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Reflections by Roger Chubb on Marek's Disease (1962-69) – A very personal perspective

This is not a traditional biography, but more of a reflection on the discovery of Marek's disease virus at the Houghton Poultry Research Station in Huntingdon, Cambs, UK by a person who was directly involved. Roger Chubb did indeed make professional contributions to the field of avian medicine and this document chronicles at least a portion of his career.

[Authors note: The views expressed here are mine alone, and may well differ from the views and perspectives of others as well as to the importance, or timing of the events described.]

The beginning of the major work at Houghton would have been the suggestion that Marek's disease and lymphoid leucosis were separate entities, and that there was a need for a special laboratory/task force to work on the two conditions. The plans for the latter

were initiated by Dr R. F. Gordon, the Director of the Institute, and Peter Biggs was appointed, soon to be followed by Jim Payne. The laboratory unit was built well away from the main station, with a completely separate staff, restricted entry, and strict quarantine procedures. There were initially two isolation wings, with restricted entry, first, into the wing, and then into the room via a door into an entry foyer. Jim initiated the construction of some Horsfall-Bauer units after I joined the lab. Special provision for cloth masks were made to hide my beard in the isolation rooms. By the time I was appointed, the laboratory was up and running, with working protocols established. Peter was still actively working at the bench, not that he was ever entirely away from it, and was always a participant in our group activities. It was he who instructed me into the world of tissue culture, and showed me how to grow Rous sarcoma virus. This merely points up that the work on lymphoid leucosis continued throughout the life of the laboratory, and was the main area of work in the initial stages because the viruses could be grown in eggs and in tissue culture. Marek's disease work was all in birds, and of course differentiation between the two diseases was based on differences in pathogenesis in experiments lasting 20 to 30 weeks. This had the effect of ensuring that bird facilities, as they developed into several more wings with positive pressure, were large, could handle the bird numbers, and accommodate them to maturity.

Peter, right from the beginning, established strong contacts with Burmester and the East Lansing laboratory in the USA, Mill Hill in London, and forged links with other workers, as well as ensuring that the credentials of the laboratory were made known. Visiting workers were also encouraged, one of the first being Sam Kenzy. Over the years, these contacts were underpinned by Peter writing reviews on lymphoid leucosis and Marek's disease thereby synthesizing the work of the past in the world literature, and especially incorporating the Houghton work and that of East Lansing. This focus highlighted the recent advances, integrated them into our current thinking at the time, and was an essential part of the Houghton laboratory's development.

It should be noted here that this was the era of the preprint which was circulated to your friends before publication, and sometimes never got published. This practice, coupled with good verbal and informal exchange of data, made writing papers extremely difficult in the reference area, and in explaining the course of events. Delays in publication were another problem, making the results appear out of sequence. This was a time of rapid advances in our understanding of both Marek's disease and lymphoid leucosis. In consequence, a perusal of the literature suggests quite a disjointed sequence of events. It also demonstrates the refinement of methodology being reported in passing. Further, it should be mentioned that some of the papers were the result of several years work.

At the time that I went over to the 'Leucosis Unit', some of the bird experiments were still being carried out in arks, outside. I well remember one occasion, while I was bleeding birds, some escaped into the adjoining field. My excuse for their escape was the extreme cold! My technician, Miss Brand, thought it a huge joke, to see me stumbling and

slipping on the ice-covered grass, in sea-boots, oilskin Macintosh and hat, breath condensing on the cold air, trying to catch the errant roosters.

These outside facilities were made redundant by the building of the new, positive pressure, isolation wings, and the advent of the three-week chick histopathology method of testing for the presence of the Marek's disease agent. We entered the new positive pressure wing, removed lab coats etc., climbed over the divide, and entered the wing. The rooms were entered via a foyer at slightly lower pressure than the room. Here, we changed into a pair of boots, put on waterproof trousers and a long waterproof gown. We then proceeded to shower the gown and trousers and hands with a hand held shower, the water from which contained a detergent, and then put on hats and, in my case, a mask. We then opened the door to the room proper, climbed over a divide, remembering to close the door. The room was usually divided into several floor pens, but the layout could be varied as the occasion warranted.

Jim Payne had just got the three-week bird test going when he exchanged for a year with Graham Purchase from East Lansing and there carried out the work with Goode and others on the histopathology of lymphoid leucosis, and showed its association with the bursa of Fabricius. Meanwhile, I carried on with mundane things like getting the Cofal test to work, inducing hamsters to breed, and producing Cofal antisera in them. Graham came hot-foot and anxious to make his mark, 'a year is a very short time' he said! But there was time for him and his wife to show us how to barbecue a steak and make pumpkin pie, [the pumpkin pie is still a dish we have on our menu]. We all took to the three-week bird assay, gathering in the post-mortem room to take down the hundreds of birds used in the experiments, {five nerves, [2x brachial plexus, 2 x sciatic plexus, vagus,] and gonad}. This was a jolly time for discussion of the news of the

day, the latest scientific idea, or whatever! The fixed specimens went to Derek Rootes et. al., on the main station, for horizontal sections of the nerves and the gonad to be processed onto one slide for each bird. About a fortnight later, the slides arrived back for microscopic observation.

Graham got on well with everybody and was very helpful. I was not very good at the histopathology of Marek's diseased nerves, so I asked him to look at the histology slides

from a titration of HPRS-14 that I had done, using the new three-week bird assay. He came back after a week, exclaiming in high dudgeon 'What did I think I was doing?!?'. He had spent hours examining the slides and there was nothing to see! We normally used citrated whole blood from birds showing paralysis as the infective inoculum. Several experiments had seemed to show, however, that plasma was also infective, although at a lower level than whole blood. I had used plasma for the titration, but, to ensure that there were no cells present, I had spun the blood twice at 1000 rpm for five minutes, let the centrifuge come to rest naturally, and then removed only the top third of plasma for use. Graham immediately repeated this procedure and showed that the virus was in the spun cells but not in the plasma. From then on the agent was considered to be wholly cell-associated – a major shift in emphasis, and crucial to the next phase of the attack on finding the agent. This new emphasis brought Peter out of his office, and had Brian Milne [his technician], and several others, scurrying about, as Peter attempted to kill the agent, release it from the cells, or tried to isolate the agent in tissue culture using the cells. It was quickly shown that putting blood cells, especially red cells, onto monolayers of cultured cells merely caused them to lift off the dish. We needed a tissue culture expert. Notwithstanding this, the incidence of Marek's disease in the field had declined, and there were the continuing problems with growing Rous sarcoma virus in tissue culture and the work on the RIF tests. Should we worry about Marek's disease?

Then acute Marek's disease arrived, and the hunt was on again. Tony Churchill was appointed, and Jim returned from East Lansing. By this time, the infected blood cells were being stored in liquid nitrogen, using calf serum and DMSO, a technique that Graham Purchase had initiated before he returned to East Lansing.

Tony had been working, amongst other things, on infectious Laryngotracheitis in tissue culture. As such he was an expert on the cultivation of epithelial cells, and of course, was used to the idea of cell associated viruses. His experience proved invaluable as he took over the tissue culture side of the hunt for the MD agent. Gradually he shifted to the use of fractions of the cells in whole blood, tumour cells, as well as cell co-cultivation of cells, which was then a ploy being discussed in the wider field of tissue culture. It didn't seem too long before he was muttering that he was 'surely seeing a cytopathic effect' especially when passaged cells were used. When he was certain, the process of evaluation against Koch's postulates began. Here again, the value of the three-week bird test cannot be over-emphasized....waiting for 20 weeks for a result, although we had been used to it, would have been wearisome! To our joy, the disease appeared in the infected-cell-culture inoculated birds, compared with the controls, and the experiments could be repeated in a matter of months. We co-opted Dr. Dourmashkin, of the Imperial Cancer Research Fund, Mill Hill, to carry out the electron microscopy. We were already suggesting a herpes virus from the histopathology and cytopathic appearance of the infected cells. The news that it was a herpes virus started a very long correspondence between Peter and Dr. Robert Huebner, who, at first, expressed disbelief!

Meanwhile, I had taken the supernatants and cells from some of the early cultures for gel diffusion, using serum from a bird being used in a long term MD experiment. I set up the tests, using microscope slides, and the same criteria that I used for poultry viruses...first time, a single precipitin line. [For me, another exhilarating morning, similar to the experience of seeing the first ILT precipitin line several years earlier. I couldn't stop re-looking at the slide]. We then showed this was related to the infection, and began to test serum samples from flocks all over the United Kingdom, and found, surprisingly, that almost all were serologically positive. How fortunate we were that the agent was not vertically transmitted, a fact already known.

Peter was by now on his way to our friends at East Lansing with the good news. He found that they, too, had isolated a virus, but that they hadn't yet got to a serological test. [It was gratifying for me, personally, at a later stage, to see that Okazaki, Purchase and Noll, at East Lansing, had repeated my work with ILT on the effects of salt concentration in the agar for the production of precipitin lines for gel diffusion when chicken serum was used, with MD as the model.]

Tony had worked previously with Bill Baxendale at the Welcome Foundation, and had passed on to him the techniques for cultivating MDV. We went to see him in his laboratory to discuss progress and he showed me his gel diffusion plates (he used small tissue culture dishes rather than slides). I was immediately impressed. He had two or three lines showing. Our questions elicited the information that Bill was in the habit of taking the whole culture from the dishes, rubbing the cells into the supernatant with a rubber policeman, freezing and thawing a couple of times, and then concentrating the mixture to make the gel diffusion antigen. Although, in the initial small batches of antigen from the early cultures of the virus we had been doing this, as the work progressed, and passage levels increased, we had changed to using supernatant fluids, usually concentrated, almost exclusively. This was partly because I had been denied the cells as they were in demand for the bird experiments and the need to establish reasonable antigen pools. Back at our laboratory, we quickly confirmed that there were three lines, one in the supernatant "A" and at least two that were cell associated "BC". I was still bereft of the cell fraction to include in the antigen for general purpose antigen preparation, and continued to get supernatant material for this purpose from cultures as they were being passaged. Much to my horror, the "A" line disappeared. A hectic few days ensued as I searched the cultures for the antigen. The result was the discovery that the "A" line was lost during passage of the virus, but the cell associated "BC" lines remained. Tony thought this may have some role to play in the disease, as his bird experiments were showing a loss of virulence for birds as the virus was passaged, and as the "A" line was lost. The virus continued to induce the "BC" precipitins. Was this attenuated virus capable of inducing resistance to the more virulent virus?

Tony and I began to plan the experiment. Almost the next day, Jim came to me to ask about looking at the serology on some experiments he was planning to do concerning the induction of resistance. He had continued to work on the comparative pathology of the

classical MD, HPRS-14 virus, compared with the acute MD, HPRS-16 virus, and had noticed that the classical strain seemed to reduce the effects of the acute strain. I immediately suggested the he and Tony should get together and design the experiments to prove that avirulent strains of MD virus could be protective against virulent strains, especially against the acute MD, with its propensity to form tumours.

While this work was progressing, I began the attempt to produce antibody free chicks in order to look at the effects of antibody on the virus. It took three attempts, using our isolation rooms, to rear small batches of birds to maturity. This shows, in hindsight, the difficulty of preventing the spread of dander. In the end, contact between staff and birds was reduced to the minimum possible, feed and water being checked, and delivered, from the doorway, only. The experiments showed that both maternal and passively administered antibody merely delayed the onset of the disease, and perhaps, reduced the incidence a little. Attempts by others, later, have shown a more positive role for cellular immune responses. Nevertheless, even today, there is no full explanation for how attenuated strains of MD virus induce resistance. The importance of the bird genotype for resistance was known then, and has been further elucidated. It was our good fortune to be able to always use the highly susceptible HPRS Rhode Island Red strain of birds for our experiments.

Our next step was for all the team to collaborate with veterinarians in the Ross Group to set up a field trial to test the resistance induced by the cell associated attenuated virus under normal conditions of exposure. Shortly after this was set up, Tony went down the road to the Elms to set up as a private manufacturer of the vaccine, and I left for Australia, principally to work on infectious bronchitis.

Conclusions. The work of a scientist is always part of a continuum which consists of several strands of communication. The first is the body of the published literature, which tells of what went before in any particular area or in related areas, how to go about the search for new knowledge, and the techniques to do it. Secondly, there is the spoken word which is the fastest way of spreading news, good or bad, the latest gossip, technical innovation etc, and which always has been a traditional part of the scientific culture. The spoken word is a social way of disseminating knowledge, at conferences, or by meeting with individuals, especially in their own work place. It is here that speculations, explanations, and analysis of facts are discussed, implications delineated, and the focusing and synthesis of ideas surrounding them carried out. Thirdly, the place of work is of importance, as it will have its own way of doing things which shape the courses of action taken. Fourthly, there is the interaction of the group with which one works, based on the collective backgrounds, training, and experience of the.-, individuals. Here, amicable socialization is of great importance...doing things together. Our place was the postmortem room, and at meal breaks etc., as well as on social occasions outside the Institute. There is a need for any leader to create these occasions and to be part of them, and these should include all the support staff, the unsung toilers who enable things to happen, but who, more often than is realized, provide the seeds of the ideas that lurk in one's mind.

In the case of the work at Houghton, one can point to the idea of setting up a special facility with quarantine and isolation facilities, the gathering together of a team with diverse backgrounds, the provision of a susceptible line of chicken, the shift to looking for cell associated virus, the three-week bird assay for infectivity, the advent of acute MD, the expertise of the team leading to the tissue culture isolation and attenuation of the virus and its relationship to serology, as being the means to the solution of the problem. However, the end result cannot be attributed solely to any single person, or event, but must be put to the amalgam of time, place, and people involved, set within the continuum of scientific effort. After all, one must have the paper, pen and ink, and be able to write, before one dots the "i"s and crosses the "t"s!

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Biography solicited by the Committee on the History of Avian Medicine, American Association of Avian Pathologists.

Additional biographical materials may be available from the AAAP Historical Archives located at Iowa State University. Contact information is as follows:

Special Collections Dept. & University Archives
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