New Knowledge and Current Problems in Human Virus Infections

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FROM his most primitive existence, man's acquisition of knowledge, while helping to resolve existing problems, has created new and, at times, seemingly more complex problems which demand solution. This has seemed to be the effect of each increment to our knowledge of virus infections. As more viruses are discovered, the work of classifying, differentiating, and establishing the clinical significance of these agents becomes more intricate.

Until 1947, 60 viruses had been cataloged as producing illness in man (*1*) but only 18 of these were strictly human pathogens. The others, including the arbor or arthropod-borne viruses as well as viruses of other lower animals (zoonotic viruses) are not obligated to man for their parasitic existence. Thus, man served as the sole reservoir for but a small fraction of his virus infections. In 1947, the enterprise of Dalldorf and Sickles (*2*) in utilizing suckling mice for isolation of a pathogenic virus, other than poliovirus, from the stools of two upstate New York cases of suspected poliomyelitis opened the door to the identification, in the ensuing 10 years, of 19 types of Coxsackie A and 5 types of Coxsackie B viruses.

Although the Maitlands, several decades ago, pioneered tissue culture for the study of pathogens, the technique was complicated, if not formidable, since an additional animal host system was necessary to demonstrate that growth of virus had occurred. The major impetus to virus isolation and culture came with the discovery by Enders and his co-workers (*3*) in 1948 that clear-cut cytopathogenic effects indicating viral activity and growth could be demonstrated in tissue cultures directly, without the need for a secondary animal indicator system.

The virus utilized was the Lansing strain of poliovirus, and the in vitro hosts were roller-tube cultures of human extraneural tissue cells. Subsequently, monkey kidney tissue cultures and a variety of other types of cells growing in vitro, including HeLa cells, have been employed with good results.

In the 12 short years since the work of Dalldorf, a tremendous number of hitherto unrecognized viruses have been identified and serologically typed. Of these, 81 new types of human viruses have been isolated and characterized physically and serologically (table 1). This number is more than all the viruses, irrespective of reservoir, recognized in the preceding half century. Numerous new viruses from other animal sources (for example, ECMO, ECBO, ECCO, and arbor) have also been isolated; nearly every month brings new identifications.

In table 1, the viruses for which man is the principal or, more frequently, the sole reservoir are presented according to the period of their discovery and their group designation. The arborviruses are not included in the table, since, as far as we know, there are no exclusively

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human parasites among them, with the possible exception of dengue. Except for the reoviruses (*4*), a new group, group designations follow the recommendations of the 1953 Conference on Virus and Rickettsial Classification and Nomenclature of the New York Academy of Sciences (*5*); the classification adopted by the Sixth International Congress of Microbiology in Rome, in 1953 (*6–8*); the definition of the enterovirus group by the Committee on Enteroviruses, National Foundation (*9*); and the definition of the adenovirus group by the principal workers in this area (*10*).

Problems in Classification

Although the earlier prototypes of these several virus groups are almost exclusively associated with specific clinical entities, such as smallpox and mumps, clinical specificity is not generally associable with each specific long-established agent and can be linked even less readily with the numerous agents discovered recently. Thus, a suitable classification cannot be predicated upon clinical entities. The virus types are categorized in groups according to size and other physical characteristics, pathogenicity for certain animal tissues or tissue cultures, antigenic relationships, and certain special biochemical and other characteristics such as the reaction of the myxoviruses with mucoproteins on the surface of cells or a complement-fixing antigen common to all the adenoviruses (*11*).

The currently accepted classification obviously is influenced somewhat by the anatomic site in which members of a particular virus group are most commonly found. This is true for the adenoviruses (formerly called the adenopharyngoconjunctival group) and for the enteroviruses. It is not true for the herpesvirus, myxovirus, and poxvirus groups, nor for most of the types in the psittacosis group. For these, the group designations are morphological, pathogenetic, or biochemical in implication.

Since viruses of the herpesvirus, myxovirus, and poxvirus groups, with the one exception of molluscum contagiosum, have a respiratory localization, some investigators (*12*) have suggested a regrouping into enterovirus and respiratory tract virus categories, with the adenoviruses, myxoviruses, and certain other viruses as subgroups. This classification would also be far from ideal, for not only can enteroviruses be isolated from the oropharynx early in an infection and adenoviruses be found in the intestinal tract, but the enteroviruses produce a substantial amount of acute respiratory illness which may even represent the bulk of the clinically apparent infections with these agents.

Even pathogenicity for infant mice, used in separating the Coxsackie from the ECHO viruses in the enterovirus group, has generated new problems of differentiation and classification. Strains of ECHO types 9 and 10 have begun to behave like Coxsackie viruses A and B (*9*). In fact, the large size of ECHO 10, its rather distinctive cytopathogenic effects, the pathogenicity for mice of some of its strains, and the respiratory disease which it apparently produces in children have led to its removal from the enterovirus category and the rest of the ECHO tribe to a new group called the reoviruses, or respiratory-enteric group (*4*), where it stands as a prototype.

Some Coxsackie viruses (A9 and B) may not be pathogenic for infant mice, and in this way they simulate the ECHO viruses. Coxackie A7 and A14 and also some ECHO viruses may produce lesions of the neurons in monkeys, thus simulating polioviruses (*13*). There is small wonder that before these characterizations were made, the Russians thought they had found a fourth type of poliovirus when they isolated a virus from the stool of a fatal case of clinical poliomyelitis and found it to be neurotropic in monkeys but not typable for polioviruses 1, 2, or 3. This virus has now been definitely characterized as Coxsackie A7 (*14,15*). These newly elicited interrelationships, despite serologic specificity of the poliovirus, Coxsackie, and ECHO tribes, are serving to obscure the demarcation lines originally drawn between the tribes. There is, therefore, increasing justification for grouping these three tribes together as enteroviruses.

Etiological and Clinical Specificity

Though these classifications tend to bring some order into the field of virology, they do not, unfortunately, simplify the problem of specific clinical differentiation and diagnosis.

Table 1. Distribution of human viral agents by type and period of discovery

Group	Known prior to 1947	Discovered since 1947 [1]
Adenovirus (*10*)	None	Types 1–18 (*12*).
Enterovirus (*9*)	Poliovirus (untyped)	Poliovirus types 1–3 (*39*). Coxsackie A types 1–19 (*40*). Coxsackie B types 1–5 (*40*). ECHO types 1–9, 11–24 (*9, 40, 41*).
Herpesvirus (*5*)	Herpes simplex Herpes zoster Varicella	None.
Myxovirus (*6*)	Influenza A and B Mumps	Influenza A prime (*42*). Influenza C (*43*). Parainfluenza group (*44*): Type 1: HA–2 (hemadsorption type 2)[2] (*45*). Influenza D (Sendai)[2] (*46*). Type 2: CA (croup-associated) (*47*). Type 3: HA–1 (hemadsorption type 1) (*45*). M–25 (*48*).
Poxvirus (*8*)	Molluscum contagiosum Smallpox	None.
Psittacosis (*5*)	Lymphogranuloma venereum	None.
Reovirus (*4*)	None	Type 1: ECHO 10 (*4, 9*). Types 2 and 3 (*4*).
Other	"Common cold" Homologous serum hepatitis Infectious hepatitis Infectious mononucleosis Measles Roseola infantum Rubella Trachoma	Respiratory syncytial virus (*49*). Salivary gland virus (*50*). JH virus (*51*). 2060 virus (*52*).
Total	18	81

[1] Since this paper was submitted for publication, Coxsackie A types 20, 21, 22, and 24; Coxsackie B type 6; and ECHO types 25, 26, and 27 have been isolated.

[2] Considered identical by investigators in the United States.

NOTE: Italicized figures in parentheses are reference numbers. References 39–52 do not appear in the text.

It has become quite clear not only that an individual member of the enterovirus, adenovirus, myxovirus, or other group can produce a diversity of clinical illnesses, but that a single clinical entity or syndrome may be produced by a number of specific virus types. Table 2 illustrates the virtual hopelessness of attempting etiological differentiation.

Although certain clinical entities have been carefully characterized, it must be remembered that the classic picture of a disease does not often obtain and that aberrant mild forms are quite common. Table 2 reveals several entities of a respiratory nature whose clinically differentiating characteristics may not always be present.

Acute undifferentiated respiratory disease (ARD) ranges from the severe form resembling primary atypical pneumonia to the mild ambulant form which, but for epidemiological and virus laboratory studies, would be classed as common colds. Within the spectrum of this entity, some illnesses may resemble influenza and others, acute pharyngitis.

Adenovirus types 4 and 7 have been incriminated as the etiological agents in military outbreaks of ARD (*16*). Although many adults eventually develop antibodies to these types, suggesting sporadic or even endemic infections, civilian outbreaks have not been documented, and these serotypes are rarely found in children. In addition, adenoviruses have not been

Table 2. Association of enterovirus, adenovirus, myxovirus, and other

Illness	Enteroviruses			
	Polio types	Coxsackie A types	Coxsackie B types	ECHO types
Paralysis (complete to slight muscle weakness)	1, 2, 3	7, 9	3, 4, 5	2, 4, 6, 16
Myocarditis or encephalomyocarditis (neonatal and early childhood).			2, 3, 4	
Epidemic pleurodynia			1, 2, 3, 4, 5	
Herpangina		2, 3, 4, 5, 6, 8, 10, 16.		
Aseptic meningitis	1, 2, 3	7, 9	1, 2, 3, 4, 5	2, 3, 4, 5,6, 9, 14, 16.
Epidemic exanthems:				
Boston exanthem and summer rash		16		4, 9, 16
Meningoencephalitis with rash				9
Summer diarrhea				18, 19
Acute febrile respiratory disease (summer grippe) most common.	1, 2, 3	Many types.	Many types.	Many types.
Acute undifferentiated respiratory disease				
Acute laryngotracheobronchitis				
Acute febrile pharyngitis				
Pharyngoconjunctival fever				
Follicular conjunctivitis				
Epidemic keratoconjunctivitis				
Viral pneumonia:				
Infants and children				
Adults				
Cytomegalic inclusion disease				

[1] Adapted from several sources *(9,12)*.
CCA—Chimpanzee coryza agent, or respiratory syncytial virus.
SGV—Salivary gland virus.

isolated from more than a very small percentage of cases of similar illnesses in civilian families (*17*). Rhodes and Van Rooyen (*18*) also feel it unlikely that more than a small percentage of colds occurring in the civilian population are due to adenoviruses. Even in military outbreaks a "large proportion of cases of typical ARD shows no evidence of infection with adenoviruses, and its etiology remains unknown" (*19*).

Furthermore, the enteroviruses, including poliovirus, are quite frequently responsible for undifferentiated febrile illness, often with respiratory symptoms (*9*).

In an extensive epidemiological and virological study of poliomyelitis in Minnesota in 1955, 1,272 fecal specimens from family contacts of poliomyelitis patients were examined for virus. Of these, 469 specimens were from family contacts of 175 patients from whom poliovirus had been isolated. One hundred and eighty-eight (40 percent) of the 469 specimens were positive for poliovirus.

In table 3, the 469 contacts are distributed according to their symptoms elicited in the course of surveillance. Only 109 showed any symptoms. The "pharyngeal" group, constituting 54 percent of the contacts showing any symptoms, included those with sore throats or history of a "cold." The "fever" group included those with fever alone or fever and other symptoms not included in the pharyngeal or meningeal complex. It is of interest that poliovirus was isolated from 63 percent of the contacts with pharyngeal symptoms.

From these several documentations and observations it is abundantly clear that the clinical illnesses of respiratory character presented in table 2 cannot be ascribed to a solitary virus group. Thus, clinical differentiation with etiological overtones is not possible without virus laboratory definition.

virus types with human illness [1]

Adenovirus types		Myxovirus types	Other types
Common	Less common		
4, 7	3, 14	Parainfluenza 1, 2, 3.	JH, 2060, CCA.
		Parainfluenza 1, 2.	
1, 2, 3, 5		Parainfluenza 3.	
3, 7a	1, 2, 5, 6, 14		
3, 7a	2, 6, 9, 10		
8 (classic)	3, 7a (mild)		
7a	1, 3	Parainfluenza 1, 3.	CCA.
4, 7	1, 3		SGV.

An analogous situation prevails for illnesses predominantly referable to the central nervous system (CNS).

In the happy, unenlightened first quarter of this century, poliovirus infections leading to paralysis were much more frequently recognized and reported than nonparalytic forms. This was so despite the careful description of abortive forms of poliomyelitis by Wickman in 1907 (*20*) and the reiteration by Lavinder, Freeman, and Frost (*21*) in 1918 that poliomyelitis very frequently occurs without paralysis.

However, by the fifth decade of this century, recognition of nonparalytic poliomyelitis based on signs of meningeal involvement during the poliomyelitis season had become universal in the United States. In Minnesota, for example, in the period 1947 to 1953 the proportion of nonparalytic to paralytic poliomyelitis reports stabilized at approximately the 1:1 level (*22*). From time to time, outbreaks of predominantly nonparalytic poliomyelitis were recorded. The Illinois Department of Public Health records for 1951 show, for instance, that of the more than 100 cases of poliomyelitis occurring in Champaign County only an extremely small percentage were paralytic. Although such outbreaks were considered most unusual, facilities were not then available in Illinois for laboratory differentiation of the viruses involved.

It was only in the late forties that the work of Dalldorf and of Enders opened up new techniques for virus isolation, and still later that workers began to isolate hitherto unknown viruses from cases of central nervous system infections and other related diseases. Causal relationships began to be clarified through these techniques, which also permitted simplified and economical antibody titration.

As late as 1955 in Minnesota, however, the actual cause of much nonparalytic disease involving the central nervous system, which was then being diagnosed as nonparalytic poliomyelitis, could not be determined. Whereas poliovirus was isolated from 75–90 percent of the cases of paralytic poliomyelitis in 1955, only 13 percent of the so-called nonparalytic poliomyelitis cases yielded poliovirus isolations. Although antibody studies verified an additional percentage of true cases of nonparalytic polio, a significant number of cases remained etiologically unexplained. In retrospect, at least some of these could have been caused by Coxsackie or ECHO viruses.

Again in the late forties, Wallgren's diagnosis of "aseptic meningitis" (*23*), originally designed to differentiate nonspecific or allergic meningitis from specific infections of the central nervous system, regained favor. The nonspecific character of the diagnosis of aseptic meningitis recently has been reaffirmed by the large number of polio, Coxsackie, and ECHO viruses found to be responsible for nonparalytic central nervous system disease (table 2). Thus, aseptic meningitis, a syndrome rather than a specific disease entity, bears no etiological significance without laboratory-determined agent designation.

To add to the problems of clinical differentiation engendered by a diversity of agents, the recent demonstration of transient mild paralysis accompanying Coxsackie A7 and A9 infections,

as well as infections with ECHO types 2, 4, 6, and 16, and the demonstration of poliolike lesions in mice and monkeys infected with Coxsackie A7 and A14 viruses (*13*) have complicated the picture of paralytic polio also. Even in the face of paralytic phenomena, a clinical diagnosis of poliomyelitis can be treacherous except under epidemiological conditions to be noted later.

Diagnosis of the time-honored exanthemata has also been affected by the problems unfolded in recent virus research. In 1951 in Boston, and again in 1954 in Pittsburgh, Neva and others (*24,25*) studied outbreaks of a febrile exanthem in which a pink maculopapular rash appeared on the face, trunk, and limbs, along with muscle pain and headache. The agent isolated on human fibroblast cultures has now been identified as ECHO 16.

In England, during the summer of 1954, viruses with the characteristics of the ECHO group were isolated from 5 of 6 stool specimens of infants, 3–14 months of age, who were exhibiting irritability, fever, a maculopapular rash, superficial lymphadenopathy, vomiting, and diarrhea. But for pleocytosis of the cerebrospinal fluid, these illnesses could readily have been confused with rubella (*26*).

Since then, a number of outbreaks have been reported in which symptoms and signs referable to aseptic meningitis were complicated by rashes variously described as rubelliform (*27–32*) and scarlatiniform (*33*). In all these outbreaks, ECHO 9 strains were isolated. Since rash was a symptom in only 30–40 percent of the cases identified as ECHO 9 infections, it is obvious that the majority of the cases presented aseptic meningitis syndrome only. Without the correlative laboratory isolations of ECHO 9 virus, outbreaks of two separate diseases might have been inferred. In fact, in the early weeks of the Minnesota 1957 outbreak of ECHO 9 aseptic meningitis, the largest to date, with 424,000 cases extrapolated from a random sample family survey (*31*), one local health officer did derive this inference; with considerable concern, he stated that in his area he had two epidemics going at the same time—one of nonparalytic polio and the other of Boston exanthem.

Table 3. Poliovirus isolations from family contacts of patients from whom poliovirus was recovered, according to symptoms of contacts, Minnesota, 1955

Symptoms of contacts	Number of contacts	Poliovirus isolations	
		Number	Percent
Any symptoms	109	72	66
Pharyngeal group	59	37	63
Meningeal group	13	6	46
Fever group	37	29	78
No symptoms	360	116	32
Total	469	188	40

SOURCE: Studies by the school of public health, University of Minnesota, and the department of bacteriology and immunology, Minnesota Department of Health.

Epidemiological Implications

It is obvious that the isolation of 24 types of Coxsackie and at least 24 types of ECHO viruses, many of which are responsible for acute central nervous system infections frequently occurring in outbreak form, has made the diagnosis of nonparalytic poliomyelitis almost impossible to establish clinically. The significance of this difficulty in the evaluation of vaccine efficacy and in epidemiological investigations in general is also quite obvious. Surveillance programs for central nervous system infections, as a result, have had to be coupled with services of a competent virus diagnostic laboratory. Only the laboratory can define an entity etiologically when the syndrome can be produced by a variety of agents. The same principle applies to the entire field of new virus infections, whether they produce CNS, gastrointestinal, or respiratory disease.

The necessity for observing the principle with CNS diseases impressed itself upon us in Minnesota in 1955, when Salk polio vaccine was applied to a virgin population. Questions of safety and efficacy required the establishment of a surveillance program for CNS infections (*22*).

Physicians and hospitals were highly cooperative, not only in reporting, but in the submission of stools and paired blood samples from their patients. Health department and county

nurses and medical students obtained clinical histories and stool specimens on all family contacts of patients with CNS infections. Stools were examined for virus, utilizing HeLa cell cultures, and paired serums were examined for antibody titer increases. Exclusion tests for mumps, St. Louis and western encephalitis, and for lymphocytic choriomeningitis were also applied. A search for Coxsackie and ECHO viruses was not part of the laboratory routine in 1955, although 27 cytopathogenic agents were isolated from patients or contacts and identified the following year.

Six hundred and forty-nine cases of CNS disease, the total State caseload, were processed in 1955. Table 4 compares the originally reported diagnoses for these cases of CNS disease with the final diagnoses established after completion of either virus isolation, or antibody titrations, or both.

The physicians who reported cases as paralytic poliomyelitis were correct 152 out of 163 times, or for 93 percent of the cases so reported. However, only 269 (59 percent) of the 457 cases originally reported as suspect poliomyelitis or nonparalytic poliomyelitis were finally designated as nonparalytic poliomyelitis. The remaining nonparalytic or suspected poliomyelitis cases were ultimately distributed among paralytic poliomyelitis (55 cases); mumps encephalitis (24 cases); lymphocytic choriomeningitis (4 cases); aseptic meningitis (45 cases); and other illnesses, including Guillain-Barré syndrome, bacterial meningitis, and transverse myelitis (60 cases).

Aseptic meningitis in this study was a diagnosis ascribed to cases which fulfilled all the following criteria:

1. No poliovirus isolated from submitted stools.
2. Absence of paralysis.
3. Absence of serum antibodies to any type of poliovirus.
4. Failure to establish a diagnosis of other etiologically definable CNS disease by serologic methods.
5. Pleocytosis in cerebrospinal fluid.
6. History of meningoencephalitic symptoms.

Of 25 cases of mumps established serologically, only 1 had been initially diagnosed clinically. In 14 of these cases, there was either a history of recent parotitis or parotitis developed shortly after report. The other 11 cases were without parotitis. Furthermore, although physicians reported infectious encephalitis 16 times, none was confirmed for western or St. Louis types.

This study indicates that although paralytic disease is still preponderantly poliomyelitis and, in the face of an outbreak of paralytic disease, the physician's clinical impression of poliomyelitis most frequently proves to be cor-

Table 4. Comparison of originally reported diagnoses with final diagnoses in CNS cases investigated during poliomyelitis surveillance in Minnesota, 1955

Originally reported diagnoses	Final diagnoses							
	Paralytic poliomyelitis	Nonparalytic poliomyelitis	Mumps	Lymphocytic choriomeningitis	Aseptic meningitis	Western and St. Louis encephalitis	Other	Total
Suspect poliomyelitis	19	85	19	1	15	0	54	193
Paralytic poliomyelitis	152	5	0	1	0	0	5	163
Nonparalytic poliomyelitis	36	184	5	3	30	0	6	264
Mumps	0	2	1	0	0	0	1	4
Lymphocytic choriomeningitis	0	1	0	0	0	0	0	1
Aseptic meningitis	0	7	0	0	1	0	0	8
Western and St. Louis encephalitis	0	4	0	0	1	0	11	16
Total	207	288	25	5	47	0	77	649

SOURCE: Studies by the school of public health, University of Minnesota, and the department of bacteriology and immunology, Minnesota Department of Health.

rect, his "batting average" for nonparalytic disease is quite disappointing.

Another important epidemiological implication of the isolation of numerous viral agents is their temporal distribution in communities. Although the newly discovered enteroviruses, adenoviruses, and myxoviruses, by direct isolations and evidence of specific antibodies in serums, have been shown to occur in all parts of the world, specific communities experience rises and declines in the amount of infection and disease caused by these agents.

Observations in Minnesota reveal a provocative reciprocal interdependence of predominating enterovirus types year by year. With continuing poliomyelitis surveillance by the Minnesota Department of Health, CNS disease is thoroughly investigated and stool and other specimens are examined for virus content. After typing, confirmations are performed by the virus laboratory of the University of Minnesota department of bacteriology and immunology under the direction of Dr. J. T. Syverton.

Table 5 presents the Minnesota enterovirus experience for the years 1955 through 1958. In each year a specific enterovirus type predominated almost to the exclusion of others. In 1955, poliovirus predominated; in 1956, Coxsackie B5 was apparently responsible for the bulk of the CNS cases; in 1957, the largest recorded outbreak of ECHO 9 meningoencephalitis with rash occurred; and in 1958, Coxsackie B5 returned. Although data on virus isolations are not complete, poliovirus was apparently in ascendancy in 1959, with preliminary reports through December 31 indicating that of 237 cases reported, 198 (84 percent) were paralytic. Furthermore, most of the recent incidences and outbreaks of poliomyelitis have been either solely, or at least predominantly, of one type.

Table 5. Isolations of enteroviruses from CNS and related cases in Minnesota, 1955–58

Virus isolated	Year			
	1955	1956	1957	1958
Polio	[1] 175	47	9	12
Coxsackie:				
A 9	0	2	4	2
B 2	11	0	2	0
B 3	4	6	1	2
B 4	0	0	1	2
B 5	0	60	18	109
ECHO:				
1	----	0	6	0
6	----	0	2	1
9	----	0	149	5
Total	190	115	192	133

[1] Excludes isolations from 188 family contacts.

The significance of these data is not clear. Is this a pure chance interdigitation of separate and distinct secular cyclings of the several agents? Or is an interference phenomenon, repeatedly documented in the laboratory for such pairs of agents as one poliovirus type and another one of its heterotypes (*34–36*), and poliovirus and Coxsackie B (*37*), operating outside the laboratory? If such interference is, in fact, occurring in nature, then the implications for vaccination, especially with mixtures of live viruses, may be quite important.

Viruses Still in Search of Disease

Referring again to table 2, it is immediately apparent that not all of the isolated new viral agents have been incriminated in human illness despite their isolation from human sources. For example, although 18 adenovirus types have been isolated, only 11 of them have been established as productive of human illness. In the Coxsackie A group, only 10 of the 19 types have been incriminated, and of the 24 ECHO types, approximately 10. Many of the viruses which have not yet been causally related are the more recent discoveries, and it is highly probable that such relationships will ultimately be established for a number of them. Furthermore, there is every reason to believe that new types will continue to be isolated and, as aberrant characteristics are elicited, new groups formed. It is likely that viruses will continue to be in search of disease.

The establishment of causal relationship between the many new virus types and human disease is a complex operation. We find ourselves at present in the same position with virology as we were with bacteriology in the early years of this century, when any bacterium isolated from a patient was considered the

cause of his disease. Time proved many organisms to be nonpathogenic; there may also be a normal viral "flora."

Merely finding an adenovirus in throat washings or an enterovirus in the stool of a sick patient does not establish a causal relationship. Their isolations may be coincidental, and several types may be isolated from the same patient during the same illness. Even a rise in antibody titer for a given viral type is not in itself proof of causation, for heterotype responses may occur. Most of the studies attempting to relate agents to disease have involved isolations from throat washings or stools, and there are those investigators who would demand viremia, rarely encountered, as proof.

There is even some evidence that virus recovered from an anatomic site of disease may not be adequate proof that the isolated virus is the cause. Melnick (*38*) cites the finding by Israeli investigators of Coxsackie virus in the spinal fluids of two patients with brain tumor and suggests that even penetration of the blood-brain barrier would thus not constitute proof of causality.

The highly frequent finding of the agent in cases of a disease with clinical distinctness and its absence from cases of other illnesses is suggested as a first step in establishing etiology. That this would not often be achieved in markedly similar syndromes has already been implied in our discussion of clinical specificity.

However, the markedly higher incidence of a given virus in patients with a given syndrome than in healthy controls selected from a comparable segment of the population would constitute strong incriminating evidence. The isolation of a virus, its production of the specific disease in volunteers, and recovery of the virus from the newly induced cases (with suitable controls) would be the ideal method but for the fact that, for a number of agents, infection takes place early in childhood and results in immunity.

No single one of these criteria, with the exception of the controlled volunteer study, would in itself constitute proof of an etiological relationship, but, in the aggregate, a strong association could be surmised. Sporadic cases, or even very small groups of cases, would rarely provide us with an aggregate of positive criteria. Since causality, in the last analysis, is a matter of inference drawn from repeated strong associations, epidemics of disease provide us with the type of material necessary for the establishment of etiological relationships. In this regard, then, not only does the epidemiologist need the virologist and the clinician, but for a long time to come, the virologist and the clinician will need the services of an epidemiologist.

At the present time, despite the tremendous gains in understanding achieved in the past 12 years, our knowledge of the relationship of the newer viral agents to illness in the human population rests just above the primitive level. We can be confident, however, that within not too many years, certain "orphans" will have found a home even though some of them will prove to be commensals without behavior problems.

REFERENCES

(*1*) Rivers, T. M., Editor: Viral and rickettsial infections of man. Philadelphia, J. B. Lippincott Co., 1948, 719 pp.

(*2*) Dalldorf, G., and Sickles, G. M.: An unidentified filterable agent isolated from the feces of children with paralysis. Science 108: 61–62 (1948).

(*3*) Enders, J. F., Weller, T. H., and Robbins, F. C.: Cultivation of Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. Science 109: 85–87 (1949).

(*4*) Sabin, A. B.: Reoviruses. Science 130: 1387–1389, Nov. 20, 1959.

(*5*) Symposium. Virus and rickettsial classification and nomenclature. Ann. New York Acad. Sc. 56: 381 (1953).

(*6*) Andrewes, C. H., Bang, F. B., and Burnet, F. M.: A short description of the myxovirus group (influenza and related viruses). Virology 1: 176 (1955).

(*7*) Von Magnus, H., Gear, J. H. S., and Paul, J. R: A recent definition of poliomyelitis viruses. Virology 1: 185 (1955).

(*8*) Fenner, F. J., and Burnet, F. M.: A short description of the poxvirus group (vaccinia and related viruses). Virology 4: 305, 314 (1957).

(*9*) Melnick, J. L., Dalldorf, G., Enders, J. G., Hammon, W. McD., Sabin, A. B., Syverton, J. T., and Wenner, H. A.: The enteroviruses; committee report. Am. J. Pub. Health 47: 1556–1566, December 1957.

(*10*) Enders, J. F., et al.: "Adenoviruses": Group name proposed for new respiratory-tract viruses. Science 124: 119 (1956).

(11) Burnet, F. M.: Animal viruses: A comparative survey. *In* The viruses. Vol. 3, Animal viruses. London, The Academic Press, 1959.

(12) Huebner, R. J.: Implications of 70 newly recognized viruses in man. *In* Symposium on perspectives in virology. Morris Pollard, Editor. New York, John Wiley & Sons, 1959.

(13) Dalldorf, G.: Neuropathogenicity of group A Coxsackie viruses. J. Exper. Med. 106: 69–76 (1957).

(14) Johnson, T., and Lundmark, C.: Identification of the "fourth type" of poliomyelitis virus. Lancet 272: 1148–1149, June 1, 1957.

(15) Horstmann, D. M., and Manuelidis, E. E.: Russian Coxsackie A7 virus ("ABIV strain"), neuropathogenicity and comparison with poliovirus. J. Immunol. 81: 32–42, July 1958.

(16) Dingle, J. H., Ginsberg, H. S., Badger, G. F., Jordan, W. S., Jr., and Katz, S.: Evidence for the specific etiology of "acute respiratory disease (ARD)." Tr. A. Am. Physicians 67: 273–278 (1957).

(17) Jordan, W. S.: The frequency of the infection with adenoviruses in a family study population. *In* Symposium on viruses in search of disease. Ann. New York Acad. Sc. 67: 273–278 (1957).

(18) Rhodes, A. J., and Van Rooyen, C. E.: Textbook of virology. Baltimore, Williams & Wilkins Co., 1958.

(19) Rowe, W. P., Huebner, R. J., and Bell, J. A.: Definition and outline of contemporary information on the adenovirus group. *In* Symposium on viruses in search of disease. Ann. New York Acad. Sc. 67: 255–261 (1957).

(20) Wickman, I.: Beitrage Zur Kenntnis der Heine-Medinschen Krankheit (Poliomyelitis acuta und Verwandter Erkrankungen). Berlin, 1907.

(21) Lavinder, C. H., Freeman, A. W., and Frost, W. H.: Epidemiologic studies of poliomyelitis in New York City and the northeastern U.S. during the year 1916. Pub. Health Bull. No. 91, Washington, D.C., 1918.

(22) Schuman, L. M., Kleinman, H., Krovetz, L. J., and Fleming, D. S.: Efficacy of poliomyelitis vaccine. J.A.M.A. 166: 1027–1035, March 1958.

(23) Wallgren, A.: Une nouvelle maladie infectieuse du systeme nerveux central? Acta paediat. 4: 158 (1925).

(24) Neva, F. A., Feemster, R. F., and Gorbach, I. J.: Clinical and epidemiological features of an unusual epidemic exanthem. J.A.M.A. 155: 544–548 (1954).

(25) Neva, F. A.: A second outbreak of Boston exanthem disease in Pittsburgh during 1954. New England J. Med. 254: 838–843 (1956).

(26) Crawford, M., Macrae, A. D., O'Reilly, J. N.: An unusual illness in young children associated with an enteric virus. Arch. Dis. Childhood 31: 182–188, June 1956.

(27) Nihoul, E., Quersen-Thiry, L., and Weynants, A.: ECHO virus type 9 is the agent responsible for an important outbreak of aseptic meningitis in Belgium. Am. J. Hyg. 66: 102–118 (1957).

(28) Davies, J. W., McDermoth, A., and Severs, D.: Epidemic virus meningitis due to ECHO 9 virus in Newfoundland. Canad. M. A. J. 79: 162–167, Aug. 1, 1958.

(29) Laforest, R. A., McNaughton, G. A., Beale. A. J., Clarke, M., Davis, N., Sultanian, I., and Rhodes, A. J.: Outbreak of aseptic meningitis (meningoencephalitis) with rubelliform rash: Toronto, 1956. Canad. M. A. J. 77: 1–4, July 1, 1957.

(30) Sultanian, I., and Rhodes, A. J.: Isolation of ECHO virus type 9 from an outbreak of aseptic meningitis with rubelliform rash: Toronto, 1956. Canad. J. Pub. Health 49: 181–185, May 1958.

(31) Kleinman, H., Rogers, D., Ellwood, P. M., Jr., Bruhl, H., Schuman, L. M., and Bauer, H.: Epidemic of ECHO 9 aseptic meningitis in Minnesota, 1957. Univ. Minn. M. Bull. 29: 306–328, Mar. 15, 1958.

(32) Sabin, A. B., Krumbiegel, E. R., and Wigand, R.: ECHO type 9 virus disease. Am. J. Dis. Child. 96: 197–219, August 1958.

(33) MacLeod, A. J., Faulkner, R. S., and Van Rooyen, C. E.: ECHO 9 virus infections in eastern Canada: Clinical and laboratory studies. Canad. M. A. J. 78: 661–665, May 1, 1958.

(34) Ledinko, N., and Melnick, J. L.: Interference between poliomyelitis viruses in tissue culture. J. Exper. Med. 100: 247–267 (1954).

(35) Sabin, A. B., Hennessen, W. A., and Winsser, J.: Studies on variants of poliomyelitis virus. I. Experimental segregation and properties of avirulent variants of three immunologic types. J. Exper. Med. 99: 551–576 (1954).

(36) Drake, J. W.: Interference and multiplicity reactivation in polioviruses. Virology 6: 224–264 (1958).

(37) Dalldorf, G.: The sparing effect of Coxsackie virus infection on experimental poliomyelitis. J. Exper. Med. 94: 65–71 (1951).

(38) Melnick, J.: Discussion (part I). *In* Symposium on viruses in search of disease. Ann. New York Acad. Sc. 67: 252–253 (1957).

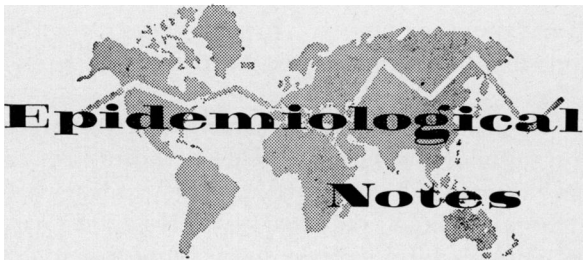
(39) Bodian, D., Morgan, I. M., and Howe, H. A.: Differentiation of types of poliomyelitis viruses. III. The grouping of fourteen strains into three basic immunological types. Am. J. Hyg. 49: 231–245 (1949).

(40) Melnick, J. L.: Advances in the study of the enteroviruses. Progress in Medical Virology 1: 59–105 (1958).

(41) Sabin, A. B.: Discussion of Huebner's paper. *In* Symposium on perspectives in virology. p. 138. (See ref. 12.)

(42) Francis, T. J., Salk, J. E., and Quilligan, J. J., Jr.: Experience with vaccination against in-

fluenza in the spring of 1947. Am. J. Pub. Health 37: 1013 (1947).
(43) Francis, T. J., Quilligan, J. J., Jr., and Minuse, E.: Identification of another epidemic respiratory disease. Science 112: 495 (1950).
(44) Andrewes, C. H., Bang, F. B., Chanock, R. M., and Zhdanov, V. M.: Parainfluenza viruses 1, 2, and 3: Suggested names for recently described myxoviruses. Virology 8: 129–130, May 1959.
(45) Huebner, R. J., Rowe, W. P., and Chanock, R. M.: Newly recognized respiratory tract viruses. Ann. Rev. Microbiol. 12: 49–76 (1958).
(46) Kuroya, N., Ishida, M., and Shiratoni, T.: Yokohama Med. Bull. 4: 217 (1953). (Referred to by T. Francis *in* The viruses. Vol. 3, Animal viruses. London, The Academic Press, 1959, p. 254.)
(47) Chanock, R. M.: Association of a new type of cytopathogenic myxovirus with infantile croup. J. Exper. Med. 4: 555–576 (1956).
(48) Johnson, K. M., Chanock, R. M., Cook, M. K., and Huebner, R. J.: Studies of a new human hemadsorption virus. I. Isolation, properties and characterization. Am. J. Hyg. 71: 81–92, January 1960.
(49) Morris, J. A., Blount, R. E., Jr., and Savage, R. E.: Recovery of a cytopathogenic agent from chimpanzees with coryza. Proc. Soc. Exper. Biol. & Med. 92: 544 (1956).
(50) Smith, M. G.: Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc. Soc. Exper. Biol. & Med. 92: 424 (1956).
(51) Price, W., Emerson, H., Ibler, I., Lachain, R., and Terrell, A.: Studies of the JH and 2060 viruses and their relationship to mild upper respiratory disease in humans. Am. J. Hyg. 69: 224–229 (1959).
(52) Mogabgab, W. J., and Pelon, W.: Problems in characterizing and identifying an apparently new virus found in association with mild respiratory disease in recruits. *In* Symposium on viruses in search of disease. Ann. New York Acad. Sc. 67: 403–412 (1957).



Carcinoma in Blackfoot Indians

During the past 5 years, 1955–59, while carcinoma of the cervix uteri and carcinoma of the breast has been occurring with about equal incidence in the United States, carcinoma of the cervix has been found in some 12 women of the Blackfoot Indian tribe of Montana. This compares with only two Blackfoot women who in the comparable time period have been found to have carcinoma of the breast. The exact significance of this difference is not understood, and may be more apparent than real since it has only been during the past 3 years that any extensive screening has been done for carcinoma of the cervix in this Indian group. Vital statistics for 1957 show about 3 to 4 deaths from cervix cancer to 1 death from breast cancer among all Indians.

The average Blackfoot Indian population on reservations during this time period has remained constant at about 4,000 enrollees, of whom approximately 1,000 were women in the age group above 20 years. The diagnoses of cervical carcinoma were all made after the women presented symptoms. Ages ranged from 24 to 89 years. One of the breast carcinomas was discovered during a routine physical examination. Both of the patients with breast carcinoma were more than 60 years old.

The members of the tribe are becoming increasingly aware of the need for periodic screening for carcinomatous conditions.

In addition to the above mentioned lesions, carcinoma of the lung, kidney, gall bladder, stomach, and tongue has been seen in members of the Blackfoot Indian tribe during the same 5-year period.—EDWARD L. KING, M.D., *health officer, Browning, Mont.*