SECTION I

The growth of virus research 1928-1978

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IT WOULD be impossible in a short essay to cover the whole ground of research on viruses, including those infecting vertebrates, insects, plants, bacteria and other organisms. Attention will accordingly be devoted particularly to those aspects of virology of interest in medicine. (This is the more appropriate as this symposium has been organized in honour of Sir Charles Stuart-Harris, who has so assiduously and profitably cultivated the borderland between clinical medicine and virology.)

In the early days of virology, the first task was to determine which infections were definitely caused by viruses. Fifty years ago they were called the filter-passing viruses or just filter-passers. A number of infections had failed to give evidence of bacterial origin and were presumed to be caused by viruses. That this was so was made certain when it was possible to filter the agent through a filter which would hold back bacteria. To do this, however, one needed a test object, perhaps a volunteer in the case of relatively minor infections, preferably an experimental animal.

Fifty years ago the viral origin of a few human infections, smallpox and vaccinia, herpes simplex, rabies, measles, dengue and poliomyelitis seemed fairly certain on this basis; and it was suspected that viruses caused mumps, rubella, varicella and influenza. There were also, of course, viruses known to infect domestic animals and plants, to say nothing of bacteriophage. Today the viruses known to infect vertebrates alone number several hundreds.

Progressively, the infections in the 'suspected' virus class have been yielding up their mysteries, until very few remain. In almost every instance in the early days, successful transmission was rapidly followed by demonstration of filterability. Important achievements were the transmission to one animal or another of influenza (Smith, Andrewes and Laidlaw, 1933), rubella (Habel, 1941), and St Louis encephalitis (Webster and Fite, 1933). This last was particularly important as it was the forerunner of the isolation of a whole host of arthropod-borne viruses, most of them infecting other species and only incidentally transmitted to man. It is of some interest that before about 1933 the rabbit was the

animal of choice for workers in virology; after that the doubtful honour passed decisively to the mouse.

Tissue culture

In more recent times it has been possible to obtain conclusive evidence of viral aetiology even when transmission to an experimental animal has not been readily achieved. In this connection, tissue culture deserves our attention. Before 1949 many viruses had been shown to multiply in tissue culture, either by titrations in animals or by demonstration of pathological changes such as inclusion-bodies in the cultivated tissues. In 1928, Maitland and Maitland had shown that elaborate culture methods were not essential: some viruses would grow in minced tissues suspended in a suitable fluid. Much of the early work on tissue culture, however, showed merely that virus multiplication could be achieved. It must be remembered that in those days tissue culture was more difficult than it is now, when antibiotics are available and the mystical surgical techniques of Carrel's time are no longer necessary. The situation was changed after 1949 by the discovery by Enders, Weller and Robbins that polio virus could be grown in various tissues, not necessarily, as had been believed, only in nervous tissues. This opened the way, not merely to successful immunization against poliomyelitis, but to the use of tissue culture to reveal the existence of a whole range of hitherto unknown viruses. It became possible to detect the presence of virus by the cytopathic effects on the cultivated cells; and the method of counting plaques introduced by Dulbecco (1952) made quantitative work much simpler.

Thus the echoviruses were detected and along with poliomyelitis and the Coxsackie viruses, later discovered by Dalldorf and Sickles (1948), they formed the enterovirus family. Soon afterwards similar methods brought to light the families of adenoviruses and reoviruses. Interference between one virus and another had been known since 1937. The phenomenon proved to have usefulness in growing otherwise elusive viruses. For example, the first proof of growth in culture of rubella was obtained by showing that rubella-infected cultures were relatively resistant to infection by another,

readily detectable, virus (Parkman, Buescher and Arnstein, 1962). The technique proved useful also in the early days of work on rhinoviruses; so, too, did the use of organ cultures.

Properties of viruses, sizes and shapes

Examination of the properties of viruses soon revealed striking differences: they varied in resistance to physical and chemical agents and in size. Elford (1931), following Bechhold and Villa (1925/6), had devised graded collodion membranes which made it possible to sort out viruses on the basis of size; some had diameters of 20 nm or less, others of over 300 nm. The findings were confirmed by centrifugation, optical and other methods. First attempts to visualize viruses employed staining methods and these revealed small round objects which might or might not have been viruses. J. E. Barnard, a hatter and amateur microscopist, constructed most ingenious microscopes using ultra-violet rays (1932). This advanced matters, but the technique was soon superseded by electron-microscopy. At the end of the war in 1945, Ruska, a pioneer in this field, was brought over from Germany to advise our workers. The author recalls that Ruska used to be brought up from his place of restraint to the National Institute at Hampstead. He was still regarded as an enemy alien, so an armed guard accompanied him, usually sitting in front with the driver, with Ruska and the guard's weapons behind. It was not long before electron-microscopy became something more than a gimmick; the first major discovery was that many phages possessed tails. The various structures revealed in different groups have greatly influenced our ideas on virus classification. Now that improved techniques have made it possible to visualize virus-like particles in such things as faeces, the technique of immune-electron-microscopy has been most useful in deciding which particles are relevant to a particular study.

One finding caused much controversy at the time; Stanley (1935) succeeded in crystallizing tobacco mosaic virus and this was later achieved for a mammalian virus, poliomyelitis. There was talk of living crystals, and wild speculation about the nature of life. Later, it was realized that very small regular bodies brought together in quantity could hardly help aggregating in crystalline form; the discovery was not quite as revolutionary as at first appeared.

Classification of viruses

Before 1950 the only serious attempts to classify viruses had been based on their affinities for different kinds of cells or on their pathological effects. Levaditi (1922) had suggested the former and the system of Holmes (1948), published as a supplement

to Bergey's Manual, was based on the latter. All this was so bad that in 1950 the International Committee for the Nomenclature of Bacteria was persuaded to set up a subcommittee to make proposals about viruses. At the International Congress held in Rio de Janeiro in that year this subcommittee proposed that virus classification should be based on such things as the size, structure and chemical composition of viruses, host-range and pathological effects being regarded as of minor importance (Andrewes, 1952). Study-groups were set up to make proposals about certain viruses, the poxes, arthropod-borne viruses, those related to influenza, insect pathogens and the psittacosis group.

At that time it was extremely difficult to persuade any virologist to take an interest in viral nomenclature and classification. Fortunately, interest was gradually awakened in the succeeding years until, today, most virologists tend to think of any virus they come across as belonging to one or other of the recognized genera or families. More and more taxa have come to be recognized and named at successive international conferences. At the Rome conference in 1953, for instance, it was decided that virus genera should have the suffix '-virus'. Also the name 'myxovirus' was born and the psittacosis group was thrown out as not having the properties of true viruses.

In 1966 the International Committee for Nomenclature of Viruses came into being in its own right: no longer was it a subcommittee of the body dealing with bacterial nomenclature. There have been warm discussions over the years; taxonomists tend to be a quarrelsome species and it has always been difficult to get the plant virologists to agree on a common line. There has been, however, agreement on the need for generic names and quite recently these have all been arranged in families bearing the suffix '-idae', but opinion has fluctuated about the desirability of using Latin binomials as specific names. The current tendency is to take no urgent action about this; viruses are probably evolving in many cases more rapidly than plants and animals, so that species boundaries are even more blurred than in other disciplines.

Immunity

The laws of immunity are much the same for bacteria and viruses. Some techniques are, however, particularly useful in virology – neutralization of infectivity, immunodiffusion and use of fluorescent-antibody. A few words are in order concerning haemagglutination. In 1941 George Hirst noticed that when influenza-infected eggs were being harvested, accidentally spilt red blood cells were apt to clump. Hare, Curl and McClelland (1941),

working in Canada, made the same observation independently. It subsequently became clear that the HA test could be used, not only for influenza, but for many other viruses; the animal source of cells, temperature and other conditions required may be quite critical but it is remarkable how general and how useful the test is.

As soon as research had made it possible to propagate a virus *in vivo* or *in vitro* and study it quantitatively, it was natural to try to immunize susceptible animals and, here again, the principles were the same as in bacteriology. Formaldehyde and other agents have been used to make inactivated vaccines, following work on bacterial toxins; and various methods have been used to attenuate viruses for purposes of immunization, following the example of Pasteur. The rival advantages of killed and attenuated vaccines have stimulated much debate, particularly in regard to poliomyelitis. The inactivated vaccine of Salk (1953) has been superseded in much of the world by the attenuated vaccine, first used by Koprowski, Jarvis and Norton (1952) and developed by Sabin (1959). Against influenza, both kinds of vaccine probably have their own usefulness, but there is not such a dramatic success to report as there is against polio. Even that is still far from satisfactory in some tropical countries.

The success of Theiler's 17D vaccine (Theiler and Smith, 1937) against yellow fever has justly earned him a Nobel prize. Its success is only surpassed by that of Jennerian vaccination against smallpox. This has been used so successfully that smallpox has apparently been banished from Asia and the rest of the world with the exception of Ethiopia and Somaliland. There, too, it was almost eliminated in 1977, and when the existing warfare there is over, it will probably be found to have been eradicated.

Interference

In 1937 Findlay and MacCallum reported that infection with Rift Valley fever virus, which is only mildly pathogenic for monkeys, can suppress a fatal infection with the immunologically unrelated yellow fever virus. This protection could not be attributed to antibodies and was the first unequivocal demonstration of the phenomenon of virus interference. This phenomenon was found to be of general occurrence and, as already mentioned, it has been usefully employed in detecting the growth of otherwise elusive viruses in tissue culture. In 1957 Alick Isaacs began a thorough study of influenza viruses growing in chick tissues. With a Swiss colleague, J. Lindenmann, he obtained evidence that when such interference was occurring *in vitro*, an interfering agent was liberated into fluids surrounding the tissues under investigation. This they called interferon (Isaacs and Lindenmann, 1957). It turned out

that they had discovered a hitherto unrecognized defence mechanism, active against all sorts of viruses and having, in addition, wide biological implications.

It was soon being studied widely. There were hopes that there was here a natural antiviral chemotherapeutic agent, with possible applications in clinical medicine. These have as yet materialized to a very limited extent; their development must await further advances in the production of interferon in quantity.

Influenza

One cannot in a short review deal with the history of every virus. Influenza does, however, deserve special mention. One may say that the influenza virus was discovered in 1901. This statement may seem surprising, but in that year Centanni and Savonuzzi (1901) succeeded in filtering the virus of fowl-plague, now known to be an avian strain of the virus. Stuart-Harris and the present author were both concerned in the early work on the human virus. The washing which first infected a ferret came from C.H.A.'s own throat and a ferret which sneezed at Stuart-Harris and gave him influenza finally settled the relation of their virus to the disease in man. Landmarks of importance were the cultivation of the virus in developing eggs by Goodpasture, Woodruff and Buddingh (1931) with improvements in technique by Beveridge and Burnet (1946) and Hirst's (1941) discovery of haemagglutination. Influenza has played a very important part in international virology. In 1947 at an international meeting in Copenhagen Dr Gautier of the WHO was persuaded to interest that organization in setting up a network of laboratories based on a World Influenza Centre at Mill Hill and a sister-centre in the U.S.A. These had the duty of monitoring the changes which take place in the antigenic make-up of the virus from time to time; for when the virus changes, its operations are on a world-wide basis. The influenza network had particular importance as it formed a model for concerted international studies of other viruses, even though these do not undergo frequent modifications as influenza does.

Although it is not possible to point a finger at any one major discovery, it has now become clear that influenza A viruses can infect many species and that strains can cross species barriers. More and more people are therefore coming to believe that pandemic strains may arise when such a species barrier is effectively transgressed or, with perhaps greater probability, when successful recombination occurs between a human virus and one from another species.

The common cold will not be considered here, as it is dealt with in the article by Tyrrell (1979).

Viruses and tumours

Fifty years ago most pathologists were very sceptical of the possible role of viruses in tumours. Peyton Rous's (1911) discovery of a filterable fowl sarcoma was dismissed as of little importance. Either, it was argued, the sarcoma was not really a tumour, or the active agent was not really a virus. In 1930, Gye and Purdy reawakened interest in the Rous sarcoma, although their main hypothesis proved unacceptable. Then came Bittner's (1936) discovery of an agent concerned with a quite typical tumour of mice, mammary carcinoma. It took some years, however, before this 'milk-factor' was accepted as being a virus. There followed Gross's (1951) discovery of a virus in mouse leukaemia, and other filterable leukaemias and sarcomata in mice, cats and other species soon came to light. Rous, returning to the cancer field, found that a rabbit papilloma discovered by Shope (1933) was apt to proceed to malignancy. Rous's studies with several colleagues (Rous and Beard, 1934) were of great importance in relation to the successive stages in the development of malignancy and to the role of carcinogenic chemicals acting in concert with a virus. Some tumours, also, turned out to be caused by the concerted action of two viruses, one acting as a 'helper', supplementing the activity of the other. Work on virus-tumours is no longer a Cinderella; it has gone on apace and is in the forefront of cancer research.

A number of viruses in the herpes group – Epstein-Barr virus, Marek's disease and several monkey viruses – have turned out to be oncogenic. In particular, it has become clear that oncogenic viruses may be integrated with the genetic apparatus of the host, where they may remain latent until some stimulus activates them, so that they are liberated as free oncogenic viruses or else carry out their nefarious deeds in a more subtle manner.

Molecular biology

Of recent years, virology has developed along new lines. It has become possible to analyse the successive steps concerned in the replication of viruses within cells. Further, many viruses have been separated into component parts and much information gained as to the functions of the various constituents. This analysis has been greatly helped by the possibility of obtaining hybrids or recombinants between related viruses. This historian, having retired from active work in the laboratory nearly 17 years ago, is not competent to review this work in detail. It does appear to him that virological research has split into two, perhaps three streams. There is first, the intimate analysis of the essential properties of a virus and its behaviour within a cell. A second stream concerns the effect of the virus on

the whole animal and includes clinical virology and pathogenesis. A third stream is that of ecology and epidemiology, the effect of viruses on communities. The second and third streams are much intermingled, but there is danger that the first stream may tend to carve out a channel of its own. One hopes that this will not be so, and that intimate knowledge of virus-cell relationships will lead us towards a successful virus chemotherapy; that is a hopeful prospect for the future, even though it has been a hopeful prospect for rather a long time.

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