The Common Cold – My Favourite Infection
The Eighteenth Marjory Stephenson Memorial Lecture

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It is a great honour to be invited to give this lecture especially as my essay does not deal with subjects to which Marjory Stephenson herself contributed, such as bacteriology. On the other hand, I am sure that, like everyone else, she had colds from time to time and would approve the subject for that reason. This leads me to apologize for using the personal pronoun in my title. Sometimes lectures like this are judicious reviews of a whole subject. As we shall see, knowledge on the common cold is now too big a subject to be encompassed by one lecture and I propose instead to emphasize certain aspects which have been studied at the Medical Research Council Common Cold Unit (CCU), Salisbury and which I happen to think are important and interesting.

A research strategy illustrated

In science, as in other human activities, no individual is indispensable. Whichever of us were to disappear, science in our field would continue to advance; but some individuals nevertheless contribute substantially while they are here and this is usually due to a combination of ability, vision and opportunity. The CCU was set up by Dr C. H. Andrewes (subsequently Sir Christopher) because he perceived the common cold as a medical problem worth solving; furthermore he envisaged a set-up through which it could be tackled by a combination of a virological laboratory and a regular supply of isolated volunteers (his human guinea-pigs) (Andrewes, 1965). He worked out how this could be achieved and vigorously pushed his idea with the Medical Research Council and the Ministry of Health. He received a cautious but basically encouraging response.

I am impressed that in 1946, at a time when the country was in dire straits economically and only beginning to repair the ravages of a terrible war, that such a project was backed; it must have been clear that there was no assurance of success and the disease was mild and unglamorous. It is difficult at this distance in time to decide whether the support owed more to Andrewes' enthusiasm and advocacy, to the discernment of Sir Charles Harington, the Director of the National Institute for Medical Research at Hampstead (where Andrewes was based) or to the philosophy of the Council and its Secretaries. Certainly their decision runs counter to many guidelines for research that I have heard more recently, e.g. goal-orientated research is bad; good medical research must be done in medical schools; isolated units always lag behind. Furthermore, research continued when early efforts to grow the virus were unsuccessful and later when every effort to produce commercially viable vaccines or antivirals failed; even so it was almost closed on a number of occasions.

After I had been supporting the concept of a Clinical Research Centre recently I was told 'It didn't need a CRC to tackle the common cold', to which I should have replied 'but it needed the CCU!', and thank goodness it was there.

Virus hunting: frustrations and joys

The Unit was set up to answer the questions of how colds are spread and what causes them. In 1946 most people thought that colds were due to the cold virus, just as influenza was due to the influenza virus, but early attempts to propagate and detect it by inoculating animals and looking for disease or inoculating embryonated eggs, as was done for influenza virus, led to confusing or
frankly negative results. With volunteers available it was possible to pass viruses from nose to nose, to prove they were present by the clinical response and so to do laboratory work with a known stock of virus. Volunteer inoculation was also a sensitive test to determine whether a virus had been propagated or not. Because of the scrupulous volunteer technique developed in the first few weeks of the Unit's life, it was possible to exclude methods of cultivation which did not work, and in the process of searching for effective methods a clear negative was very valuable.

After the first studies had shown that classical methods using animals or eggs were ineffective, the methods developed by Enders and his colleagues to grow the poliovirus were tried. Nasal washings were added to tube cultures of human embryo lung explants. The cells did not degenerate but the culture medium caused colds up to the 10th serial passage when given as nasal drops to volunteers (Andrewes et al., 1953). This success came only after years of work and was only partial, since the results were not reproduced when the experiment was repeated with tissue from other embryos. However in retrospect one can realize that the basic methodology had been validated. In the U.S.A. and elsewhere, secretions from patients with colds and other diseases were dropped into tissue cultures of the types used to propagate other viruses, such as poliomyelitis and influenza viruses, and a number of important new pathogens were discovered, such as adenoviruses, respiratory syncytial (RS) virus and parainfluenza viruses because they produced obvious changes in the cells (Chanock & Parrott, 1965). However, the viruses that usually cause colds produce no changes in such cultures. Methods for propagating them were discovered by putting cold-producing washings into various cultures, removing the medium a few days later and testing for its ability to produce colds by giving it as nose drops to volunteers (Tyrrell et al., 1960). Thus it was found that rhinoviruses needed selected susceptible human cells (human embryo kidney at first; later, other workers discovered susceptible cultures of human diploid or HeLa cells) and appropriate medium, for example adjusted for pH. When conditions were satisfactory, a cytopathic effect appeared and could be used to detect the presence of virus. We also followed the very simple idea that respiratory viruses ought to grow in normal respiratory epithelium, and found that human foetal tracheal or nasal cells in suitable organ cultures would grow not only all the respiratory viruses known at the time (Hoorn & Tyrrell, 1965) but also further rhinoviruses and organisms which had not been recognized before (Tyrrell & Bynoe, 1965) namely coronaviruses, though one type of these (229E) was independently discovered in the U.S.A. (Hamre & Procknow, 1966).

There was real excitement in these discoveries. First, we no longer had to regard the basic cause of colds as mysterious. We could grow viruses, characterize them and type them. The techniques for growth of virus meant that others could also perform epidemiological surveys, study immunity, develop antiviral substances or vaccines. Furthermore, we found the organisms themselves fascinating. We had expected 'cold' viruses to resemble an influenza virus but the rhinoviruses turned out to be biologically close to polioviruses. On the other hand, the coronaviruses turned out to be viruses only seen before in animals. Morphology, immunology and chemical study showed they had a characteristic structure and a long positive-stranded RNA genome within a lipid envelope: a new class of organisms fully justifying their new name, coronaviruses (Tyrrell et al., 1978). Those related to the type (229E) isolated by Dr Hamre's group in the U.S.A. grow in tissue cultures and have been very useful in studies in volunteers.

There is still a need for virus hunting, and virus hunters! The work is again back at the slow and unglamorous stage. Some years ago we applied all the available techniques to nasal specimens collected from members of the staff on the Northwick Park Clinical Research Centre site who had colds. Viruses were recovered from the expected proportion and those that were negative to culture were tested by inoculating them into volunteers and about half produced mild but typical colds (Larson et al., 1980). However they proved unstable on storage so we had to repeat the study and conserve all material at liquid nitrogen temperature. We now have washings which we know produce colds in volunteers and we are inoculating them into organ cultures of human ciliated respiratory epithelium as a first effort to propagate them in the laboratory.
On the other hand there is a great deal of active research on the molecular genetics and structure of both rhinoviruses and coronaviruses. It is all of great interest and some of it impinges on work at the CCU as will be described below.

The hunt for viruses as causes of colds has been exciting and varied, with numerous unexpected twists and turns, and we are not at the end yet, more delights are surely in store.

**Resistance to colds**

I can remember how exciting it was to realize when we had grown our first few rhinoviruses that we could now develop a neutralization test and would be able to measure antibodies to them and work out whether they belonged to different serotypes. We now had laboratory methods by which to test for immunity to common cold viruses. In case I wasn’t sufficiently interested I remember Sir Christopher Andrewes saying to me ‘You shouldn’t be interested in why volunteers given viruses get colds, but why so many don’t!’ About this time, following the work of Tomasi there was great interest in the U.S.A. in secretory antibody, and suggestions that this was the main explanation for resistance. We didn’t agree with this, but equally there were paradoxes: volunteers with high titres of specific antibody in the inoculum might nevertheless get infection and illness after they were given a virus.

In recent years our group has developed ELISA tests which provide simple and very sensitive assays for antibodies to coronaviruses (Kraaijeveld et al., 1980), mainly directed against the glycoprotein forming the characteristic surface peplomers and responsible for neutralization of virus infectivity. We have adopted the simple approach of inoculating a group of volunteers with a coronavirus, having first collected serum and nasal secretions, we then documented any signs and symptoms and whether they shed virus or showed an immune response. All these results were then correlated with immunological and other assays on the serum and secretions. The overall results were very informative. They showed for instance that, allowing for correlations between the various measurements, secretory and serum antibodies both had an effect on the volunteer response and reduced the risk of infection and illness (Callow, 1985). However the total protein content of the secretions was also associated with resistance to infection, and we wonder whether this is connected with earlier work which showed that breast milk and other secretions contain a factor, probably glycoprotein, that prevents plaque formation by a number of viruses (Matthews et al., 1976). This clearly requires further investigation.

We already have some further evidence of the significance of these findings for we have shown that in the volunteer group (as had been shown before in field studies) women were more susceptible than men, and those who had had a cold in the previous 6 months were more resistant than those whose last cold occurred earlier; it was found that women and volunteers with recent colds had higher antibody titres in both their secretions and their circulation and more protein in their nasal washings than men or those with distant colds. Thus the fact that women have more colds than men may not be because they are more exposed to children but because their immune system is ‘set’ differently. Finally it was found that high titres of serum or secretory IgE are found in those infected volunteers who have symptoms (Callow et al., 1987). This suggests that some sort of allergy or associated difference in immunological responsiveness determines whether the infection produces a cold. Of course, we do not know whether this is true only of coronavirus infections so such observations will have to be repeated and extended. We already have evidence that whether a cold develops or not depends on a complex mixture of immunological parameters. We suspect that others remain to be discovered; it is clear that the secretions of the nasal cavity contain T and B cells (Holmes et al., 1987) and that antibody-dependent cellular cytotoxicity against coronavirus-infected cells can be demonstrated with human serum and peripheral blood lymphocytes (Holmes et al., 1986), so at some time we ought to examine their protective role, if any. There is already evidence that cytotoxic T cells can reduce the shedding of influenza A viruses and it could well be that they help to defend us against other viruses too.

**Virus detection, new style**

I have indicated the key role played by virus cultivation but the use of such methods has disadvantages. Although they can be very sensitive they require supplies of special tissue culture
cells and results often come slowly. Years ago it was shown in pioneering experiments that respiratory viruses can be detected in clinical specimens, especially of children, by immunofluorescence. The method is often called rapid, because it can yield results within a day, much more rapidly than virus cultivation (Gardner & McQuillin, 1980). However it actually requires a lot of bench time and meticulous, expert microscopy, and in these senses is not particularly rapid. We have therefore been attracted by the idea of using other methods, for instance detecting virus nucleic acid by nucleic acid hybridization and virus peptide antigens by ELISA. For this purpose Dr A1-Nakib, who did the work, drew on the resources of the Unit in virus strains and carefully documented material from infected volunteers and also on collaborators in other centres, for instance to supply cloned cDNA for rhinoviruses. Considerable progress has been made already. For example, it has been established that a cDNA probe derived from the 5' end of the genome detects virus RNA by dot hybridization, and that there is considerable homology with viruses of many other serotypes (A1-Nakib et al., 1986b). Nucleic acid can be detected in clinical specimens by suitable modification and development of this method. It may also be possible to detect virus in cultures of the virus in microtitre wells or by in situ hybridization detected with a biotinylated probe. Work to develop such techniques is in progress.

On the other hand, ELISA tests for rhinovirus antibodies have also been developed and are being adapted to detect virus antigens in clinical material such as nasal washings. The method used is a capture assay in which antigen is bound to an antibody-coated surface and its presence is then shown in a second detection step by exposing it to labelled antibody. Under favourable circumstances it can detect a few hundred infectious doses of a virus and so this test is also being evaluated on volunteer material. It could possibly be adapted to detect the common C antigenic determinant of the virus. At the moment it is being used with reagents which react relatively specifically and would pick up efficiently only the homologous virus serotype. We have also used an ELISA test for coronavirus detection in the past.

So part of the pleasure and excitement of working on common colds is that it gives us an opportunity to use new technologies to develop simpler and more rapid tests for virus infections and there is a good chance that such tests will be readily adapted to a wide range of infections.

Psychology

I now think that common colds provide a wonderful opportunity to study the effect of the psyche on what is, in the usual meaning of the term, a straightforward organic disease. But I have to confess that when it was first suggested that we should study the role of psychological factors in colds the most I thought of was that attitudes and personality might alter the way in which our volunteers reported their symptoms. The very first study with the psychologist Richard Totman was done to test the idea that if a subject chose their medicine (a placebo) it would do them more good than if it was issued to them. No such effect was detected, but the results did suggest that the stress of the trial and the possession of an introverted personality made the colds worse objectively, e.g. as judged by virus excretion (Totman et al., 1977).

A result as strange as this had to be repeated and extended, so in a further study we examined whether volunteers who were going through adverse life events at the time they came to the Unit got worse colds. The results confirmed the effect of personality and showed that changes in life, even if they were basically agreeable, predisposed to bad colds (Totman et al., 1980).

We collaborated with a further group of psychologists in a third set of studies which we conducted 'pick-a-back' on our regular series of trials. Volunteers came to the Unit to take part in experiments on the effects of viruses and antiviral drugs but we also studied their psychological status by giving them questionnaires before they were challenged and relating their symptoms etc. to their answers. These showed again that introverts had worse colds and certain symptoms, which are believed to indicate personal stress, also predicted bad colds (Broadbent et al., 1984).

Although we are finally convinced that personality type and psychological 'stress' predispose to colds we have little idea how they are mediated. We are at present involved in a further study in collaboration with the Carnegie-Mellon University, Pittsburgh, Pa., U.S.A. in part of which
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we shall be investigating whether these psychological measures are predicting differences in the immune system that account for the volunteers' responses.

The mechanisms of illness

It is obvious that the starting point of illness in our volunteers is the deposition of virus on the nasal mucosa, but we have no very detailed knowledge of the link between that and the symptoms and signs that follow. There is an incubation period of about 2 days and towards the end of that time virus is found in the nasal washings and increases in concentration. We believe that ciliated cells are damaged and shed (they can be found in nasal washings) but we could only speculate what the mucosa looks like at the electron microscopical level or what the cause of nasal secretion, nasal obstruction or the constitutional symptoms of illness might be.

In collaboration with others we have made some initial studies. Microscopy of nasal biopsies shows that at the time of illness the ciliated cells are damaged and functional tests show that nasal clearance by the mucociliary apparatus is greatly prolonged (Wilson et al., 1987). We speculated that there might be an increase in the amount of mediators of allergic inflammation, such as leukotrienes or histamine, but none was found. However we have now looked at the effect of an inhibitor of mast cell degranulation (nedocromil) and this diminished the severity of symptoms in a double-blind placebo-controlled trial in colds due to a coronavirus. We think more work on these lines is justified; it may lead us to a single important mediator of local inflammation and possibly to a useful medicament.

We know that some peptide mediators are released, for instance interferon α (IFN-α) can be found in the nasal secretions of virtually all children with colds (Isaacs et al., 1981). In addition, although IFN-α is difficult to detect in the nasal washings of volunteers it can also be found in the circulation of patients and volunteers with influenza virus infections. We can also deduce that interleukin 1 is released since an acute phase reactant (SAA), which is secreted by the liver in response to interleukin 1, is found to increase in the circulation in response to experimental colds and influenza.

We have also studied the effect of these localized infections on the whole person and on the performance of the central nervous system in particular. Volunteers coming to the Unit have participated in a variety of performance tests under the direction of Dr A. Smith of the MRC Perceptual and Cognitive Performance Unit at the University of Sussex. A series of experiments is in progress and only the first few have so far been analysed. However, already, interesting results have emerged (Smith et al., 1987). In the first place he has resolved the dispute between those who believe that people with colds only feel that their performance deteriorates and those that believe that the execution of skilled tasks is objectively worse during a cold or influenza. The second group are right. For instance the ability to do a tracking task in which, using a joystick, the subject has to direct a spot at a moving target on a screen, deteriorates substantially during a cold. Likewise subjects with experimental influenza do badly at a task requiring them to watch for the appearance of a particular number on a screen and respond by pressing a key. In addition we should mention that colds and influenza have quite large effects compared with other factors such as ambient noise or alcohol ingestion, and furthermore the effects are in some measure specific: influenza infections did not impair the tracking task and colds had no effect on the attention/response task.

So far we have little idea how these effects are mediated. The characteristic effects of a ‘cold’ seem to be the same whether it is induced by a coronavirus, a rhinovirus or RS virus, and so we think it is likely to be due to an element in the pathophysiological response rather than to a component of the virus. We wonder whether the effect of influenza might be due to circulating interferon and this possibility is the subject of further experiments.

Antiviral prophylaxis or treatment

This is a large subject which has recently been reviewed (Al-Nakib & Tyrrell, 1987). We have been interested in the problem since the early 1960s. Over most of that period the general opinion has been that little progress was taking place. Nevertheless as someone involved in the research I am sure that a number of important ‘thresholds’ have been reached and crossed. In the
first place substances of high antiviral activity and low cytotoxicity have been found. At one time we thought that inhibition of rhinoviruses by a few micrograms of a substance in a tissue culture tube represented high potency but when given to volunteers they conferred no benefit. Recently much more potent substances have been found and it is now clear that interferon, which was the first substance we studied with a wide antiviral activity and little in vitro toxicity is actually extremely potent; there are about 10^8 units of anti-rhinovirus activity per milligram. Nevertheless we have been able to administer sufficient amounts of antivirals by nasal spray to be effective. The first example was an experiment in 1973 in which repeated intranasal sprays of relatively crude human interferon prevented infection and illness produced by subsequent drops containing a rhinovirus (Merigan et al., 1973). This was confirmed using pure human IFN-α from various sources and recent family studies (Scott & Tyrrell, 1985) have shown that such a spray given daily reduces by about 70 or 80% the number of rhinovirus colds which occur after an individual with a rhinovirus cold is introduced into the family (e.g. Douglas et al., 1986).

However, there is still more work to do. In the first place, we have yet to show substantial protection by intranasal sprays of a synthetic antiviral and we need to identify and test more potent molecules. Furthermore, it turns out that intranasal IFN-α produces too much nasal stuffiness, minor nose bleeds, etc. to be acceptable for long term use. There are however many possible ways of getting over such difficulties. Other natural interferons or modified IFN molecules could be used and there can be a powerful synergy in vitro between interferons and synthetic anti-rhinovirus molecules which must be tested to see whether it can be applied to volunteers’ colds (Ahmad & Tyrrell, 1986).

There have been problems in finding antiviral molecules that are well absorbed and then excreted into the respiratory tract. We have several examples of substances which reach active concentrations in the serum but then cannot be detected in respiratory secretions. We thought it possible that, nevertheless, molecules might accumulate in the respiratory epithelium and be effective there. However, when volunteers who had taken drug by mouth were challenged there was no evidence of protection (Al-Nakib & Tyrrell, 1987).

However, the early studies on amantadine showed that this molecule could be given by mouth and protect the respiratory tract against influenza A virus infections. We have recently studied a related molecule (ICI, 130685) a secondary amine linked to a nonane ring structure, and find that it appears in higher concentrations in nasal secretions than in the blood. It is a potent prophylactic against experimental influenza A virus infections. Furthermore, studies of this compound showed what had already been demonstrated with amantadine, namely that it is still effective when administration is delayed until symptoms have developed, i.e. it acts therapeutically as well as prophylactically (Al-Nakib et al., 1986a). This represents another important threshold for at least two reasons. One is that there was a presumption that by the time respiratory disease was obvious, virus replication was almost complete and could not be significantly modified by an antiviral compound and this is further evidence that this is not true for a mild human respiratory tract infection. The other is that as colds are infrequent and mild the expense and risks of unwanted effects will increase with long administration so it is likely that prophylactic antivirals will only be practicable or worthwhile in a limited range of clinical situations. Thus, if a therapeutic drug regime is possible, it would be more acceptable, less expensive and therefore more widely used.

Thus, although we still have no antivirals ready for general use so much progress has been made that it seems likely that they will become available in years to come.

**Molecular strategies for preventing colds**

We are now in a period during which rapid progress is being made in describing in precise chemical terms the structure and, to a lesser extent, the function of rhinoviruses and other picornaviruses. The nucleic acid of a number of viruses has been copied into DNA, cloned and sequenced and the sequence of the viral peptides has been deduced. Furthermore, virus crystals have been analysed by X-ray crystallography and, as a result, we have the three-dimensional structure of a key rhinovirus, human rhinovirus type 14 (HRV14) (Rossmann et al., 1986).
Monoclonal antibodies against HRV14 have also been produced and used to select neutralizing antibody-resistant mutants. When the genomes of these viruses were sequenced, single or limited amino acid substitutions were found and these have now been mapped on the three-dimensional model to show where the key antigenic sites are found.

Proceeding by analogy it is now possible for us to identify peptides in other viruses which have been sequenced (such as HRV2) and to synthesize peptides which may evoke antiviral antibodies. Such oligopeptides have been made to mimic key sequences of foot-and-mouth disease virus. These viruses resemble rhinoviruses in many ways even though they produce a different range of diseases, but the important fact to note is that such peptides not only evoke antibodies but also protect animals against experimental infections (Bittle et al., 1981).

In the past we showed that a parenteral injection of HRV2 vaccine prepared from inactivated virus particles protected volunteers against intranasal virus challenge (Scientific Committee on Common Cold Vaccines, 1965) so it is possible to imagine an injection of a mixture of peptides which could protect against colds due to a range of rhinoviruses. Obviously we need much more information if this is to be done rationally; for instance we need to know the epitopes against which protective circulating and secretory antibodies of humans are directed. These can now be ascertained using the ELISA tests I mentioned earlier. We also need to know how to formulate and present such peptides so that they are effective and safe antigens for humans. But after a period of 20 years during which we thought that there would never be a practical technology for vaccinating against these viruses we now find that there are many possibilities and that the subject is on the rise again.

Another interesting approach is being made in the U.S.A. This is to use monoclonal antibodies against the host cell to identify the two receptor sites through one of which all rhinoviruses attach to and enter cells (Tomassini & Colombo, 1986). A preliminary report shows that such monoclonal antibodies reduce infection when given as an intranasal spray to volunteers. The approach has the advantage that, although there are at least 100 serotypes of rhinovirus, they each use one of only two receptors. Monoclonal antibodies would probably not prove to be a satisfactory medicament but alternative molecules may well be found.

Finally, it is possible to develop precisely defined live virus vaccines. Of the many possibilities that might be used we have been involved in trials of temperature-sensitive (ts) mutants of RS virus. Early killed vaccines enhanced rather than prevented the disease, and previous live attenuated ts vaccines were either inadequately or excessively attenuated and also were genetically unstable. Professor C. Pringle (personal communication) is preparing mutants in which the site of mutation is being defined and he has evidence that strains carrying several mutations are more stable. An initial trial shows that in adult volunteers one double mutant is partly attenuated, but still immunogenic, and we are thus encouraged to try to develop it further. Although RS virus causes colds in adults it produces severe lower respiratory disease in infants and is a worldwide problem. Inhalation of aerosolized ribavirin has been shown to have some therapeutic effect, but vaccine prophylaxis against RS virus is desirable and at the moment we do not know which of the many possible methods will prove successful. Studying its behaviour as a common cold virus may give us facts which we can exploit in our search for a rationally designed and effective vaccination.

Conclusion

The study of common colds, especially in volunteers, has occupied me for much of my scientific career and has constantly provided new and fascinating insights to someone like myself who is interested in human disease and how it is produced. But the study has also led me to investigate the biology of some fascinating organisms and to work with novel scientific ideas and methods. Progress has been real, though at times spasmodic, and has introduced me to many interesting and agreeable colleagues and collaborators both here and abroad, who, of course, have done most of the actual work. Whatever the public may think, we have passed a number of significant thresholds. I believe that eventually the prevention and specific treatment of colds will become practical and I hope to be around to see that day.
REFERENCES


