. His research interest is the pathogenicity of influenza, in particular the origin and nature of 1918 Spanish influenza strain, which he combines with conducting clinical trials using new influenza vaccines and antiviral drugs. This research has been featured on Science TV programs recently in the UK, USA, Germany and Holland. He was awarded communicator of the year for the influenza pandemic by the Society for Applied Microbiology and top communicator by the Society of Journalists, and placed among the 1,000 most influential Londoners by the Independent and Evening Standard newspapers. He has recently been awarded a D.Sc. for his research work on virology and as a science communicator, and elected a Fellow of the Royal College of Physicians of Edinburgh (FRCPE).

During those years in Sheffield there were several epidemics of rubella and in the USA alone there were over 20,000 rubella syndrome babies. In the 1960s it was still not easy to obtain a termination of pregnancy. In fact, even birth control was still frowned upon by many. It was therefore not too surprising to me when the Zika virus, related to the Togavirus rubella, was also shown this year to be teratogenic. As with the MMR vaccine for rubella, I am optimistic for a Zika vaccine.

After 5 years at Sheffield I applied for a Fellowship at the John Curtin School of Medical Research where Graeme Laver and Rob Webster were just starting their work on the avian origin of pandemic influenza. In fact, with Jean Downie they isolated a virus from a migrating bird on an island near the Great Barrier Reef. In the next laboratory P. Doherty was working on a project that no one in the influenza section understood at all but he was awarded the Nobel Prize! He was rather modest and told me that he and his coworker R Zinkernagel only started the work off in a short *Nature* paper and that most of the work was done by excited scientists around the world.

Graeme Laver was restless but he was the most accomplished experimenter I have ever known. He showed me how to do peptide mapping on huge squares of blotting paper in glass aquaria containing vicious buffers charged with thousands of volts. "Just add a pinch of salt," he would say, "and maybe a small pinch of this as well." He would later spray ninhydrin and expose the dots of several amino acids. He and Rob Webster showed that in the influenza HA all the antigenic change was on the HA1 portion, which he had isolated on cellulose acetate strips.

In contrast Universal Influenza Vaccines have the HA2 portion; joining two EU grants, Unisec and Influvac, I have now returned to the study of the stalk of the HA. The EU is crucial to medical research in the UK.

I was very content to work on the proteins of influenza B and then on the influenza RNA polymerase. A chemist Doug Perrin persuaded me that the influenza enzyme was a metalloenzyme and we did experiments using a mass spectrometer which coincidentally was used for examining moon rock which had been brought back by the American astronauts. I was given a small plastic vial containing moon powder but later mislaid it! We synthesized a number of semi carbazone molecules which inhibited the virus RNA polymerase enzyme and so I entered the world of antiviral chemotherapy. A very recent antiviral drug targets this enzyme of influenza and in theory could block both influenza A and B.

When I returned to the UK from Australia I became even more involved in the standardisation of influenza vaccines with G. Schild and J. Wood at NIBSC. We used monoclonal antibodies to pressurise for influenza mutants and to select and characterize vaccine strains which could be more efficacious. At the same time, Philip Minor at NIBSC researched polio and discovered that only a few mutations were needed to attenuate such a virus and, also unexpected, that back mutation could cause a revertency to virulence. That is why in the final WHO push for polio eradication at the moment the traditional Salk killed vaccine is being used.

Lars Haaheim arrived to work with Geoffrey Schild on the techniques of immune double diffusion and single radial hemolysis with J.L. Vireliziev from Paris and they published papers in the *J. Exp. Medicine* on B and T cells involved in the immune response. A key person in the team was Bob Newman, who was the laboratory maestro. Importantly he would spend hours each week purifying influenza viruses on sucrose gradients and we must have had the world's most plentiful and diverse supply. T. Corcoran was my dedicated technician.

I finally realized the scientific importance and strength of international collaboration when an unlikely trio of Mrs. Thatcher, President Reagan and their Russian counterpart signed a tri partite agreement to exchange influenza scientists. I would only have to phone up the Home Office and say please book me to Vector in Novosibirsk for 2 weeks and they would! Then a Russian scientist would return to the UK for the same time. We all had a fine time and I even met P. Palese in Red Square. The Russians were excellent with virus vaccines. In fact, the live polio vaccines of Sabin were first tested there. I published studies of the RNA of field influenza strains with Y. Ghendon and S. Klimov. Professor A. Smorodintsev in Leningrad always gave me a personal lecture on influenza when I visited, and he began work on attenuated influenza vaccines a few years after the war. His vaccine has taken 50 years to develop and is

only now licensed in India, for example, while the American equivalent is now licensed in the UK and is used in children to break onward spread to the grandparents. V. Zhanov as Minister of Health in the USSR proposed at the WHO Conference in Alma Ata that smallpox should be eradicated. Later he was Director of the Ivanovsky Institute of Virology in Moscow. Whenever I met him in the laboratory he could be spotted from afar in a black surgeon's gown while everyone else was in white.

In 1990 I accepted an offer from the medical microbiologist D. Williams to take a Chair of Virology. My predecessor at the London Hospital was Leslie Collier and for the past 25 years we have written and rewritten our acclaimed Oxford University Press textbook, *Human Virology*. Leslie was very modest and he had made a significant contribution for the WHO smallpox eradication campaign by discovering a heat stabiliser for the live vaccine which would preserve it in hot climates. When he died, his family invited me to the funeral to explain exactly what he did. These days many researchers publish in the *The New Yorker* or *The Times*.

I remember my first days at the London Hospital sitting in that Chair of Virology and wondering how I would raise funding to equip a virology laboratory. But George Jackson was already there from the USA as a visiting Professor and we applied for an MRC grant to study the newly discovered HIV. The immunologist Tony Pinching had a ward full of AIDS patients and our study was to search for drug resistant mutants of AZT.

At this time I met a molecular biologist and City finance person, R. Holmes, and he suggested we should set up a small company and so formalise collaborations with pharma groups such as Roche. Lars Haaheim gave us the name, Retroscreen, "you are screening for anti HIV drugs."

Retroscreen started as a small academic off shoot. I had a very hard working staff, including a PhD student Esther Race who had green fingers for cell culture, and she discovered how to safely inactivate HIV to make a killed vaccine, a little like polio. In fact in the 90 s I met up with J. Salk who had also patented a spike stripped particle HIV vaccine. I feel ours was better, which cross linked the gp120 spike using mild formaldehyde and which killed the virus with BEI and slightly split it open with detergents. We tried, with help from Instituto Sierro Vaccinegino Italiano (ISI) to develop the vaccine, but I feel in retrospect that we had all seriously under estimated the opposition of scientific consensus which felt that these approaches were old fashioned and that a vaccine would be found quickly by the molecular biologists. Unfortunately I am still waiting for molecular break 20 years later and would still like my vaccine to have a go.

However I wanted to return to my work with influenza and in 1997 the virology group in Hong Kong described the first humans infected and killed by an avian influenza, H5N1. Retroscreen continued to help with the development of new anti HIV drugs but now become influenza oriented. In the 1980s I had worked with D. Tyrrell, Sylvia Read and Paul Beare at the Common Cold Research Unit, Salisbury. It was a unique hospital on the outskirts of Salisbury where volunteers came from all over England to be infected with common cold viruses and influenza. The unit showed that interferon could abrogate common cold, while our own study of 100 volunteers there showed that a single mutation in influenza virus in the HA near the receptor binding site could attenuate virulence. With this scientific background and know how, the London Hospital Ethics Committee gave me permission for a series of influenza experiments with volunteers at Retroscreen. At first we used hotels to infect volunteers but more recently the Medical School fitted out a dedicated unit with individual volunteer rooms and air control. This has evolved now and includes asthma patients and also allows analysis of human gene switching during infection. The idea is to identify unique host genes vital for virus replication, which can be targeted for a new generation of antivirals.

The company with college help and the dedicated attention of 2 college appointees to the Board, C. Perrin and J. Travers-Clarke, braved the dangerous but exiting world of commerce. A decade later the company appointed a brand new management team of K. Denny and D. Norwood and made the company (now hVivo) fully commercial by raising money in the city. My strong advice in retrospect to potential academic inventors is to bring in fully commercial expertise early although it does remove some of the joy.

As well as being Scientific Director of Retroscreen, I kept up my academic work. I was surprised by a call from R. Daniels to say he had visited the Pathology Department at the Royal London Hospital and noticed lung blocks there from victims of the 1918 pandemic. With the help of a team of students I found the pathology blocks and the case notes and with molecular expertise of J. Taubenberger in Washington we detected flu RNA from the Spanish influenza. The nucleotide sequence of the HA from our London policeman who died in 1919 was remarkably similar to his own from a soldier 3000 miles away in the USA in 1918. To date no single gene can be shown responsible for the deaths of 50 million people around the world. A combination of bacterial super infection and overcrowding may carry responsibility. We both joined a Canadian team who had located a gravesite in Spitzbergen, near the North Pole, with permafrost frozen bodies of a group of Norwegian coal miners who had died of Spanish influenza in 1918. J. Skehel and R. Webster joined the team as heavyweight virologists and I raised finance from Roche, which was matched by NIH funding. It is no easy task to exhume bodies from permafrost, which is like concrete, but we had an experienced exhumation company called Necropolis. In the event the bodies were in the top active layer and had thawed at 4°C and frozen to −20°C each year from 1918 to 1998. The bodies were skeletal. But on return to the UK the Necropolis team described how they had recently exhumed a well preserved body in a sealed lead coffin from a churchyard in the UK. We advertised for families of lead encased victims of the 1918 pandemic and have had permission to exhume 4, but none were perfect because of the cracks in the lead. I continue the search now for a lead encased coffin in a crypt rather than in a soil burial.

When I started in virology I could not imagine how emerging viruses like SARS, MERS, Ebola and Zika would come to the attention of the world. I did not realize either that by doing TV I would be recognized at airports and receive letters sometimes in their hundreds asking for help for the most obscure diseases and offering assistance. But most importantly of all I had not appreciated that my virology colleagues from around the world would become my friends and that all this would last a lifetime. We would even meet up and go on holiday together. I have also met wonderful families who gave permission for us to exhume their long dead relatives

from the Spanish influenza in 1918. I have learnt of acts of heroism from persons who risked their own lives to look after their infected friends and relatives. So I commissioned an abstract stained glass window for the Church of St Augustine and St Philip, now the library of the London Hospital Medical School. It is a tryptic and changes color throughout the day according to the angle of the sun. It is based on the Gauguin painting, "Where have we come from, Who are we and Where are we going." It is dedicated to the Home Front in 1918 where courage was acted out on a daily basis. This inspires me today as with the Ebola volunteer nurses from the NHS and will continue to do so. But I also see how a few talented persons can lift a young student toward a lifetime of dedicated research work and I will be forever grateful to C. H. Stuart Harris, G. Schild and to the writer of *Microbe Hunters*.