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Career thoughts and recollections: 50 years publishing in The Journal of General Virology

I was both delighted and honoured to receive a letter recently informing me that I had been elected as an honorary member of the Microbiology Society. I have been a member of the society (which I still refer to as SGM from force of habit) since 1972, and the Microbiology Society has had an important influence on me during most of my research career; I owe it a lot. I was surprised to learn in that letter that I had co-authored 50 papers in the Journal of General Virology between 1969 and 2015. In fact, when I checked, the actual number is 51 and this encouraged me to look back and reflect on the immense changes that have occurred in my work personally and in the field of virology generally during the 50 years since my first JGV paper.

I cut my teeth virologically in 1964 when I took up my first post in Fred Brown's laboratory at the Animal Virus Research Institute (now the Pirbright Institute) following my post-graduate studies at the University of Southampton. Fred was both my boss and mentor for many years after I joined his group and it was at Pirbright that I was exposed to the weird, wonderful and sometimes frightening world of foot-and-mouth disease. A passion for research into its causative agent, foot-and-mouth disease virus (FMDV), that was engendered while working in Fred's group has remained with me ever since and I am still actively involved to this day.

As in all spheres of scientific endeavour, the advances made over the past 50 years (the course of a single lifetime career in my case) have been amazing. These occurred through both small incremental steps and in quantum leaps, the latter often being driven by major technological developments. It is almost embarrassing now to look back at the impoverished level of understanding we had when I published my first JGV paper in 1969[1]. Virus purification techniques, involving mostly ultracentrifugation procedures had been established by then but we had little idea of the antigenic structure and protein composition of the virus and determination of the nucleotide sequence of the viral genome was a far off dream. Serological experiments in those days involved the complement fixation test – has anybody heard of that now? In the early 70s major developments were made in techniques for protein separation and analysis. The era of SDS polyacrylamide gel electrophoresis had arrived and enabled us to demonstrate for the first time that the FMDV particle comprised multiple proteins[2]. I can remember bets being taken at scientific meetings during that period as to how many SDS PAGE profiles each speaker would present.

Much of our work in the 70s was devoted to applying biophysical techniques to compare and contrast the properties of viruses within the picornavirus family in order to establish groupings for classification purposes. Methods such as nucleic acid base composition and buoyant density determination were used to define four groupings within the picornaviruses[3] – compare this with the latest score of 47 picornavirus genera[4] resulting from increased virus isolations combined with genetic sequencing and bioinformatics. Methods to study virus replication and protein processing were heavily reliant on radiochemical labelling, often involving pulse-chase approaches. These methods enabled the unravelling of the complexities of processing of the picornavirus polyprotein and mapping of the genetic structure of the genome despite the lack of sequence information[5].

The 80s saw the introduction of three technical developments which had huge influences on virology generally and my personal interests in particular. The first of these, monoclonal antibodies, facilitated mapping of antigenic features of viruses such as FMDV. The second was molecular cloning and sequencing, which enabled the determination and manipulation of viral genomes. Finally, advances in X-ray crystallography meant that the structure of virions, such as picornaviruses, could be resolved in near atomic detail. A combination of these methods enabled us to identify a distinctive feature on the surface of the FMDV particle, the VP1 G-H loop, which was the target of a high proportion of the neutralising antibodies present in anti-FMDV sera. This information enabled us, in collaboration with Richard Lerner's group at the Scripps Institute, to explore the (then) incredibly exciting possibility of producing chemically

synthesised vaccines. Indeed, we showed for the first time that small laboratory animals could be protected from challenge with virulent FMDV by immunisation with synthetic peptides representing the VP1 G-H loop sequence[6]. The crystal structure of the virus, determined with Dave Stuart's group in Oxford, showed why peptide vaccines for FMDV were more successful than with other systems as the major epitope appears as a mobile loop on the virion surface, much like a synthetic peptide[7, 8] (Fig. 1). Although practical synthetic vaccines against FMD were not realised, for a variety of reasons, their investigation opened up a number of important aspects of immunology and vaccine development. In attempting to optimise the immune response to purely synthetic immunogens, we were able to demonstrate the importance of Th cell epitopes[9]– immunological concepts that were in their infancy then. We were also able to demonstrate the immunogenic potential of presenting antigenic epitopes in the context of particulate carriers (nanoparticles in modern parlance) by fusing peptide epitopes to the self-assembling hepatitis B core protein[10].

Cloning and sequencing of the FMDV genome was, and is, of huge importance for progressing our understanding of how the virus works. The FMDV genome was found to have a number of unusual/unique features that we are still trying to understand to this day. For example, a seventh of the genome comprises the untranslated 5' end, which contains at least 5 distinct domains, and there are 3 copies of the RNA primer protein, VPg[11, 12, 13]. We still do not understand the roles of several of these unusual features, and the desire to resolve some of these intriguing problems is why I cannot give up yet.

A new departure for me in the 90s was hepatitis C virus (HCV). Although the existence and importance of this cryptic human pathogen had been suspected for many years it was not finally identified until 1989 by Michael Houghton's group[14]. This was a triumph of the new molecular approaches to virus identification when the agent could not be grown using conventional culture techniques. The full significance of the virus for human health only became apparent following its formal identification and the development of diagnostic tests. The realisation that HCV infection usually leads to persistent infection with serious consequences in later life meant that it was an ideal target for antiviral intervention and the race was on to develop chemotherapeutic agents. I, together with a significant proportion of the virology community, joined the fray to discover vulnerable features of HCV replication which were suitable for therapeutic intervention. While with the Wellcome Foundation in the early 90s we worked on NS3, the viral protease[15] and following my move to the University of Leeds in 1996 and teaming up with Mark Harris, we extended our interests to include the hepatitis C virus non-structural proteins and the putative viroporin, P7[16, 17, 18].

More recently, I have returned to practical work on FMDV. This was finally made possible when the regulatory authorities agreed that replicons are not viruses and pose no threat through accidental release of important pathogens to the environment. In collaboration with my colleague Nicola (Nic) Stonehouse here in Leeds we are capitalising on this decision and have an active programme of work addressing some of the unanswered questions (e.g. the functions of RNA elements within the UTRs, the complexities of polyprotein processing and the structure and functioning of replication complexes) about this remarkable virus that I alluded to at the start of these reminiscences.

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David J Rowlands

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The author declares that there are no conflicts of interest.

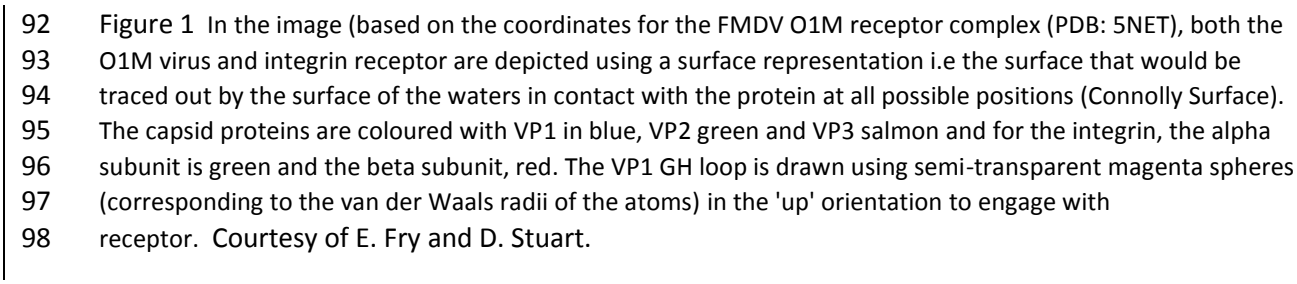

Figure 1 In the image (based on the coordinates for the FMDV O1M receptor complex (PDB: 5NET), both the O1M virus and integrin receptor are depicted using a surface representation i.e the surface that would be traced out by the surface of the waters in contact with the protein at all possible positions (Connolly Surface). The capsid proteins are coloured with VP1 in blue, VP2 green and VP3 salmon and for the integrin, the alpha subunit is green and the beta subunit, red. The VP1 GH loop is drawn using semi-transparent magenta spheres (corresponding to the van der Waals radii of the atoms) in the 'up' orientation to engage with receptor. Courtesy of E. Fry and D. Stuart.

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