CHAPTER 8

Coronaviruses

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1. Introduction

Coronaviridae are a monogeneric family of RNA-containing agents that have been associated etiologically with respiratory illnesses in man and with a number of other diseases in laboratory and domestic animals. They also have been associated with diarrheal disease and other conditions in humans, although for differing reasons these relationships cannot be considered etiologic. The name for the family was adopted to describe the characteristic fringe of crownlike projections seen around the viruses by electron microscopy; these spikes are petal shaped rather than sharp or pointed as is the case with the myxoviruses. Like the myxoviruses, the coronaviruses contain essential lipids and are 80–160 nm in diameter.[28] Unlike them, the coronaviruses are positive-stranded.[66,79] Whereas the animal strains are readily isolated in several different systems, recovery of the human strains has posed major problems, partially related to species specificity.[120] 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 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 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.[60] 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 a 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.[116]

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 WI-38 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 fourfold rise in neutralization antibody titer against 229E.[37]

It became clear that these "novel" viruses were 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.[74] The B814 and 229E strains were soon also

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A. S. Evans et al. (eds.), *Viral Infections of Humans*

demonstrated to have a similar structure on electron microscopy and to develop in infected cells by budding into cytoplasmic vesicles.[1,4,36] 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.[6] The name *coronavirus* was adopted for the group to describe the fringe of projections seen around them on electron microscopy.[28]

Except for 229E, none of the human coronaviruses had been successfully propagated in a system other than organ culture. McIntosh *et al.*[72] 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. The 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[53] 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 epidemiologic studies, since it was simple and reproducible.

Subsequent developments included adaptation of OC38 and OC43 to growth in cell monolayers; either mouse brain or organ culture material could be used as sources of virus.[14] Not only was CPE available for reading of neutralization tests, but also the OC38 and OC43 viruses were found to hemadsorb red cells of rats and mice, making available a more precise means of evaluating endpoints in tests involving these organ-culture-derived strains.[51] The other OC strains that could not be adapted to mouse brain resisted adaptation to cell culture. Finally, immune electron microscopy was added to the methods available for identifying the presence of coronaviruses. 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.[52] More recent studies have begun to explain the species specificity of the viruses in terms of cellular receptors.[120]

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 respiratory infection. This situation may be a reflection of the limited number of investigations carried out and the difficulty in isolating the virus. It is known that these agents frequently infect small children and reinfect adults, including persons with chronic respiratory disease.[96] 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. The original 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 were 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.[35,37] Employee groups were the source of specimens in the NIH[50,77] and in the studies at Charlottesville, Virginia.[41] Infection was also evaluated in children's homes[56] and boarding schools,[60] among military recruits,[119] and among children hospitalized for severe respiratory illnesses in various parts of the world.[50] Serological methods were used to detect occurrence in persons with

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

Location	Population	Virus studied
Chicago, IL[35]	Medical students	229E
Washington, DC[50,77]	Hospitalized children	229E, OC43
Bethesda, MD[50,77]	Adult employees	229E, OC viruses
Atlanta, GA[54,56]	Institutionalized children	229E, OC43
Charlottesville, VA[41]	Working adults	229E, OC43
Tecumseh, MI[23,86]	General community	229E, OC43
Brazil[21]	Nonhospitalized children	229E
Denver, CO[75]	Hospitalized asthmatic children	229E, OC43
N. and S. Carolina[119]	Military	229E, OC43

acute exacerbations of asthma[75] or chronic obstructive respiratory disease.[96] Patterns of coronavirus infection were identified among the general population residing in the Tecumseh, Michigan, community as part of a longitudinal study of respiratory illness.[23,86] 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.[10,11]

3.3. Serological Surveys

Relatively simple serological techniques are available for two coronaviruses (229E and OC38 or OC43), and surveys of antibody prevalence have been carried out in various parts of the world. Many surveys formed a part of studies directed mainly toward determination of incidence of infection. Information on the prevalence of antibody is available for populations in the United States,[23,41,77] Britain,[11] Brazil,[21] and other parts of the world. 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.[39] 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.[58,59] 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.[82] The 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 (WI-38), in which it has been maintained.[37] However, this cell line is not a reliable system for primary isolation of 229E-like agents. Human embryonic intestine (MA177) has proven a suitable cell system, but it is available only in limited quantities.[50] Human coronaviruses not related to 229E were originally isolated in organ cultures of human trachea or lung.[38,74,116,117] The presence of virus was usually detected by electron microscopy, or sometimes by fluorescent antibody (FA) staining of impression smears.[115] 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.[14,51,72] 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.[9,12] This last finding has not been confirmed by other workers.[14] Similarly, MRC-C cells have been used for 229E-like viruses and human rhabdomyosarcoma cells for propagating 229E and OC43.[99,105]

Since it was conceivable that special conditions of cell cultures were required for primary isolation of these agents, various additives to media were examined[87]; this would be similar to the strict requirements for propagation of the rhinoviruses before the availability of WI-38 cells.[95] The situation is in sharp contrast to that found with the cornoaviruses of animals. Although they are species-specific in their *in vitro* growth characteristics, especially on primary isolation, such isolation is easily accomplished.[80,98,102,111]

Recently a technique for direct detection of the 229E virus by nucleic acid hybridization has been described.[91] The method involves creating a cDNA copy of the nucleocapsid gene. It could be predicted from the sequence data that significant cross-hybridization would not occur with OC43 and that the probe would be able to detect 50 pg of viral RNA. When tested on specimens collected from human volunteers artificially infected with 229E, the probe was shown to be as sensitive but no more sensitive than cell culture.[92] Ease of use may be the major advantage at the moment.

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 cultures.[74] 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. WI-38 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.[8,12,14] Hemadsorption rather than CPE can be used for identification of endpoints with the BS-C-1 cell line.[15,16]

Most seroepidemiologic 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.[37] By this method, the CF test detected antibody in low titer and for only 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.[21] 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.[8] 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.[57]

It was found that CF tests can be satisfactorily performed with OC43 virus using infected suckling mouse brain as antigen.[77] 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.[53] 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 cations to attach virus to glutaraldehyde-treated red cells.[43]

The enzyme-linked immunosorbent assay (ELISA) for antibody has been adapted for use with coronavirus antigens.[62,103] A number of methods have been used to recognize a significant change in antibody titer between specimens. The test has been performed with both 229E and OC43 viruses as antigens. When sera from individuals infected artificially with other coronaviruses, including organ culture viruses, are tested against these antigens, they are found to cross-react with one or the other but not both. Thus, it is possible that the ELISA test with 229E and OC43 antigens may be able to detect infection with most, if not all, human coronaviruses.[68] Other serological tests have been developed that have been used more in antigenic analyses of the different coronaviruses than in epidemiologic studies. With the indirect FA technique, characteristic cytoplasmic inclusions were demonstrated with 229E, OC43, and even the other coronaviruses grown in organ culture.[69,88] The last were prepared for testing by making smears of fragments of the infected trachea.[76] An example of granular cytoplasmic fluorescence exhibited by OC43 in LLC-MK2 infected cells is shown in Fig. 1. 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[8] in tests with hyperimmune animal or human serum, but others have identified only one such line.[55]

4. Biological Characteristics of the Virus

A growing body of information is available on the relationship of coronavirus structure to patterns of antigenicity and infectivity. Much of the work has been done on the animal strains, principally because of their role in producing a number of economically important diseases, although some investigations have involved 229E and OC43. The viruses contain a single continuous strand of RNA that is about 30 kilobases in length.[108] The nonsegmented genome is of positive polarity.[61,66,79,114]

There are three major proteins. The nucleocapsid protein, N, is enclosed within the viral envelope with the RNA in a helical nucleocapsid. The other two, both glycoproteins, are the membrane glycoprotein, M, and the large spike glycoprotein, S. The latter protein, which forms the distinctive projections of the virus, has been associated with N, HA, and CF tests.[106] Antibodies elicited by it are thought to be associated with protection.[113] OC43 along with some animal viruses also contain a third glycoprotein, the hemagglutinin esterase (HE), which forms smaller spikes on the viral envelope.[46] No neuraminidase has ever been detected and there have been reports of phylogenetic relationships of the HE gene of OC43 to influenza type C.[36,55,121] The lipids associated with the envelope have been well defined.[97]

Recent studies on the cellular receptors involved with coronavirus infection help in understanding the phenomenon of species specificity. The receptor for the mouse hepatitis viruses, MHV-A29, has been identified and cloned. It is a member of the carcinoembryonic family. Nonmurine cells, which had been resistant to infection, when transfected with the receptor became susceptible.[31] Antibody to the receptor also prevented infection.[27] More recently, a receptor for 229E has been identified to be human aminopeptidase N, a cell surface metalloprotease present on certain epithelial cells. This receptor is specific for 229E and not for OC43.[120]

The total number of serological types that infect man has not been defined. Here again, the problem revolves

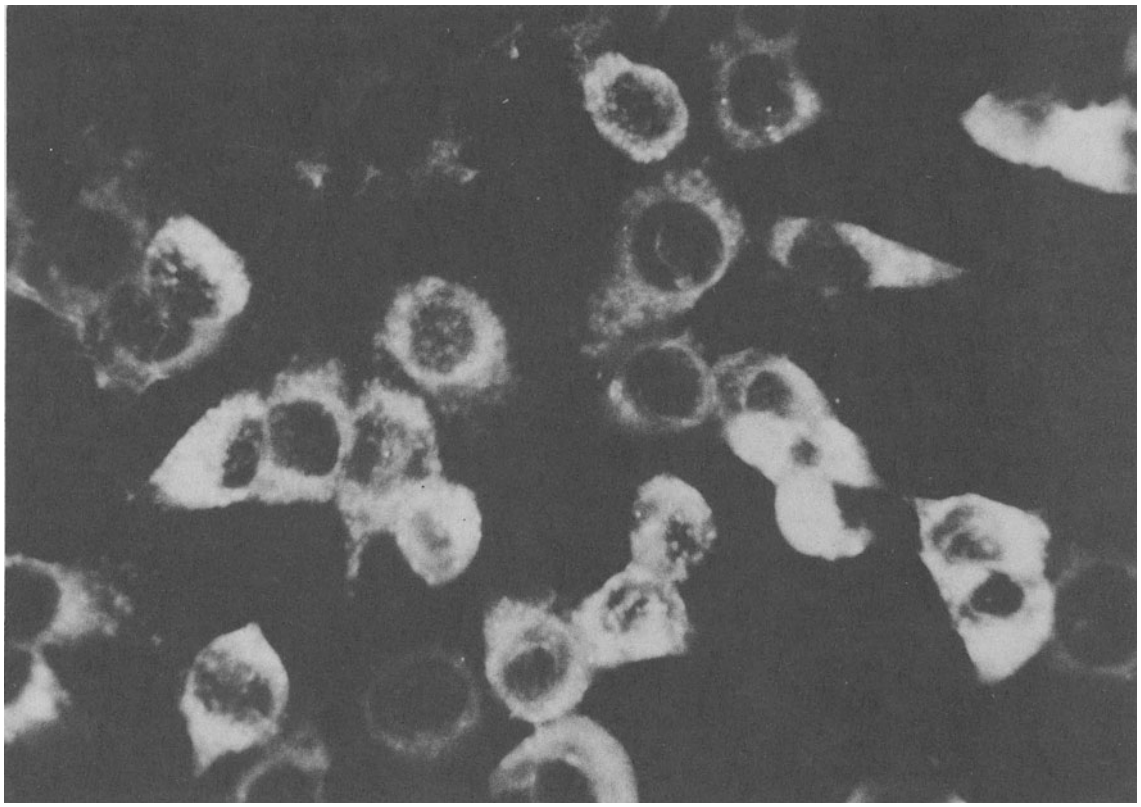

Figure 1. Cytoplasmic fluorescence produced using the indirect fluorescent antibody procedure in LLC-MK2 cells infected with OC43 virus.

around the difficulties encountered is 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 identities of types that grow only in organ culture as compared with those that grow in cell culture. Unfortunately, the situation does not seem to be improving; although there are newer organ culture isolates, the older ones have not been propagated for many years and may actually be lost. The N, CF, HI, gel-diffusion, and immunofluorescent techniques have been used in the antigenic analyses of the older strains by McIntosh *et al.*,[76] by Bradburne,[8] and by Bradburne and Somerset.[11] 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 original groups of human coronavirus isolates 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

Table 2. Serological Relationships of the Human Coronaviruses

Group	Strains tested with animal antisera		Strains tested with human antisera
I	229E	Closely related	
	229E-like isolates	but not	
	LP, Linder	identical	
	EVS (probably)		
II	OC38	Nearly identical	OC44
	OC43		
	MHV		
Others	B814		OC16
			OC37
			OC48

from individuals naturally infected or from volunteers challenged artificially. Such sera would be expected to be considerably less specific than animal antisera.

There are many additional 229E-related isolates, some originally recovered in cell culture and others in organ culture.[99] The 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 three other viruses are listed together by exclusion, i.e., not because of any demonstrated relationship to one another but rather because they are less closely related to viruses in the first two groups. Some low-level reactions with the agents in these two groups have been shown to be present, with OC16 virus being the most distinctly different strain.

As indicated above, much of the information on the behavior of 229E and OC43 viruses in populations 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 to help in interpreting results. 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.[13] Of greater practical relevance is the occurrence of cross-reactions between OC43 and the other organ culture viruses. It was postulated that rises in titer detected when using OC43 antigen in seroepidemiologic studies might result either from OC43 infection itself or from infection with one of these related viruses.[86] Indirect evidence that the infecting agent might not be OC43 itself was the dissociation seen between the CF and HI test 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.[86]

Recent work involving the ELISA test has expanded the above observations. When 229E or OC43 was used as antigen, sera from individuals infected with a wide variety of coronaviruses showed a rise in titer to one or the other virus but not to both.[68] Sera were mainly obtained from studies involving artificial challenge of volunteers, and all the infecting viruses were found by ELISA to fall into either group I or II, as shown in Table 2. Of particular interest is the fact that new organ culture isolates, which could not be easily adapted to cell culture, all had produced a rise in antibody titer to OC43. This confirms the earlier observation that rises in titer to OC43 by CF and HI might be produced by distinct but related strains. A similar situation exists with 229E-like strains; a number of additional isolates have been identified that are antigenically distinct, and the differences among these related strains may have implications for cross-protection from reinfection.[99] Although the ELISA results suggest that all coronaviruses fall into groups I and II, it is still possible that viruses such as OC16 may prove to be unrelated, since the classification of it and other older viruses cannot now be reevaluated. Work at the molecular level is also helping to determine the relationships among the entire coronavirus family.[45,64]

Data that demonstrate the etiologic role of coronaviruses in respiratory infections are derived 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.[10,11,99] 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.[23] Similarly, 229E infection among Chicago medical students was statistically associated with illness when those with rises in titer were used as their own controls.[35]

5. Descriptive Epidemiology

5.1. Incidence and Prevalence

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

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

Study	Mean incidence of infection with 229E	Mean incidence of infection with OC43
Chicago medical students[35]	15/100/yr	—
Tecumseh, MI[23,86]	7.7/100/yr	17.1/100/yr
	Proportion of illnesses associated with 229E	Proportion of illnesses associated with OC43
Charlottesville, VA, employees[36]	1.7% of illnesses	2.4% of illnesses
Atlanta, GA, children[54,56]	4.3% of illnesses	3.3% of illnesses

number of seasons of high viral activity included in a particular study. Table 3 presents a summary of results obtained in four such studies.

Another approach toward developing a minimal estimate of the total role of coronaviruses in respiratory illnesses comes from a study involving exhaustive laboratory examination, including organ culture, of specimens from 38 common colds. Coronaviruses were isolated from 18.4% of the specimens, but an additional 13%, which were negative in the laboratory, produced colds when given to volunteers.[65] Based on these results, which came from a limited age group, it has been estimated that coronaviruses are responsible for at least 14% of all respiratory illnesses in a general population.[88]

5.1.1. Incidence and Prevalence of 229E Virus. Frequency of 229E illness and infection has been determined in several large-scale investigations. 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 twofold 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.[35]

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. 2. 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. Both CF 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.[23]

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 a 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

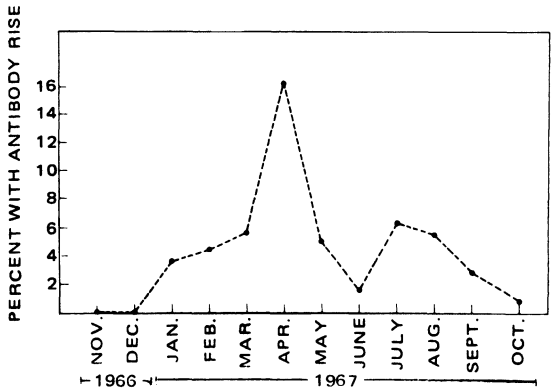

Figure 2. Serological incidence by CF of infection with 229E virus in Tecumseh, Michigan, 1966–1967.

differences in the number of specimens tested from various years did not permit complete identification of cyclical patterns.[41] 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.[50,77] 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 nonill 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.[54]

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.[25,41,77] Children under 10 years of age exhibited lower mean antibody titers than older children or adults.[23,77] Individual sera from normal healthy adults collected serially in Britain from 1965 through 1970 were tested by Bradburne and Somerset.[11] 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 suggests 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.*[56] used the group of institutionalized children in Atlanta, Georgia, 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 nonill individuals indicated that an additional equal number of OC43 infections were occurring without the production of symptoms.[56] 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.[41]

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 etiologic. This finding was in contrast to that seen with 229E, in which no rises in titer were detected in such cases.[73,77]

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 of 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 HI 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, whereas the 1967–1968 activity was caused by 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.[86]

Surveys of antibody prevalence have been conducted in several settings using OC43 antigens. McIntosh *et al.*[77] 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.[41,47,56] In Britain, Bradburne and Somerset[11] 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.[11]

5.2. Geographic Distribution

Occurrence of coronavirus infection has now been documented, by either isolation or serology, throughout the world. 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 many regions of the country.[93,96] Extensive studies have been carried out by the Common Cold Research Unit, which have demonstrated the presence of the agents in Britain. The activity of 229E virus has been documented in Brazil in an early 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, whereas 26% of adults were antibody-positive.[21] Later investigations have confirmed the worldwide distribution of these agents.[40,47] 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 respiratory viruses.[84] 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, DC.[24,50]

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, although this event has been more common in recent studies involving more sensitive techniques.[104] This is the portion of the year in which isolation rates for rhinoviruses and other respiratory viruses often reach their low. An exception to this rule is a study in which frequent rises in titer were detected by ELISA in summer as well.[67] In addition, a cyclical pattern may be discerned when individual virus types are considered. In Fig. 3, data are summarized from five longitudinal studies of coronavirus activity carried out in different parts of the United States. In all 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. The findings overall suggest that the serological techniques used detected infection with the various variants of 229E listed in Table 2.

With OC43, the situation is quite different. As with 229E, in no investigation did 2 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 using the serological test employed.

The viruses related to OC43 are apparently more diverse than those related to 229E but still exhibit cross-

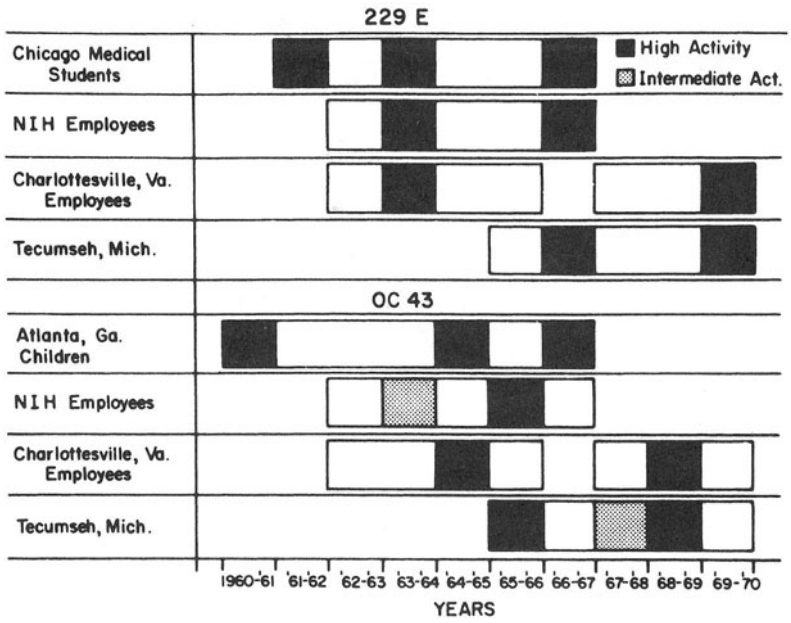

Figure 3. Cyclic behavior of 229E and OC43 viruses observed in five longitudinal studies.

reactions in serological tests; 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 area. 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.[18]

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.[86] This finding is quite different from the situation that exists with other respiratory agents, such as respiratory syncytial virus, in which a more distinct decrease in infection rates can be observed with increase in age.[85] 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.[23] This suggests 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.[83] Similarly, female volunteers appeared to be more susceptible to infection with 229E-like strains than males in artificial challenge studies.[19]

In the study by Candeias *et al.*[21] of antibody prevalence, the results were examined by sex, but no significant differences could be observed. There are no data available 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 children's institutions, has been reported.[50,60,119] 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.[119] 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.[23,104]

Although 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. In fact, in one study, coronaviruses were the most common agent involved in episodes of wheezy bronchitis.[34,75,81,96]

6. Mechanism and Route 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.[10,117] The virus is most stable at pH 6 0 and low temperature appears to protect it against varied relative humidity.[48,63] No other route of transmission for coronaviruses seems involved in man, although animal coronaviruses are infectious by the fecal–oral route.[111] There is currently no direct evidence to aid in identifying the main mechanisms of transmission. However, it is possible to compare the epidemiologic 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.[23] 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 as well as by large droplets, which would explain its ability to spread quickly.[29] Rhinoviruses, on the other hand, are thought to be transmitted by large droplet and may at times spread via fomites.[42] 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.[30]

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 to avian IBV in sera of poultry workers but not of controls.[82]

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 (range, 2–4 days).[10,117] Following exposure, the virus apparently multiplies superficially in the respiratory tract in a manner similar to that in which multiplication occurs *in vitro*. Nasal airway resistance and temperature of the nasal mucosa increases.[5] 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. Fewer than half of those infected with 229E showed a CF rise. It is not clear how the existence of titer or preinfection antibody affects the mag-

nitude 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.[8,11] Use of the ELISA test has given added sensitivity in antibody detection; it is not as yet clear if decreased specificity should be a concern.

An important characteristic of the coronaviruses is their apparent high rate of reinfection, which in volunteers has now been documented to be possible within a year of prior infection.[20] In the Tecumseh study, 81.5% of those infected with OC43 actually possessed prior N antibody.[90] Possession of circulating OC43 HI antibody among the Atlanta children did not appear to play a role in modifying severity of a subsequent illness.[56] With 229E virus, Hamre and Beem[35] demonstrated that the frequency of rise in titer 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 had in fact been demonstrated with a swine coronavirus[7] and subsequently with 229E in humans experimentally infected.[19]

8. Patterns 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.[10] Sore throat has been somewhat less common and in children has been associated with pharyngeal injection.[46] 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.[30] The mean duration of coronavirus colds, at 6.5 days, is shorter than that seen in rhinovirus colds, at 9.5 days.[10]

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.[77] Mufson *et al.*[90] 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 etiologic 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 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.[23] In Atlanta children, OC43 virus produced illness in about 50% of those infected.[56] 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, often in high frequency, and therefore a role in etiology of acute enteric disease has been suggested.[94] This would not be surprising in view of the clear involvement of certain strains in severe diarrheal disease of domestic animals. A general problem in most studies has been the inability to isolate the viruses; typically they have been identified directly by electron microscopy of stool specimens. Only rarely has it been possible to concentrate a specimen sufficiently to characterize the agent contained as a coronavirus.[100] Questions have been raised concerning whether all coronaviruslike particles in stool are truly coronaviruses; in certain situations it is clear that they are not.[107] In addition, although in some studies coronavirus particles are more commonly detected in stools from ill individuals than from healthy controls, in other studies this has not been the case.[22,25,33,109,118] In one study, excretion of these particles were associated more with various exposures than with symptoms of diarrhea.[71]

Multiple sclerosis is another disease entity concerning which evidence has been presented suggesting a role for coronaviruses. Coronaviruses were reported to be cultured from brain tissue of two multiple sclerosis patients.[17] Questions about the species origin of such isolates have been raised.[32] Gene expression of 229E viral RNA in brain tissue of multiple sclerosis patients but not in tissue of controls has also been reported.[112] Some serological results confirm this association, but others do not.[51,70,101] Again, the behavior of certain neurotropic animal coronaviruses is given as support for this association. Finally, 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.[2]

9. Control and Prevention

It is premature at present to think in terms of control of coronavirus infection by vaccination. 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 future studies may allow further characterization of truly protective antibodies. Work on vaccines for the animal viruses is in progress, and these studies may help in understanding issues of protection.[110] Chemoprophylaxis and related measures may be a more practical approach; it has been shown that recombinant α interferon can prevent infections artificially produced in volunteers,[44] and other approaches have been under investigation.[3,26,35,44] There remains environmental control of infection; such efforts have rarely been useful for other respiratory agents, but they may be more efficacious if a practical barrier to transmission can be devised.[78]

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 will quickly be filled. A partial solution would be direct detection of the virus through an ELISA or nucleic acid hybridization; this approach may soon be available.[30,92] Only serological tools are available now for most epidemiologic 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 in those with chronic respiratory disease, the viruses are important respiratory pathogens simply in terms of numbers of illnesses produced. They may also be involved in other important diseases. Only through further understanding of the behavior of these agents will it be possible to determine the scope of their activity and the most appropriate means by which control can be attempted.

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