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SPECIAL ARTICLE

What is the true nature of epidemic influenza virus and how do new epidemic viruses spread?

It might seem very late to suggest, nearly 400 years after the first clinical description of influenza and 54 years after its isolation (reviewed by Stuart-Harris, Schild & Oxford, 1985), that many fundamental questions remain to be answered about the virus itself. However the precise antigenic and biochemical structure of the natural field virus has not been established. If so much remains to be learned concerning the nature of the virion then perhaps it may be less surprising that there are some conflicting theories as regards influenza epidemiology. Such questions are raised in the current volume of the journal where Hope-Simpson & Golubev (pp. 5–54) propose a major role for virus persistence in the human disease and, a lesser role for a linked chain of acute infection spreading influenza around the world (see also Hope-Simpson, 1979; 1981). This would be a minority view of the epidemiology of influenza A at present and is most definitely in conflict with the orthodox idea of person to person spread in an endless chain.

A central theme of the Hope-Simpson and Golubev's hypothesis is that most influenza is caught from symptomless excretors who themselves contracted influenza, and perhaps had a clinical attack, a year or more previously. In other words a proportion of the population at large and unbeknown to themselves harbour influenza viruses. An unknown stimulus, itself associated with natural phenomena such as climatic conditions, causes the virus to emerge from these persistently infected individuals and influenza spreads to contacts and a typical epidemic is caused. The theory attempts to explain antigenic drift on the basis that after infection the immunity of the persistently infected person suppresses the emergence of the original infecting virus but allows the emergence of variants' or neutralization 'escape' mutants. Similarly, the phenomenon of antigenic shift is accommodated because certain persons would be persistently infected with older viruses which could, at an appropriate time, recycle. Thus the occurrence of pandemic influenza A (H3N2) in 1968 could be explained by a re-emergence from a person infected originally in an epidemic during the last years of the nineteenth century or, at the latest, during Edwardian times. Hope-Simpson rather dismisses ideas of new pandemic influenza A viruses emerging from birds or animals by noting that '*man is most in contact with man*'. However, as anticipated with such a hypothesis, and such a complex disease, there are many questions left open, not the least being the clinical and laboratory data whereby persons have been infected artificially with laboratory strains of influenza and develop clinical influenza and infect others immediately. So if the new hypothesis is even partially correct it would not be an all-embracing theory.

I suspect Hope-Simpson's interest in virus persistence or even latency comes from his detailed studies of the varicella-zoster virus carried out almost three

decades ago (Hope-Simpson, 1954) and yet still retaining their interest today as a classic investigation of a general practitioner-naturalist interested in the wider aspects of viral disease.

But to return to the question of the precise nature of the infecting virion itself, a crucial series of papers was published by Burnet and his group in Melbourne (Burnet & Bull, 1944). These workers had, a little earlier, pioneered the use of the embryonated hens egg in which to grow influenza virus. The use of such a strange substrate in which to grow a human virus may have inadvertently misled succeeding generations of virologists into concluding that egg-grown influenza viruses are closely related, or even identical, in their antigenic and biological properties to natural epidemic influenza virus. Burnet noted that the different routes of inoculation of a single egg (e.g. via the amniotic or allantoic routes) resulted in the selection of virus populations, with differing biological characteristics. A mixture of biological variants must have been present in the original clinical specimen but which was the important one? Three decades later Yewdell, Webster & Gerhard (1979) were able to demonstrate conclusively, using immunosuppression experiments with monoclonal antibodies, that many antigenic variants of influenza could exist in even a cloned pool of infective allantoic fluid. These observations would concur with current ideas of RNA viruses existing, at least in the laboratory, as dynamic mixtures of countless variants with perhaps one virus population dominating (Holland *et al.* 1982). Finally, our recent studies (Schild *et al.* 1983; Oxford *et al.* 1987) have almost completed the circle because we have shown, at least indirectly, that influenza A and B viruses replicating in mammalian cells are more akin in the antigenic structure of the HA to natural epidemic viruses than are their egg-grown counterparts. But we certainly do not know yet if they are identical to the natural unpassaged virus. The HA of egg-grown virus is very different antigenically to the HA of mammalian cell-grown viruses and this has led us to investigate the potential of using mammalian cells for innovative influenza vaccines and also for sero-epidemiological studies. Nucleotide sequence analysis of the HA of MDCK cell-grown and egg-grown viruses cultivated from the same clinical specimen has shown that one or two amino-acid substitutions differentiate the two viruses and that these occur at or near the receptor binding site on the HA molecule (Robertson *et al.* 1985; R. D. Daniels, unpublished data). Differential receptor binding properties can explain the varying affinities of the two virus populations for egg or mammalian cells and concomitant antigenic changes occur because of the physical location of the substituted amino acids which intrude on, and hence alter, important epitopes on the HA. Most of these studies have been carried out using MDCK cells but we have performed some preliminary experiments with influenza virus infected human diploid MRC-5 cells (Jacobs, 1970). Interestingly, and of particular relevance to the question of viral persistence, we have been able to chronically infect these cells with influenza A (H1N1) (H3N2) or B viruses and still subculture them over six generations during a period of 4 weeks (T. Corcoran & J. S. Oxford, unpublished data). The cells release influenza viruses intermittently during this time. Other laboratories have described *in vitro* systems of influenza virus persistence such as A/WSN in MDCK cells where a number of phenotypic changes are detectable in the released virus (Frielle, Huang & Youngner, 1984).

Sir Christopher Andrewes has suggested a slogan for influenza virologists: 'back to the pig' (Andrewes, 1984). In the 1950s Shope had noted how outbreaks of swine influenza A virus occurred simultaneously in a number of farms some distance from each other, with no communication between them. Shope thought that the virus may have been seeded into the herds and 'activated' subsequently by a meteorological stimulus. Dr Hope-Simpson's hypothesis extends these ideas into the human population. Therefore perhaps Sir Christopher's slogan could be rephrased to 'back to the human'. Can molecular virology uncover yet more wonders for us in unpassaged clinical samples from influenza-infected persons?

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