

SECTION II

Studies of rhinoviruses and coronaviruses at the Common Cold Unit, Salisbury, Wiltshire

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SIR Christopher Andrewes has outlined the history of virology in general and I have been asked now to give a rather more detailed account of certain subjects with which I have been involved for the past 20 years. Because of this I shall take the liberty of giving it, to a large extent, from my own personal point of view.

Medical research often starts with a clinical problem and common colds were the problem in the present case. These illnesses were shown by Kruse to be due to a filterable virus – he produced colds in volunteers and published his results in Munich at the beginning of the first world war. More rigorous experiments were done by Dochez in New York in the 1930s – he used chimpanzees as well as human beings. Andrewes repeated some of these experiments at St Bartholomew's Hospital a few years later; he was able to show that volunteers given filtered nasal secretions developed colds but he could not confirm that the methods which had been described for growing the virus in the laboratory were effective – material from the inoculated cultures did not cause colds when he gave them to volunteers (Tyrell, 1965). In 1946 he grasped the opportunity of using the huts which formed the American Red Cross-Harvard Hospital, Salisbury in Wiltshire; he and his colleagues adapted them for use as a centre for doing experiments on volunteers in isolation. Such volunteers cannot catch colds accidentally as do a proportion of volunteers who are allowed to mix with the general public. The methods used for the handling and clinical observation of volunteers have been almost unchanged for the last 30 years (Beare and Reed, 1976) and during that time the Unit has been used on the general lines which he conceived originally – namely, volunteers are used as sensitive test objects for the presence of virus, so that samples resulting from various laboratory procedures can be examined to see whether they contain virus (Andrewes, 1953). Over 10 000 volunteers have come to the Unit to

help in the work. Having detected the viruses the next objective was to cultivate them in the laboratory, and it is an indication of how difficult and complicated the problem is that experiments to achieve this are still going on – I prefer this explanation to the alternative, namely the incompetence or laziness of the staff!

In the first years a valuable foundation was laid in showing that earlier claims to have grown a cold virus, in fertile eggs for example, were unfounded. It is a little recognized fact that it may take many more years of good work to disprove a false claim than it took of bad work to make it. Sir Christopher has already mentioned what an important turning point the discovery of the growth and detection of poliovirus in tissue cultures was. This was quickly taken up by Pereira and workers at Salisbury and they were able to show that a cold virus could apparently be passed in explant cultures of human embryo lung (Andrewes, 1953). The cultures looked normal but the medium from them produced colds in volunteers. Having published this claim they were unable to repeat their own experiment, and after exploring numerous alternatives came to the conclusion that there was something special about the embryo which provided the tissue (H. G. Pereira, personal communication).

Rhinoviruses

American workers were exploiting tissue cultures in a different way, inoculating respiratory materials into the sort of cultures in which poliovirus grows, for example human tumour (HeLa) cells, or monkey kidney cells. In this way they detected adenoviruses, which were associated with sore throats and respiratory syncytial and parainfluenza viruses (Chanock and Parrott, 1965). The latter are usually isolated from children, but we gave them to volunteers at Salisbury and found that they produced colds – similar results were obtained by American workers at the NIH under R. M. Chanock. Price (1956) and Pelon *et al.* (1957) used monkey kidney cells and in them they cultured viruses (called JH and

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2060 respectively) which came from patients with typical colds and caused colds when given to our volunteers – they turned out to be identical serologically. However, simple tests showed that the materials available at Salisbury which produced typical colds contained none of these viruses. We therefore undertook a series of systematic experiments in which we looked for a *combination* of cultural conditions which would enable us to propagate and detect these viruses. We found that human embryo kidney cells in a slightly acid medium in roller tubes at 33°C would support the growth of virus, and cause the cells to degenerate; it was quickly possible to show that with this method we could grow a virus from about 25% of adults with typical colds, and recognize them by the characteristic degeneration they produced. The viruses turned out to be closely related to the enteroviruses (of which polioviruses are the best-known members) and they are now known as rhinoviruses because they are particularly adapted to grow and flourish in the nose (Tyrrell, 1968). There was a strong faction in the U.S.A. which was impressed by their close relationship to enteroviruses and wished to include them in that group – in fact the JH.2060 viruses were called echovirus type 28 for a while. They were later renamed rhinovirus 1A, although they are rather unusual members of the group since they are much easier to grow than typical rhinoviruses.

It is good to be able to report that in the succeeding years many different rhinoviruses were isolated. We showed that separate serotypes existed, but a well organized and extensive study, largely based in the U.S.A., and under the aegis of the WHO, showed that there were scores of serotypes; the study also established agreed type strains and produced and distributed antisera (Kapikian *et al.*, 1967). Eventually the D.C. virus grown at Salisbury in the early 1950s was identified as a strain of rhinovirus type 9. It was at this time that the Common Cold Unit became a WHO Reference Centre.

However, there were many other outstanding questions. A common cold vaccine seemed an attractive idea, and several of these were made and were shown in fact to be effective if given by injection (Doggett, Bynoe and Tyrrell, 1963) and in the U.S.A. as a nasal spray (Perkins *et al.*, 1969). However, it became clear that there were so many serotypes and they were so different from each other than no cross protection was possible; a vaccine would have to contain scores of antigenically distinct strains, and would not be practicable.

The rhinoviruses produced changes in cells that were much like those of poliovirus and other enteroviruses and many of their physical properties were similar also. We stumbled on some significant

differences, such as their adaptation to growth at lower temperatures and their susceptibility to inactivation at low pH, but it was natural to ask basic biological questions such as 'What sort of organisms are these?' We were fortunate that we were allowed to tackle such basic problems and a series of young workers showed that rhinoviruses do contain RNA (Dimmock, 1966), that they can be grown in *in vitro* systems much like poliovirus (Stott and Tyrrell, 1968) and that the 4 peptides of which they are built are similar in size to those of the enteroviruses (Stott and Killington, 1972). We also tackled the question of which particular step in virus multiplication came to a stop when the temperature was raised too high (Killington, Stott and Lee, 1977). In other centres there have been excellent studies of how the virus attaches to and enters cells, and how the nucleic acid replicates the virus (Butterworth *et al.*, 1976). It is interesting that the virus used for many of these studies is rhinovirus type 2 – strain HGP – named after Dr H. G. Pereira who preceded me at the Unit and who had the cold from which the virus was cultivated.

The resources of the Common Cold Unit enabled us to study in great detail a few specimens from patients with colds. However it was also of interest to find out to what extent we could use the techniques and the viruses we had discovered to find causes for the common respiratory diseases which are encountered in general practice and in the children's wards of hospitals – in both places a substantial proportion of the disease seen is due to respiratory infections. In collaboration with other laboratories and clinicians we therefore set out to study this question (Working Party, 1965). The results showed that rhinoviruses caused only a part of the total disease. They did not bring children into hospital, but they were an important cause of colds in children and adults outside. Only later was it discovered that rhinoviruses can also initiate relapses in patients with chronic bronchitis.

However, it was clear at the time that even using the greatest care viruses could be isolated from only about a quarter of patients with common colds. There were 2 possible explanations for this. One was that three quarters of the patients were not shedding the virus that was causing the illness. The other was that they were infected with viruses which could not be cultivated by the methods then in use. We therefore gave washings from such patients to volunteers and found they produced colds; so clearly the tests did not detect some viruses and our next research objective was clear – we had to find a more sensitive method of virus culture.

At this stage occurred one of those fortunate 'accidents', rather like the one by which we used acid culture medium and found the right conditions for

producing a cytopathic effect with rhinoviruses. This time it was an indirect request from a Swedish ENT surgeon, Dr B. Hoorn, to use a new method he had developed for growing viruses in organ cultures of respiratory epithelium. After some rather haphazard correspondence he arrived in an old car with all his equipment in the back and within days had set up successful cultures in our laboratory. Using known viruses we found that any virus which would grow in the trachea of an intact animal or man would grow in the same organ in culture – it looked as though this was the universal culture system for respiratory viruses (Hoorn and Tyrrell, 1965). We therefore tested it by adding some of our ‘unculturable’ cold viruses. It turned out that they grew. In some cases they were rhinoviruses, and by passing them a couple of times in human embryonic nose or trachea cultures they were modified and would grow with a cytopathic effect in tissue cultures. A few would not; however, one was studied in detail because its presence could be detected because it destroyed the cilia on epithelial surfaces (Hoorn *et al.*, 1966). It was called HS, indicating ‘Hoorn’s cold’ in Swedish.

Coronaviruses

Thus, some rhinoviruses and some parainfluenza viruses which could not be grown in tissue cultures were grown in human organ cultures. But there was also a cold virus, B814, which seemed to be different and ‘new’ in some ways. It was ether-labile, which indicated that it contained an essential lipid structure, presumably an outer envelope, yet volunteers inoculated with it did not seem to get antibodies against any of the known viruses with this characteristic – for example, influenza or parainfluenza viruses. It produced colds which were distinct from those produced by rhinoviruses, for instance, the incubation period was longer and there was less cough. However it did not destroy the cilia and its growth could only be detected by inoculating culture medium into volunteers and producing colds. At this point, Dr J. D. Almeida showed that viruses could be detected in organ cultures by examining broken cells from them under the electron microscope. This method revealed that the B814 had an unusual appearance (Almeida and Tyrrell, 1967); it resembled infectious bronchitis virus (IBV) of chickens and mouse hepatitis virus (MHV) and also a virus which had just been detected in the U.S.A. by Dr D. Hamre by inoculating specimens from students with colds into tissue cultures (Hamre and Procknow, 1966). The appearance suggested a sort of halo of projections surrounding a flexible but basically rounded particle, and the name was coined from the Latin for a halo – *corona*. The characteristic club or petal-

shaped projections were quite distinct from the spikes of the influenza and para-influenza viruses.

As far as the human disease is concerned some years of further work have not brought us much progress in studies of these coronaviruses. There is no substitute for human fetal tracheal organ cultures for the culture of many of them, and so we have learned little of their frequency or serological types. They are all capable of causing colds in volunteers, and they seem to have some antigenic relationship to the one serotype which can be grown in tissue cultures as first shown by Dr Hamre (Bradburne, Bynoe and Tyrrell, 1967). About 15% of colds are due to coronaviruses which are recognizable by present methods – they may occur in children or adults. There is little evidence that they cause more serious respiratory disease.

It has turned out that coronaviruses are very widespread in the animal kingdom, and cause a variety of diseases (Tyrrell *et al.*, 1975). For example, IBV (infectious bronchitis virus) of chickens causes not only respiratory disease but egg drop owing to infection of the oviduct, and also a form of nephritis. In several species a coronavirus causes gastroenteritis, and transmissible gastroenteritis of pigs is a serious economic problem, as is bluecomb of turkeys in the U.S.A. Feline infectious peritonitis is also a clinical problem due to a coronavirus. Antibodies against a virus in one species may be found in another – for example mouse hepatitis virus antibodies are found in man. This may be because the viruses of animals are related antigenically to those of man and so antibodies against them appear, although there is no evidence that the viruses themselves infect man – in other cases the virus found in one species may spread to others.

The structure of some of the viruses of man and animals is now being investigated in some detail. It seems that they are unusual as enveloped viruses in that they have ‘positive stranded’ and infectious RNA as their genome (Macnaughton and Madge, 1977; Schochetman, Stevens and Simpson, 1977). The characteristic peptides are being separated, but they seem to be of rather different sizes in different viruses and there is more work to be done before they and the antigens they carry are properly identified. Thus we find ourselves treading common ground with investigators of many different types of disease in various species. This has led to interesting international contacts to gather together all the information needed for comprehensive reports on the various viruses in the group (Tyrrell *et al.*, 1975).

Since 1970, the Common Cold Unit has been part of the Division of Communicable Diseases at the Clinical Research Centre (CRC), Harrow. The strategy has therefore been to do at Salisbury

things which are best done there, particularly studies based on volunteers. On the other hand, work needing techniques such as biochemistry and electron microscopy has been done at the CRC, which is well equipped and staffed for this.

The strategy of research on colds

It is possible now to see some broad patterns in the way this work has gone. It was clearly a wise decision to set up a specialized unit, at Salisbury, in which the central problem, 'How to grow the cold viruses', could be studied. It is also an interesting example of an occasion when the voluntary help of many members of the general public played a key role. As is usual in science, workers in various other laboratories contributed but it was the techniques for virus culture developed at Salisbury which made it possible to broaden the work, to study the viruses without the use of volunteers and so to investigate precisely and in depth the epidemiology in the field, the mode of spread, the microanatomy and the biochemistry of these viruses, and the way they produce disease. With volunteers and the viruses now available, Dr S. E. Reed made detailed studies of the effect of some psychological factors, particularly 'stress', on colds (Totman, Reed, and Craig, 1977) and also the effect of rhinovirus infections on ascorbic acid metabolism and lung function. In the first years of the Unit, Dr J. Lovelock studied the ways in which colds might spread using mock nasal 'drips' containing dyes, but more recently the spread of real viruses has been studied in experimentally infected volunteers - these viruses usually travel in sneezed out droplets of nasal secretion but they can also survive on fingers (Buckland, Bynoe and Tyrrell, 1965; Reed, 1975). This work has also been extended by producing colds in explorers in the Antarctic, and seeing how they spread (Holmes *et al.*, 1976).

It is commonly said that research on the common cold has made little progress and that we are still no nearer the longed-for common cold cure. However it seems to me that things have changed enormously over the past 25 years. We are no longer asking, 'What causes colds?' for we know they are due to certain viruses, particularly rhinoviruses and coronaviruses, and knowing this we are now able to ask other questions such as 'How do these viruses do it?' and 'How can we stop them?'. The change in the questions is the best sign of our progress and I am hopeful that the next 25 years will provide answers to these and that we will move on to ask still more difficult ones, for that is the way science goes.

Many of the questions in the minds of the early workers have now been answered. I believe we know the causes of most colds and how they produce

their effects. We still lack, however, any effective way of preventing or treating these conditions and repeated attempts, all unsuccessful, were made to show an anti-cold effect using substances which would stop rhinoviruses or other organisms from growing in tissue culture cells. One important positive experiment was done in which human leucocyte interferon was given by repeated nasal spraying about the time of a rhinovirus infection and this clearly prevented the occurrence of a common cold (Merigan *et al.*, 1973). This result indicates that appropriate treatment can influence a nasal virus infection. We are still looking for an appropriate antiviral agent, which is cheaper and more readily available, with which to extend these observations; but more active synthetic substances and more convenient sources of interferon may yet be obtained and these might then be used to reduce the severity of colds.

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