CHAPTER 7

Coronaviruses

Arnold S. Monto

1. Introduction

The coronaviruses are a group of RNA-containing agents that have been associated with respiratory illnesses in man and with a number of other diseases in laboratory and domestic animals. The name for the group was adopted to describe the characteristic fringe of crownlike projections seen around the viruses by electron microscopy; these projections are rounded, rather than sharp or pointed as is the case with the myxoviruses. Like the myxoviruses, the coronaviruses contain essential lipid and are 80–160 nm in diameter.[18] While the animal strains are readily isolated in several different systems, recovery of the human strains has posed major problems. A number of these strains have been isolated only in organ culture of the human respiratory tract. This factor has made it difficult to determine the relationship among isolates and has complicated efforts to understand the role of these viruses in human respiratory illness. Therefore, much of the information on the epidemiology of the agents has come from serological studies.

2. Historical Background

The first human coronaviruses were isolated by different techniques in the United States and Britain at approximately the same time. The British Medical Research Council's Common Cold Research Unit had been studying fluids collected from persons with natural respiratory infections and by standard cell-culture isolation methods and by inoculating them into human volunteers. Rhinoviruses or other cytopathogenic agents could be recovered from a portion of the fluids.[36] There was an additional substantial portion from which no agents could be isolated but that could still cause colds in the volunteers. Organ cultures of human embryonic trachea or nasal epithelium were then used in an effort to detect the recalcitrant viruses present in the fluids. A specimen, B814, that had been collected in 1960 from a boy with a common cold had not yielded a virus on inoculation into cell culture. After the specimen had been passaged serially three times in human tracheal organ culture, it could still cause colds on inoculation into volunteers, which indicated that replication had taken place.[58]

In Chicago during the winter of 1962, five agents were isolated in primary human kidney-cell cultures from specimens collected from medical students with common colds. The viruses were ultimately adapted to W138 cultures and exhibited a type of cytopathic effect (CPE) not previously seen. A prototype strain, 229E, was selected for characterization and was found to be RNA-containing, ether-labile, and 89 nm in diameter, but distinct serologically from any known myxo- or paramyxoviruses. Sera collected from the five medical students all exhibited a 4-fold rise in neutralizing antibody titer against 229E.[23]

It became clear that these "novel" viruses were

Arnold S. Monto · Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan.

A. S. Evans (ed.), *Viral Infections of Humans*

of more than passing significance when organ-culture methods were added to standard cell-culture techniques in a study of acute respiratory infections of adults conducted at the National Institutes of Health (NIH). Six viruses were found that grew in organ but not cell culture and were ether-labile; on electron microscopy, the agents were shown to resemble avian infectious bronchitis virus (IBV) in structure.[39] The B814 and 229E strains were soon also demonstrated to have a similar structure on electron microscopy and to develop in infected cells by budding into cytoplasmic vesicles.[1,2,22] As a result of the similarity of the human agents to IBV and also to mouse hepatitis virus (MHV), they were collectively considered to represent a group of vertebrate viruses distinct from the myxoviruses antigenically and structurally.[3] The name *coronavirus* was adopted for the group to describe the fringe of projections seen around them on electron microscopy.[18]

Except for 229E, none of the human coronaviruses had been successfully propagated in a system other then organ culture. McIntosh *et al.*[37] reported successful adaptation of two of the NIH isolates, OC (organ culture) 38 and OC43, to the brains of suckling mice. These strains were shown to be essentially identical antigenically but quite distinct from MHV. Only OC38 and OC43 could be so adapted; the other four OC strains resisted such attempts. IBV was known to exhibit hemagglutination under certain conditions, but no such phenomenon had been demonstrated for the human strains until OC38 and OC43 were adapted to mice. Kaye and Dowdle[32] found that the infected brain preparations would directly and specifically agglutinate red cells obtained from chickens, rats, and mice. This technique greatly expanded the ability to do epidemiological studies, since it was simple and reproducible.

Other more recent developments have included adaptation of OC38 and OC43 to growth in cell monolayers; either mouse brain or organ-culture material could be used as source of virus.[11] Not only was CPE available for reading of neutralization tests, but also the OC38 and OC43 virus was found to hemadsorb red cells of rats and mice, making available a more precise means of evaluating end points in tests involving these organ-culture-derived strains.[30] The other OC strains that could not be adapted to mouse brain resisted adaptation to cell culture. Finally, immune electron microscopy has been added to the methods available for identifying the presence of coronaviruses in organ-culture harvests. This highly sensitive technique should improve the ability to detect virus, but it is obviously unsuitable for use in all but the most specialized studies.[31]

3. Methodology

3.1. Sources of Mortality Data

Coronaviruses that infect domestic and laboratory animals produce illnesses that are sometimes fatal. In contrast, there is no documented report yet on record of human coronaviruses being involved in a lethal infection. This situation may be a reflection of the limited number of investigations carried out as yet. It is known that these agents frequently infect small children and reinfect adults, including persons with chronic respiratory disease.[53] It would be logical to assume that deaths could occasionally occur in these most susceptible segments of the population, but they are probably not very frequent.

3.2. Sources of Morbidity Data

Since coronaviruses usually produce respiratory illnesses indistinguishable from those caused by many other types of viruses, it is not possible to obtain data on morbidity in the absence of laboratory identification of infection. The viruses are difficult to isolate, so most workers have relied on serological techniques to increase the numbers that can be studied. Investigations into coronavirus infection have usually formed part of overall evaluations of the role of viruses in general in respiratory illnesses. As indicated in the partial listing in Table 1, a variety of different open and closed populations have been used for these studies. The 229E strain was originally isolated from medical students in Chicago as part of a long-term study of respiratory illnesses in young adults.[21,23] Employee groups have been the source of specimens in the NIH[29,42] and in the studies at Charlottesville, Virginia.[26] Infection has also been evaluated in children's homes[34] and boarding schools,[36] among military recruits,[60] and among children hospitalized for severe respiratory illnesses in various parts of the world.[29] Serological methods

Table 1. Longitudinal Studies on the Epidemiology of Coronavirus Infection in Humans

Location	Population	Virus studied
Chicago, Ill.[21]	Medical students	229E
Washington, D.C.[29,42]	Hospitalized children	229E, OC43
Bethesda, Md.[29,42]	Adult employees	229E, OC viruses
Atlanta, Ga.[34,35a]	Institutionalized children	229E, OC43
Charlottesville, Va.[26]	Working adults	229E, OC43
Tecumseh, Mich.[16,49]	General community	229E, OC43
Brazil[15]	Nonhospitalized children	229E
Denver, Colo.[40]	Hospitalized asthmatic children	229E, OC43
N. and S. Carolina[60]	Military	229E, OC43

have been used to detect occurrence in persons with acute exacerbations of asthma[40] or chronic obstructive respiratory disease.[53] Patterns of coronavirus infection have been identified among the general population residing in the Tecumseh, Michigan, community as part of a longitudinal study of respiratory illness.[16,49] Volunteers have continued to be employed, especially to determine characteristics of illness not yet well defined in natural infection because of problems associated with isolation of the viruses.[7,8]

3.3. Serological Surveys

Although relatively simple serological techniques are now available for two coronaviruses (22E and OC38 or OC43), extensive surveys of antibody prevalence have not been carried out. When done, the surveys have often formed a part of studies directed mainly toward determination of the incidence of infection. Information on the prevalence of antibody is available for populations in the United States,[16,26,42] Britain,[8] and Brazil.[15] A special situation is the presence in man of antibody against coronaviruses of animals. The finding of mouse hepatitis antibodies in military recruits and in children and adults from the general population was surprising when first described in 1964.[25] It is now recognized that this does not indicate past experience with MHV, but rather with human coronavirus strains that are known to cross-react with it. Similarly, antibodies in human sera against the hemagglutinating encephalomyelitis virus of swine and the coronavirus of calf diarrhea also appear to represent cross-reactions with OC43 or related strains.[35b,c] In contrast, in a survey of antibodies to avian IBV, none could be found in a military population. Low-level antibodies were detected only in a portion of subjects who had close contact with poultry.[45] This virus is not known to cross-react with the human strains.

3.4. Laboratory Methods

3.4.1. Viral Isolation. Only the 229E strain was originally isolated in cell culture. It was eventually adapted to human embryonic lung cells (W138), in which it has been maintained.[23] However, this cell line is not a reliable system for primary isolation of 229E-like agents. To date, human embryonic intestine (MA177) has proven the most suitable cell system, but it is available only in limited quantities.[29] All other known human coronaviruses were originally isolated in organ cultures of human trachea or lung.[24,39,58,59] The presence of virus was usually detected by electron microscopy, or sometimes by fluorescent antibody (FA) staining of impression smears.[57] Two strains that are essentially identical, OC38 and OC43, have been adapted to suckling mouse brain and to primary monkey-kidney and BS-C-1 cell cultures.[11,30,37] Another cell system, L132, a heteroploid human lung line, has been reported to be suitable for primary isolation of 229E, a related virus (LP), and the B814, the first-described organ-culture agent.[6,9] This last finding has not been comfirmed by other workers.[11]

It is conceivable that special conditions of cell culture are required for primary isolation of these agents; this would be similar to the strict requirements for propagation of the rhinoviruses before the availability of W138 cells.[52] The situation is in sharp

contrast to that found with the coronaviruses of animals. While they are rather species-specific in their *in vitro* growth characteristics, especially on primary isolation, such isolation is easily accomplished.[44,54–56]

3.4.2. Serological Tests. Neutralization (N) tests of varying degrees of complexity can be performed for all described coronavirus types. The most involved procedure must be used for those viruses that up to now have never been adapted to systems other than organ culture.[39] This technique involves incubating serum with known virus and inoculating the mixture into cultures of human trachea. Evidence of N manifest by a reduction in viral yield is determined by electron microscopy. For those coronaviruses adapted to cell cultures, tube- or plaque-reduction N tests are available. W138 or L132 cells may be used for both methods with 229E virus; a number of cell lines including primary monkey kidney and BS-C-1 have been used for N tests involving the OC38–43 virus.[5,9,11] Hemadsorption rather than CPE can be used for identification of end points with the BS-C-1 cell lines.[12,13]

Most seroepidemiological studies have not used N but rather complement-fixation (CF) or hemagglutination-inhibition (HI) tests as sources of their data. The method of preparing a CF antigen for 229E directly from cell-culture harvests was reported along with the original description of the viruses by Hamre and Procknow.[23] By this method, the CF test detected antibody in low titer and only for a short time after infection. This observation was subsequently confirmed in a large study, and it was suggested that the presence of CF antibody in a population could be interpreted as evidence for recent activity of the virus.[15] However, it was also learned that if the antigen was highly concentrated, antibody could be detected at a higher titer, and this antibody persisted in the population so that the CF method could be employed in surveys of prevalence.[5] An indirect HI test for 229E virus using tanned sheep erythrocytes has also been described. The procedure appears to be highly sensitive, and no cross-reactions with OC43 virus were observed.[35]

It was found that CF tests can be satisfactorily performed with OC43 virus using infected suckling mouse brain as antigen.[42] The same mouse-brain material can also be used in the HI test for OC43 antibody. In this test, the hemagglutination titer was higher for rat than for chicken erythrocytes, but was sufficient with the chicken cells so that they could generally be employed; this is of particular importance in view of the spontaneous agglutination that often complicates working with rat erythrocytes. Serum to be tested did not require treatment with receptor-destroying enzyme, but rather standard heat inactivation at 56°C. The agglutination took place equally at various temperatures including room temperature.[32] In addition, a single radial hemolysis test has been developed. It can be used not only for OC43 but also for the nonhemagglutinating 229E by using chromic cations to attach virus to glutaraldehyde-treated red cells.[28a]

Other serological tests have been developed that have been used more in antigenic analyses of the different coronaviruses than in epidemiological studies. With the indirect FA technique, characteristic cytoplasmic inclusions were demonstrated with 229E, OC43, and even the other coronaviruses grown in organ culture.[49a] The last were prepared for testing by making smears of fragments of the infected trachea.[41] It has also been possible to demonstrate precipitin lines on gel-diffusion tests with coronavirus antigens concentrated 10- to 50-fold. Two or three precipitin lines were observed by Bradburne[5] in tests with hyperimmune animal or human serum, but others have identified only one such line.[33]

4. Biological Characteristics of the Virus

Very little information is available on the relationship of coronavirus structure to patterns of infectivity and antigenicity. The viruses, although originally thought to be similar to the myxoviruses, are in fact quite different. They contain a single continuous strand of RNA, associated with proteins as a ribonucleoprotein. Biochemical studies on both human and animal strains clearly indicate that they are positive-stranded.[36a,b,43a,56a] The spikes of the virus are distinctive in appearance and are reported to contain glycopolypeptides; both HA and CF antigens have been associated with the surface of the virion and are presumably located in the projections. No neuraminidase has been demonstrated, and therefore it has been concluded that the antigens belong to a single species present on the surface. By analogy, antibodies to these antigens should be associated with protection.[28,33]

The total number of serological types that infect man has not been defined. Here again, the problem revolves around the difficulties encountered in isolating the human coronaviruses. Consequently, there is no way of estimating the proportion of existing types that have already been isolated. It is also difficult to determine the separate antigenic identity of types that grow only in organ culture as compared with those that grow in cell culture. N, CF, HI, gel-diffusion, and immunofluorescent techniques have been used in the antigenic analyses by McIntosh *et al.*,[41] by Bradburne,[5] and by Bradburne and Somerset.[8] As would be expected, results have differed by each of these procedures, with N tests the most specific. However, cross-reactions were commonly demonstrable even by this method using animal antiserum or immune ascitic fluid, indicating that there must be many shared antigens.

An attempt at placing the human coronaviruses in broad groups is shown in Table 2; MHV is included because of its frequent interrelationships with the human strains, and avian IBV is omitted because it is antigenically distinct. The unadapted organ-culture strains have been listed separately; it has not been possible to prepare animal antisera against them, and they have been tested only against pairs of sera obtained either from individuals naturally infected or from volunteers challenged artificially. Such sera would be expected to be considerably less specific than animals antisera. It has been clearly shown in several laboratories that 229E is quite different from OC38 and OC43 not only in growth characteristics but also antigenically; cross-reactions can be shown by N tests, but these are demonstrable only using very sensitive procedures. LP virus was originally isolated in organ culture and not in cell culture, but is closely related antigenically to 229E. OC43 virus has a low-level cross-reaction with MHV; in some reports, this has been reciprocal and in some one-way. Although B814 virus is quite different from OC43, they both share some antigens in common; again, cross-reactions with 229E are rare. Among the additional viruses, OC44 is closely related antigenically to OC38 and OC43, but has never been successfully adapted to mouse brain or cell cultures. The four other viruses are listed together by exclusion, i.e., not because of any demonstrated relationship to one another, but rather because they are not closely related to viruses in the first three groups. Some low-level reactions with the agents in these three groups have been shown to be present, with OC16 virus being the most distinctly different strain.

Table 2. Serological Relationships of the Human Coronaviruses

Group	Strains tested with animal antisera		Strains tested with human antisera
I	229E, LP	Closely related but not identical	
II	OC38, OC43, MHV	Nearly identical	OC44
III	B814		
Others			OC16 OC37 OC48 EVS

As indicated above, much of the information on the behavior of 229E and OC43 viruses has come from CF and HI tests. In view of the sharing of antigens among many of the viruses listed in Table 2, the specificity of these procedures must be carefully considered. Cross-reactions between 229E and OC43 have been reported only rarely when tested by CF against animal sera. With human serum, heterologous rises in antibody titer have been observed occasionally, but not frequently enough to create problems in studies involving significant numbers of specimens.[10] Of greater practical concern is the occurrence of cross-reactions between OC43 and the other organ-culture viruses. It is possible that rises in titer detected when using OC43 antigen in seroepidemiological studies may result either from OC43 infection itself or from infection with one of these related viruses. Indirect evidence that the infecting agent may not be OC43 itself is the dissociation seen between the CF and HI tests for OC43 during a particular period of time. Rises in titer by CF should usually be accompanied by rises in titer by HI in the same serum pairs. If this does not ordinarily occur during one time period, but does during a second period, it suggests that a related virus but not OC43 was circulating during the first period.[49]

Data that demonstrate the etiological role of coronaviruses in respiratory infections derive from laboratory and field studies. The viruses do interfere

with the action of cilia in tracheal organ culture, which suggests that they should have the same effect *in vivo*. In addition, volunteers have been inoculated with essentially all available strains with production of illness.[7,8] It has also been possible with 229E to demonstrate that natural infection was statistically related to the production of illness. During the 1967 outbreak of 229E infection in Tecumseh, Michigan, illness was significantly more common among those with infection than among matched subjects without infection.[16] Similarly, 229E infection among Chicago medical students was statistically associated with illness when those with rises in titer were used as their own controls.[21]

5. Descriptive Epidemiology

5.1. Incidence and Prevalence

Evidence is steadily mounting that the coronaviruses are of major importance in common respiratory infections of all age groups. The total impact of coronavirus infections on the general population cannot be calculated at present because not all viral types have been identified. Only 229E and OC43 are amenable to large-scale serological studies; infection rates for other distinct types such as OC16 cannot be determined. The assumption must be made that the former two types are typical of the other viruses. Incidence of infection with these agents exhibits a marked cyclical pattern, so it is to be expected that reported rates will vary based on the number of seasons of high viral activity included in a particular study. Table 3 presents a summary of results obtained in four such studies.

5.1.1. Incidence and Prevalence of 229E Virus. The activity of 229E was found to be of high prevalence in 3 out of 6 years of a study among Chicago medical students. The mean annual incidence of infection during the total period was 15%, based on person-years of observation. The criterion for identification was a reproducible 2-fold seroconversion determined by CF. There was marked year-to-year variation in infection frequency, ranging from a high of 35% of those tested in 1966–1967 to a low of 1% in 1964–1965. However, nearly 97% of the infections occurred during the months from January to May, often at a time when isolation of rhinoviruses was at a low, and seroconversions for 229E were only rarely accompanied by a rise in titer for another respiratory agent.[21]

The serological study of 229E activity in the community of Tecumseh, Michigan, initially covered 2 years, which included one period of high prevalence. As with the study in Chicago, routine blood specimens were collected so that infection rates could be determined; however, the study group was composed of individuals of all ages living in their homes. Over the 2 years, infections were detected in 7.7% of individuals tested by CF, as shown in the curve in Fig. 1. However, this appeared to be an underestimate of the actual activity of the virus. Serum specimens had been collected on a regular basis, 6 months apart; rises in titer by CF occurred most frequently in those pairs in which the second specimen was collected in April 1967, clearly indicating the peak period of viral dissemination. CF-

Table 3. Reported Frequency of Infection or Illness with 229E and OC43 in Four Locations

Study	Mean incidence of infection with	
	229E	OC43
Chicago medical students[21]	15/100/yr	—
Tecumseh, Mich.[16,49]	7.7/100/yr	17.1/100/yr
	Proportion of colds associated with	
	229E	OC43
Charlottesville, Va., employees[26]	1.7% of illnesses	2.4% of illnesses
Atlanta, Ga., children[34,35a]	4.3% of illnesses	3.3% of illnesses

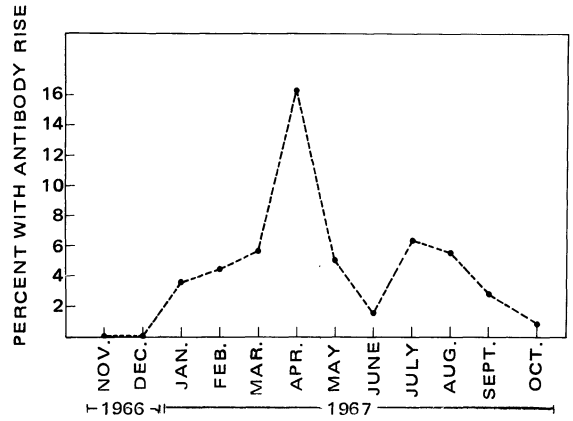

Fig. 1. Serological incidence by CF of infection with 229E virus in Tecumseh, Michigan, 1966–1967.

and the more sensitive N-test results were combined to give an overall infection rate for the population studied; this rate, 34%, was remarkably similar to the 35% observed in Chicago at the same time. Because of the limited period of viral activity, it was possible to compare illness rates of those infected with persons not infected matched by age and sex; it was estimated that 45% of the infections had produced clinical disease. Thus, the rate of 229E-associated illnesses during the outbreak was 15 per 100 persons studied. Activity in all age groups was apparent, including children under 25 years of age.[16]

In other investigations of 229E activity, attention has been directed mainly toward study of associated illnesses; in such studies, sera have been collected before and after the illness, rather than continually on a routine basis as done to determine infection rates. Employees at State Farm Insurance Company in Charlottesville, Virginia, were studied during an 8-year period for rises in titer for both 229E and OC43. By CF, 229E infection could be related to 3% of the colds that occurred in the winter–spring and to 0.4% of colds that occurred in the summer–fall. There was some year-to-year variation in activity, but differences in the number of specimens tested from various years did not permit complete identification of cyclical patterns.[26] Employees of the NIH with respiratory illness were studied by both isolation and serology for 229E infection over a 6-year period. Again, attention was specifically directed toward certain segments of the 6 years, and no specimens were tested during other segments. Of particular interest once more is the segment from December 1966 to April 1967. Isolation of rhinoviruses and myxoviruses was uncommon at this time, but respiratory illness continued to occur. During this period, 24% of those persons with colds studied had rises in titer for 229E. As part of the same investigation, paired blood specimens collected from infants and children admitted to the hospital with acute lower respiratory disease during the 1967 period of 229E activity were tested for rise in antibody against the virus, but none was found.[29,42] Healthy children institutionalized in Atlanta, Georgia, were studied from 1960 to 1968; antibody response to 229E was determined by the indirect hemagglutination test. The investigation involved collection of serum specimens related to illness and also routine collection of sera from some non-ill individuals. Frequency of infection showed marked variation from year to year. Overall, 4% of colds could be associated with 229E infection, with greatest association in autumn, winter, and spring.[35a]

Surveys of prevalence of 229E antibody have also been carried out to document past history of infection, often as parts of longitudinal studies. A general finding is that antibody is present in a significant portion of adults who, despite possessing this antibody, can go on to have reinfection and illness. Reports of antibody prevalence in adults in the United States have varied from 19 to 41%, depending on the type of test used to determine antibody and the time of collection of serum.[16,26,42] Children under 10 years of age exhibited lower mean anti-

body titers than older children or adults.[16,42] Individual sera from normal healthy adults collected serially in Britain from 1965 throught 1970 were tested by Bradburne and Somerset.[8] It is of interest that there was a buildup in sera positive by CF from approximately 17% in specimens collected in October–December 1966 to 62% in those collected in July–September 1967. This would suggest that the spring 1967 outbreak that occurred in several parts of the United States may have taken place in Britain as well.

5.1.2. Incidence and Prevalence of OC43 Virus. Populations employed to study infection and illness caused by OC43 virus have generally been the same ones employed to study the occurrence of 229E virus. Kaye *et al.*[34] used the group of institutionalized children in Atlanta, Georgia, in which to identify infection by means of their HI test. Infections with the agent were detected in all years of the study but with definite cyclical variation. Seasons most involved were the winter and spring. Overall, 3% of the illnesses recorded in the 7-year period could be associated with OC43 infection, with a high of 7% in 1960–1961. Interestingly, testing of the sera collected routinely from non-ill individuals indicated that an additional equal number of OC43 infections were occurring without the production of symptoms.[34] The Charlottesville study of adult employees was of both OC43 and 229E infections. Here, too, the emphasis was on illness, and it was found in all years studied that OC43 was associated with 5% of colds in the winter–spring and with no illnesses in the summer–fall. Again, there was cyclical variation from year to year in the number of rises in titer detected.[26]

The original isolations of OC38 and OC43 were made in December and January 1965–1966 as part of the study carried out among NIH employees with colds. Testing of sera collected from these employees indicated that during this period, up to 29% of the colds studied were accompanied by rise in titer for OC43. In the children hospitalized with lower respiratory disease, up to 10% of illnesses during this period were associated with such a titer rise. However, it was impossible to show that the relationship to disease was truly etiological. This finding was in contrast to that seen with 229E, in which no rises in titer were detected in such cases.[38,42]

In the Tecumseh study, occurrence of OC43 infection was determined in the community population over a 4-year period. CF and HI tests were used on all specimens, and N tests were used as an aid in evaluating these results in selected specimens. During the total period, OC43-related infection was detected in 17.1% of the 910 persons studied for 1 year. Most of the infections took place in the winter–spring months of 1965–1966, 1967–1968, and 1968–1969. The only winter–spring period without such activity was in 1966–1967, when the 229E outbreak had taken place. There was good agreement between the CF and HI tests for the 1965–1966 and the 1968–1969 periods, but not for 1967–1968. The N test was used to clarify the situation. It was found that most rises in titer for the periods in 1965–1966 or 1968–1969, whether they had occurred by CF or HI or both, were also accompanied by rises in N antibody. In 1967–1968, most CF rises in titer were not accompanied by rises in titer in the other test, nor was the reverse true; significant change in N antibody in this period was exceedingly rare. It was concluded that the outbreaks of infection in 1965–1966 and 1968–1969 were probably caused by agents closely related to OC43, while the 1967–1968 activity was due to one of the other OC viruses that share some antigens with OC43 but are more distantly related to it. The 1968–1969 outbreak of OC43 infection was nearly as widespread as the prior 229E outbreak, with 25.6% of the population studied showing evidence of infection. Of special note was the fact that children under 5 years of age had the highest infection rates.[49]

Surveys of antibody prevalence have been conducted in several settings using OC43 antigens. McIntosh *et al.*[42] found that children began to acquire antibody to this virus in the first year of life. By the third year of life, more than 50% had antibody present. Among adults, 69% could be demonstrated to have antibody; this indicates, in view of the high incidence of infection with the agents in all age groups, the frequency with which such infections must represent reinfection. The high prevalence of antibody has been confirmed in other studies.[26,34] In Britain, Bradburne and Somerset[8] followed prevalence of antibody for OC43 over time, as they also had done with 229E. Each year, the greatest prevalence of antibody was found in the winter–spring period. The single highest point in antibody prevalence was in January–March 1969, at the same time the OC43 outbreak was occurring in some parts of the United States.[8]

5.2. Geographic Distribution

Occurrence of coronavirus infection has been documented, by either isolation or serology, from coast to coast in the United States. In addition to the studies listed in Table 1, a 229E-like virus has been isolated in California, and OC43 and 229E have been demonstrated to be present in Vermont by serological methods.[51,53] Extensive studies have been carried out by the Common Cold Research Unit, which has demonstrated the presence of the agents in Britain. The activity of 229E virus has been documented in Brazil in a study of children and adults with and without respiratory illness. Significant rises in antibody titer accompanied nonhospitalized respiratory infection in the children. Prevalence of antibody was determined by CF, and like the situation in some studies in the North Temperate Zone, children had little antibody, while 26% of adults were antibody-positive.[15] These findings suggest that coronaviruses are worldwide in distribution and cause similar types of illness in different localities; such a situation has been noted with many other repiratory viruses.[47] An attempt was actually made to detect rises in antibody titer for 229E in paired sera collected from small children with lower respiratory infection in many tropical parts of the world. No evidence of infection was found, which is hardly surprising, since no rises in titer were found in similar sera collected as part of the same study in Washington, D.C.[17,29]

5.3. Temporal Distribution

Because most illnesses caused by coronaviruses are similar to those caused by other respiratory viruses, it is impossible to identify epidemic behavior of the viruses. There is, however, great variation in the frequency of infection on both a seasonal and a cyclical basis. Isolation and rises in antibody titer for all types of coronaviruses have been rare events outside the period from December through May. This is the portion of the year in which isolation rates for rhinoviruses and other respiratory viruses often reach their low. In addition, a cyclical pattern may be discerned when individual virus types are considered. In Fig. 2 are summarized data from five longitudinal studies of coronavirus activity carried out in different parts of the United States. In all

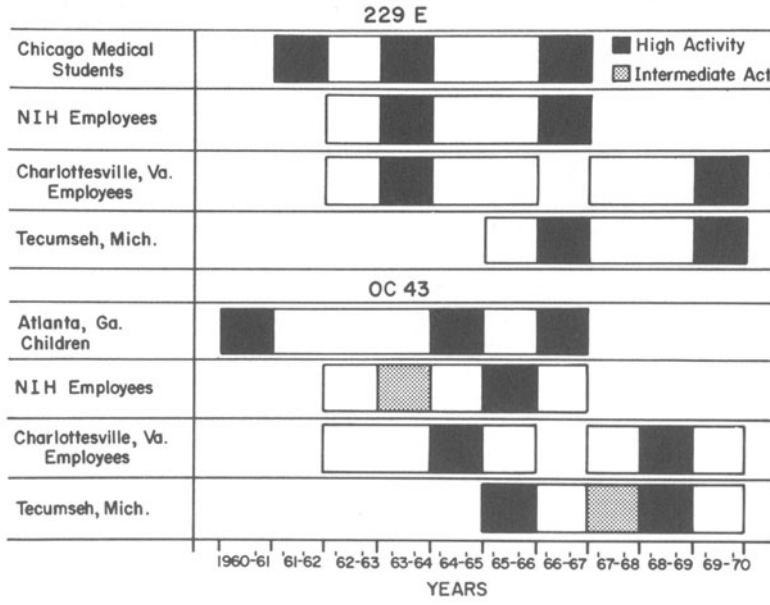

Fig. 2. Cyclic behavior of 229E and OC43 viruses observed in five longitudinal studies.

studies, some sporadic activity did occur in nearly all years studied, but rises in antibody titer were concentrated in certain years that far exceeded the means for the entire studies. Those periods are indicated as solid black boxes in the figure. The times during which specimens were collected in each investigation are indicated in the figure by the white boxes. Activity of 229E was detected in all four studies at the same time, even though two were in the Midwest and two in the eastern United States. It seems possible, on the basis of these data, to postulate a 2- to 3-year cycle for this agent. The greatest number of infections in Chicago was seen in 1967, after absence of the agent for 3 years, which would suggest a role of herd immunity in determining the time of reappearance of the agent.

With OC43, the situation is quite different. As with 229E, in no investigation did two years with high rates of infection or illness follow one another. A possible exception was in the Tecumseh study. However, the agent that caused the rises in titer in 1967–1968 did not appear as closely related serologically to OC43 as the agent involved in the other two outbreaks. This observation indicates a problem in identifying cycling of OC43. The virus undoubtedly shares more antigens with other identified or perhaps unidentified coronaviruses than does 229E (see Table 2), and these other viruses may well have cycles of their own that may confuse the situation. In 1964–1965, high activity occurred in Atlanta and Charlottesville. However, in Bethesda, just a short distance away, high activity was not seen in that year, but in 1965–1966, the same time as high activity occurred in Michigan, many miles away. In 1968–1969, Charlottesville and Tecumseh data did agree with very high activity in both areas. Thus, cycling of the agents was found in all studies, but the cycles did not agree on specific years. This may be a result of actual differences in patterns of occurrence or a result of differences in the serological techniques used to identify infection, which are of greater importance with OC43 because of the problem of cross-reactions. That cycling of coronaviruses does exist and occurs every 2–4 years with production of many infections suggests that the number of truly different coronaviruses may be relatively small. This situation is unlike that seen with the rhinoviruses, in which cycling has been more difficult to demonstrate, in part because of the large number of serotypes.[14]

5.4. Age

All age groups are involved in infection with OC43 virus. High rates have been noted in children and adults during studies separately examining both groups. In the Tecumseh study, a total population group was followed. During the 1968–1969 outbreak, infection rates were relatively uniform for all age groups, varying from a high of 29.2 per 100 person-years in the 0–4 age group to 22.2 in those over 40 years of age.[49] This finding is quite different from the situation that exists with other respiratory agents, such as respiratory syncytial virus, where a more distinct decrease in infection rates can be observed with increase in age.[48] The reversal of the pattern of age-specific infection rates customarily associated with the respiratory viruses becomes complete with 229E. Infection with this virus has been more difficult to demonstrate in small children than in adults. In Tecumseh, during the 1966–1967 outbreak, highest age-specific infection rates by CF were found among those 15–29 years of age, following a steady increase in infection frequency from the 0- to 4-year-olds. However, when neutralization tests were used to detect infection, the 15- to 19-year-olds still had high infection rates, but the serial increase to that point among younger age groups was much less steep.[16] This would suggest that the apparent sparing of small children with 229E may be an artifact resulting from the relative insensitivity of the young to the serological procedures commonly employed. It would be surprising if two different coronavirus serotypes behaved so differently.

5.5. Other Factors

There is little evidence of a sex differential in infections with the coronaviruses simply because the data have rarely been examined in such a manner. In Tecumseh, adult females experienced higher infection rates with OC43 than adult males, which is in conformity with the usual patterns of all respiratory illnesses.[46] In the study by Candeias *et al.*[15] of antibody prevalence, the results were examined by sex, but no significant differences could be observed. There are no data available data on occupational or racial susceptibility to infection or on the role of socioeconomic status in influencing rates. Occurrence of infection in closed or special populations, such as military recruits or residents of chil-

dren's institutions, has been reported.[34,36,60] However, it is at present difficult to determine, based on the relative paucity of information on the behavior of the virus in open populations, whether they exhibit any unique features in other settings. There is a suggestion that OC43 virus might cause acute respiratory disease in military recruits.[60] If this finding is confirmed, it would represent a distinct departure from the types of illness customarily associated with that virus in young civilian adults. The role of the school-age child in dissemination of coronavirus has not yet been clearly defined, but it would be surprising if these infections differed in their transmission pattern so markedly from that documented with the other agents. Because of the high frequency of infection in older children and adults, other sites of dissemination may also be of significance. It has been possible to show that the family unit is of importance in transmission, since clustering of 229E and OC43 infections in families was observed in the Tecumseh study.[16]

While nutritional and genetic factors have not been associated with susceptibility to coronavirus infections, there are clear indications that the viruses are associated with exacerbations of chronic obstructive respiratory disease. Such a finding is hardly surprising in view of the high infection rates that have been observed in unselected older adults. It has not yet been demonstrated whether this represents true increased susceptibility to infection or simply a more severe form of expression of the infection when it occurs in an already compromised host. In addition to the situation in older individuals, there is evidence that both OC43 and 229E may trigger acute attacks of wheezing in young asthmatics.[20a,40,53]

6. Mechanisms and Routes of Transmission

The coronaviruses are presumably transmitted by the respiratory route. It has been possible to induce infection experimentally in volunteers by inoculating virus into the nose.[7,59] No other route of transmission for coronaviruses seems involved in man, although animal coronaviruses are infectious by the fecal–oral route.[56] There is currently no direct evidence to aid in identifying the main mechanisms of transmission. However, it is possible to compare the epidemiological behavior of the coronaviruses with that of other respiratory agents the transmission mechanisms of which have been more directly studied. Large-scale outbreaks of coronavirus infections have taken place, as in Tecumseh in 1967.[16] This is much more analogous to the situation seen with influenza than to that with the rhinoviruses. It is likely that the former agent can be transmitted by aerosol in addition to large droplet, which would explain its ability to spread quickly.[19] Rhinoviruses, on the other hand, are thought to be transmitted by large droplet and may at times spread via fomites.[27] It is therefore probable that human coronaviruses can be spread by aerosol as well as by large droplet. Aerosol transmission of avian IBV has actually been documented in poultry.[20]

There is no evidence that any animal reservoir or vector is involved in the maintenance of infection or transmission of the human coronaviruses. Each animal coronavirus appears to be restricted to its own species. The only known exception is the finding of antibody of avian IBV in sera of poultry workers but not of controls.[45]

7. Pathogenesis and Immunity

The incubation period of coronavirus colds is relatively short. In studies involving volunteers, the mean period from inoculation of virus to development of symptoms was from 3.2 to 3.5 days depending on the strain, with a range of 2–4 days.[7,59] Following exposure, the virus apparently multiplies superficially in the respiratory tract in a manner similar to that in which multiplication occurs *in vitro*. Virus excretion usually reaches a detectable level at the time symptoms begin and lasts for 1–4 days. The duration of the illness is from 6 to 7 days on the average, but with some lasting up to 18 days. Serological response either to induced or to naturally acquired infection has been quite variable depending on the infecting strain and the serological test employed. For example, among those experimentally infected with OC38 or OC43 virus who had a cold produced, only 46% had rises in titer by HI and 23% by CF. Less than half those infected with 229E showed a CF rise. It is not clear how the existence of titer or preinfection antibody affects the magnitude of the response detected by these tests.

Rises in N antibody titer are easier to detect and have been found with sensitive techniques in all volunteers experimentally infected.[5,8]

An important characteristic of the coronaviruses is their apparent high rate of reinfection. In the Tecumseh study, 81.5% of those infected with OC43 actually possessed prior N antibody.[49] Possession of circulating OC43 HI antibody among the Atlanta children did not appear to play a role in modifying severity of a subsequent illness.[34] With 229E virus, Hamre and Beem[21] demonstrated that frequency of rises in titers detected by N was inversely proportional to preinfection levels of N antibody, which would indicate that this antibody exerted some protective effect. However, the importance of this N antibody could not be confirmed when infection was detected by CF. Thus, circulating N antibody as measured at present may bear a relationship to modification of infection, but this association is not a very strong one. Since coronavirus infections involve mainly the surface of the respiratory tract, it is likely that secretory IgA antibody plays a more direct role in protection; this has in fact been demonstrated with a swine coronavirus.[4]

8. Pattern of Host Response

The coronaviruses generally produce a coldlike illness that on an individual basis is difficult to distinguish from illness caused by other respiratory viruses. In both induced and natural infections, the most prominent findings have been coryza and nasal discharge, with the discharge being more profuse than that customarily seen with rhinovirus colds.[7] Sore throat has been somewhat less common and in children has been associated with pharyngeal injection.[34] Experimental colds caused by B814 virus were about as severe as those caused by 229E; however, natural OC43 infections caused illnesses with considerably more cough and sore throat than did 229E infections.[26] The mean duration of coronavirus colds, at 6.5 days, is shorter than that seen in rhinovirus colds, at 9.5 days.[7]

There is no clear evidence yet available that coronaviruses cause severe lower respiratory illness in infants and young children. In fact, such infections were more common in one study among the control group than among the diseased.[42] Mufson *et al.*[50] have associated coronavirus 229E and OC43 infection with acute lower respiratory infections in children at Cook County Hospital. The lack of a comparable control group makes assignment of an etiological role to these viruses hazardous at present, but the relationship should be sought in the future. The association of OC43 with the acute respiratory disease (ARD) syndrome in military recruits should also be viewed as tentative.

Clinical disease occurred in no more than 45% of those infected with 229E in Tecumseh during the 1967 outbreak.[16] In Atlanta children, OC43 virus produced illness in about 50% of those infected.[34] It is likely that with increase in age and concomitant experience with these agents, the ratio of clinically apparent to inapparent infection will decrease. As with other respiratory agents, a continuum of severity of symptoms exists among those in whom infection results in disease, and this may also be related to past experience with the viruses.

Coronaviruslike particles have been identified in stools of persons with diarrhea, and therefore a role in etiology of acute enteric disease has been suggested. This would not be surprising in view of the clear involvement of certain strains in severe diarrheal disease of domestic animals.[15a] However, the association with human disease has not been observed in a number of other studies. Coronaviruslike particles have also been observed in renal biopsies from cases of endemic (Balkan) nephropathy. A slow coronavirus infection acquired from pigs has been suggested as being involved.[1a]

9. Control and Prevention

It is premature at present to think in terms of control of coronavirus infection. Not all viral types have been identified, and some known agents cannot be easily propagated in the laboratory. Thus, preparation of vaccines of the conventional types is impossible. The frequency of reinfection observed with these agents is so high that control by vaccination may not be practical, but it is possible that further studies may allow identification of truly protective antibodies. There remains environmental control of infection; such efforts have been useful only rarely for other respiratory agents and they are not likely to be more efficacious for the coronaviruses.[43]

10. Unresolved Problems

The major immediate need in coronavirus research lies in the laboratory. If a practical system can be found for isolation and propagation of the viruses, the gaps in understanding the behavior of these agents would quickly be filled. Only serological tools are available now for most epidemiological studies, and even these can be applied to only two different coronavirus types. Therefore, many of the data that have been so laboriously gained give only partial evidence on the total dimensions of the problem—and the problem is almost certainly a very large one. Coronaviruses have been isolated and outbreaks identified in periods of the winter and spring when rhinoviruses and myxoviruses are uncommon. It appears that during these times, the coronaviruses cause a significant portion of respiratory illnesses. Even discounting suggestions of production of severe disease in young children and those with chronic respiratory disease, the viruses are important pathogens simply in terms of numbers of illnesses produced. Only through further understanding of the behavior of these agents will it be possible to determine the means by which control can be attempted.

11. References

1. Almeida, J. D., and Tyrrell, D. A. J., The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture, *J. Gen. Virol.* **1**:175–178 (1967).

1a. Apostolov, K., and Spasic, P., Evidence of a viral aetiology in endemic (Balkan) nephropathy, *Lancet* **2**:1271–1273 (1975).

2. Becker, W. B., McIntosh, K., Dees, J. H., and Chanock, R. M., Morphogenesis of avian infectious bronchitis virus and a related human virus (strain 229E), *J. Virol.* **1**:1019–1027 (1967).

3. Berry, D. M., Cruickshank, J. G., Chu, H. P., and Wells, R. J. H., The structure of infectious bronchitis virus, *Virology* **23**:403–407 (1964).

4. Bohl, E. H., Gupta, R. K. P., Olquin, M. V. F., and Saif, L. J., Antibody responses in serum, colostrum, and milk of swine after infection or vaccination with transmissible gastroenteritis virus, *Infect. Immun.* **6**:289–301 (1972).

5. Bradburne, A. F., Antigenic relationships amongst coronaviruses, *Arch. Gesamte Virusforsch.* **31**:352–364 (1970).

6. Bradburne, A. F., An investigation of the replication of coronaviruses in suspension cultures of L132 cells, *Arch. Gesamte Virusforsch.* **37**:297–307 (1972).

7. Bradburne, A. F., Bynoe, M. L., and Tyrrell, D. A. J., Effects of a "new" human respiratory virus in volunteers, *Br. Med. J.* **3**:767–769 (1967).

8. Bradburne, A. F., and Somerset, B. A., Coronavirus antibody titres in sera of healthy adults and experimentally infected volunteers, *J. Hyg.* **70**:235–244 (1972).

9. Bradburne, A. F., and Tyrrell, D. A. J., The propagation of "coronaviruses" in tissue culture, *Arch. Gesamte Virusforsch.* **28**:133–150 (1969).

10. Bradburne, A. F., and Tyrrell, D. A. J., Coronaviruses of man, *Prog. Med. Virol.* **13**:373–403 (1971).

11. Bruckova, M., McIntosh, K., Kapikian, A. Z., and Chanock, R. M., The adaptation of two coronavirus strains (OC38 and OC43) to growth in cell monolayers, *Proc. Soc. Exp. Biol. Med.* **135**:431–435 (1970).

12. Bucknall, R. A., Kalica, A. R., and Chanock, R. M., Intracellular development and mechanism of hemadsorption of a human coronavirus, OC43, *Proc. Soc. Exp. Biol. Med.* **139**:811–817 (1972).

13. Bucknall, R. A., King, L. M., Kapikian, A. Z., and Chancok, R. M., Studies with human coronaviruses. II. Some properties of strains 229E and OC43, *Proc. Soc. Exp. Biol. Med.* **139**:722–727 (1972).

14. Calhoun, A. M., Jordan, W. S., Jr., and Gwaltney, J. M., Jr., Rhinovirus infections in an industrial population. V. Change in distribution of serotypes, *Am. J. Epidemiol.* **99**:58–64 (1974).

15. Candeias, J. A. N., Carvalho, R. P. de S., and Antonacio, F., Seroepidemiologic study of coronavirus infection in Brazilian children and civilian adults, *Rev. Inst. Med. Trop.* **14**:121–125 (1972).

15a. Caul, E. O., Pavel, W. K., and Clarke, S. K. R., Coronavirus particles in faece from patients with gastroenteritis, *Lancet* **1**:1192 (1975).

16. Cavallaro, J. J., and Monto, A. S., Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan, *J. Infect. Dis.* **122**:272–279 (1970).

17. Chanock, R., Chambon, L., Chang, W., Goncalves Ferreira, F., Gharpure, P., Grant, L., Hatem, J., Imam, I., Kalra, S., Lim, K., Madalengoitia, J., Spence, L., Teng, P., and Ferreira, W., WHO respiratory disease survey in children: A serological study, *Bull. WHO* **37**:363–369 (1967).

18. Coronaviruses, *Nature (London)* **220**:650 (1968).

19. Couch, R. B., Douglas, R. G., Jr., Lindgren, K. M., Gerone, P. J., and Knight, V., Airborne tranmission of respiratory infection with Coxsackievirus A type 21, *Am. J. Epidemiol.* **91**:78–86 (1970).

20. Geilhausen, H. E., Ligon, F. B., and Lukert, P. D., The pathogenesis of virulent and avirulent avian in-

fectious bronchitis virus, *Arch. Gesamte Virusforsch.* **40**:285–290 (1973).

20a. Gump, D. W., Phillips, C. A., Forsyth, B. R., McIntosh, K., Lamborn, K. R., and Stouch, W. H., Role of infection in chronic bronchitis, *Am. Rev. Respir. Dis.* **113**:465–474 (1976).

21. Hamre, D., and Beem, M., Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance, *Am. J. Epidemiol.* **96**:94–106 (1972).

22. Hamre, D., Kindig, D. A., and Mann, J., Growth and intracellular development of a new respiratory virus, *J. Virol.* **1**:810–816 (1967).

23. Hamre, D., and Procknow, J. J., A new virus isolated from the human respiratory tract, *Proc. Soc. Exp. Biol. Med.* **121**:190–193 (1966).

24. Harnett, G. B., and Hooper, W. L., Test-tube organ cultures of ciliated epithelium for the isolation of respiratory viruses, *Lancet* **1**:339–340 (1968).

25. Hartley, J. W., Rowe, W. P., Bloom, H. H., and Turner, H. C., Antibodies to mouse hepatitis viruses in human sera, *Proc. Soc. Exp. Biol. Med.* **115**:414–418 (1964).

26. Hendley, J. O., Fishburne, H. B., and Gwaltney, J. M., Jr., Coronavirus infections in working adults, *Am. Rev. Respir. Dis.* **105**:805–811 (1972).

27. Hendley, J. O., Wenzel, R. P., and Gwaltney, J. M., Jr., Transmission of rhinovirus colds by self-inoculation, *N. Eng. J. Med.* **288**:1361–1364 (1973).

28. Hierholzer, J. C., Palmer, E. L., Whitfield, S. G., Kaye, H. S., and Dowdle, W. R., Protein composition of coronavirus OC43, *Virology* **48**:516–527 (1972).

28a. Hierholzer, J. C., and Tannock, G. A., Quantitation of antibody to non-hemagglutinating viruses by single radial hemolysis: Serological test for human coronaviruses, *J. Clin. Microbiol.* **5**:613–620 (1977).

29. Kapikian, A. Z., James, H. D., Jr., Kelly, S. J., Dees, J. H., Turner, H. C., McIntosh, K., Kim, H. W., Parrott, R. H., Vincent, M. M., and Chanock, R. M., Isolation from man of "avian infectious bronchitis virus-like" viruses (coronaviruses) similar to 229E virus, with some epidemiological observations, *J. Infect. Dis.* **119**:282–290 (1969).

30. Kapikian, A. Z., James, H. D., Jr., Kelly, S. J., King, L. M., Vaughn, A. L., and Chanock, R. M., Hemadsorption by coronavirus strain OC43, *Proc. Soc. Exp. Biol. Med.* **139**:179–186 (1972).

31. Kapikian, A. Z., James, H. D., Jr., Kelly, S. J., and Vaughn, A. L., Detection of coronavirus strain 692 by immune electron microscopy, *Infect. Immun.* **7**:111–116 (1973).

32. Kaye, H. S., and Dowdle, W. R., Some characteristics of hemagglutination of certain strains of "IBV-like" viruses, *J. Infect. Dis.* **120**:576–581 (1969).

33. Kaye, H. S., Hierholzer, J. C., and Dowdle, W. R., Purification and further characterization of an "IBV-like" virus (coronavirus), *Proc. Soc. Exp. Biol. Med.* **135**:457–463 (1970).

34. Kaye, H. S., Marsh, H. B., and Dowdle, W. R., Seroepidemiologic survey of coronavirus (strain OC43) related infections in a children's population, *Am. J. Epidemiol.* **94**:43–49 (1971).

35. Kaye, H. S., Ong, S. B., and Dowdle, W. R., Detection of coronavirus 229E antibody by indirect hemagglutination, *Appl. Microbiol.* **24**:703–707 (1972).

35a. Kaye, H. S., and Dowdle, W. R., Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children, *Am. J. Epidemiol.* **101**:238–244 (1975).

35b. Kaye, H. S., Yarbrough, W. B., and Reed, C. J., Calf diarrhoea coronavirus, *Lancet* **2**:509 (1975).

35c. Kaye, H. S., Yarbrough, W. B., Reed, C. J., and Harrison, A. K., Antigenic relationship between human coronavirus strain OC43 and hemagglutinating encephalomyelitis virus strain 67N of swine: Antibody responses in human and animal sera, *J. Infect. Dis.* **135**:201–209 (1977).

36. Kendall, E. J., Bynoe, M. L., and Tyrrell, D. A. J., Virus isolation from common colds occurring in a residential school, *Br. Med. J.* **2**:82–86 (1962).

36a. Kennedy, D. A., and Johnson-Lussenburg, C. M., Isolation and morphology of the internal component of human coronavirus, strain 229E, *Intervirology* **6**:197–206 (1975/1976).

36b. Lomniczi, B., and Kennedy, I., Genome of infectious bronchitis virus, *J. Virol.* **24**:99–107 (1977).

37. McIntosh, K., Becker, W. B., and Chanock, R. M., Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease, *Proc. Natl. Acad. Sci. U.S.A.* **58**:2268–2273 (1967).

38. McIntosh, K., Bruckova, M., Kapikian, A. Z., Chanock, R. M., and Turner, H., Studies of new virus isolates recovered in tracheal organ culture, *Ann. N.Y. Acad. Sci.* **174**:983–989 (1970).

39. McIntosh, K., Dees, J. H., Becker, W. B., Kapikian, A. Z., and Chanock, R. M., Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease, *Proc. Natl. Acad. Sci. U.S.A.* **57**:933–940 (1967).

40. McIntosh, K., Ellis, E. F., Hoffman, L. S., Lybass, T. G., Eller, J. J., and Fulginiti, V. A., The association of virual and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children, *J. Pediatr.* **82**:578–590 (1973).

41. McIntosh, K., Kapikian, A. Z., Hardison, K. A., Hartley, J. W., and Chanock, R. M., Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses, *J. Immunol.* **102**:1109–1118 (1969).

42. McIntosh, K., Kapikian, A. Z., Turner, H. C., Har-

TLEY, J. W., PARROTT, R. H., AND CHANOCK, R. M., Seroepidemiologic studies of coronavirus infection in adults and children, *Am. J. Epidemiol.* **91**:585–592 (1970).

43. MCLEAN, R. L., General discussion, International Conference on Asian Influenza, *Am. Rev. Respir. Dis.* **83**(Part 2):36–38 (1961).

43a. MCNAUGHTON, M. R., AND MADGE, M. H., The genome of human coronavirus strain 229E, *J. Gen. Virol.* **39**:497–504 (1978).

44. MEBUS, C. A., STAIR, E. L., RHODES, M. B., AND TWIEHAUS, M. J., Pathology of neonatal calf diarrhea induced by coronavirus-like agent, *Vet. Pathol.* **10**:45–64 (1973).

45. MILLER, L. T., AND YATES, V. J., Neutralization of infectious bronchitis virus by human sera, *Am. J. Epidemiol.* **88**:406–409 (1968).

46. MONTO, A. S., HIGGINS, M. W., AND ROSS, H. W., The Tecumseh study of respiratory illness. VIII. Acute infection in chronic respiratory disease and comparison groups, *Am. Rev. Respir. Dis.* **111**:27–36 (1975).

47. MONTO, A. S., AND JOHNSON, K. M., Respiratory infections in the American tropics, *Am. J. Trop. Med. Hyg.* **17**:867–874 (1968).

48. MONTO, A. S., AND LIM, S. K., The Tecumseh study of respiratory illness. III. Incidence and periodicity of respiratory syncytial and *Mycoplasma pneumoniae* infections, *Am. J. Epidemiol.* **94**:290–301 (1971).

49. MONTO, A. S., AND LIM, S. K., The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection, *J. Infect. Dis.* **129**:271–276 (1974).

49a. MONTO, A. S., AND RHODES, L. M., Detection of coronavirus infection of man by immunofluorescence, *Proc. Soc. Exp. Biol. Med.* **155**:143–148 (1977).

50. MUFSON, M. A., MCINTOSH, K., CHAO, R. K., KRAUSE, H. E., WASIL, R., AND MOCEGA, H. E., Epidemiology of coronavirus infections in infants with acute lower respiratory disease, *Clin. Res.* **20**:534 (1972).

51. OSHIRO, L. S., SCHIEBLE, J. H., AND LENNETTE, E. H., Electron microscopic studies of coronavirus, *J. Gen. Virol.* **12**:161–168 (1971).

52. PELON, W., Classification of the "2060" viruses ECHO28 and further study of its properties, *Am. J. Hyg.* **73**:36–54 (1961).

53. PHILLIPS, C. A., MCINTOSH, K., FORSYTH, B. R., GUMP, D. W., AND STOUCH, W. H., Coronavirus infections in exacerbations of chronic bronchitis, in: *Twelfth Interscience Conference on Antimicrobial Agents and Chemotherapy*, Atlantic City, New Jersey, Abstr. No. 6 (1972).

54. PURCELL, D. A., AND CLARKE, J. K., The replication of infectious bronchitis virus in fowl trachea, *Arch. Gesamte Virusforsch.* **39**:248–256 (1972).

55. SAIF, L. J., BOHL, E. H., AND GUPTA, R. K. P., Isolation of porcine immunoglobulins and determination of the immunoglobulin classes of transmissible gastroenteritis viral antibodies, *Infect. Immun.* **6**:600–609 (1972).

56. STAIR, E. L., RHODES, M. B., WHITE, R. G., AND MEBUS, C. A., Neonatal calf diarrhea: Purification and electron microscopy of a coronavirus-like agent, *Am. J. Vet. Res.* **33**:1147–1156 (1972).

56a. TANNOCK, G. A., AND HIERHOLZER, J. C., Presence of genomic polyadenylate and absence of detectable virion transcriptase in human coronavirus OC-43, *J. Gen. Virol.* **39**:29–39 (1978).

57. TYRRELL, D. A. J., AND ALMEIDA, J. D., Direct electron microscopy of organ cultures for the detection and characterization of viruses, *Arch. Gesamte Virusforsch.* **22**:417–421 (1967).

58. TYRRELL, D. A. J., AND BYNOE, M. L., Cultivation of a novel type of common-cold virus in organ cultures, *Br. Med. J.* **1**:1467–1470 (1965).

59. TYRRELL, D. A. J., BYNOE, M. L., AND HOORN, B., Cultivation of "difficult" viruses from patients with common colds, *Br. Med. J.* **1**:606–610 (1968).

60. WENZEL, R. P., HENDLEY, J. O., DAVIES, J. A., AND GWALTNEY, J. M., JR., Coronavirus infections in military recruits: Three-year study with coronavirus strains OC43 and 229E, *Am. Rev. Respir. Dis.* **109**:621–624 (1974).

12. Suggested Reading

BRADBURNE, A. F., AND TYRRELL, D. A. J., Coronaviruses of man, *Prog. Med. Virol.* **13**:373–403 (1971).

HAMRE, D., AND BEEM, M., Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance, *Am. J. Epidemiol.* **96**:94–106 (1972).

KAYE, H. S., MARSH, H. B., AND DOWDLE, W. R., Seroepidemiologic survey of coronavirus (strain OC43) related infections in a children's population, *Am. J. Epidemiol.* **94**:43–49 (1971).

MCINTOSH, K., KAPIKIAN, A. Z., TURNER, H. C., HARTLEY, J. W., PARROTT, R. H., AND CHANOCK, R. M., Seroepidemiologic studies of coronavirus infection in adults and children, *Am. J. Epidemiol.* **91**:585–592 (1970).

MONTO, A. S., AND LIM, S. K., The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection, *J. Infect. Dis.* **129**:271–276 (1974).