R. M. TAYLOR*

Section of Epidemiology and Preventive Medicine, Yale University School of Medicine

STUDIES ON THE ECOLOGY OF ARTHROPOD-BORNE (ARBOR) VIRUSES**

It is a curious fact that the first disease in man proven to be caused by a filterable virus is arthropod-borne—I refer of course to yellow fever—and yet today our knowledge of this category of viruses, particularly as concerns the epidemiology of human infection, is less complete or more obscure than any of the other main virus families.

There are reasons for this: the infection does not pass directly from one human being to the next and therefore cannot be traced by this simple means; there is always an intervening vector or arthropod, and in most instances one or a number of intervening lower vertebrates. Indeed, with a few notable exceptions, man is not involved in the cyclic propagation of these viruses. His infection is tangential, fortuitous, and usually dead-end. It may be compared to that of a bystander at a ball game who gets hit by a foul ball. His social rank, his economic status, his food, his physical state, and his association with his fellow man are of little moment in the epidemiology of his infection. What counts is his exposure to the bite of an arthropod carrying the virus.

Thus, to understand the epidemiology of these infections in man, we must know when and where they occur and the arthropods and lower vertebrates involved in their propagation—in short, we must be familiar with the ecology or life cycles of these viruses.

In our opinion the outstanding problems to be tackled for a better understanding of these viruses are the following:

Search for as yet unknown viruses in this category. It is only in recent years that concerted efforts have been made to search for these viruses in their arthropod vectors and lower vertebrate hosts. These efforts have been rewarding, particularly in the humid tropics. New ones are constantly being discovered and further search with improved methods will undoubtedly yield more. Also, it should be remembered that the exact etiology of a large

* Lecturer in Epidemiology and Preventive Medicine.

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portion of human encephalitis occurring each year in the United States remains undetermined. The clinical manifestations and the cytological response of many of these cases suggest a viral infection. It is quite likely that some of them may be caused by as yet undiscovered arthropod-borne viruses.

Identification of the arthropods and vertebrates actively involved in the cycles of these viruses and discovery of means of their survival during the inactive transmission season. A good deal is known about the ecology of some of the long recognized arthropod-borne viruses, but even with these our knowledge lacks precision, and the natural history of all of the newly discovered ones remains to be unraveled.

Classification and geographical distribution are essential in order to appreciate the interrelation and the global epidemiology and to evaluate the potential threat of these infections. There is reason to suspect that the birthplace and permanent home of many of these viruses is in the tropics, and that they have been secondarily emplanted in the temporate zones. An example will be given in discussing the ecology of Eastern equine encephalomyelitis (EEE).

Manner of dissemination. It is known that birds are hosts to many of these viruses, and it is suspected that they may play an important part in their long-range implantations. This concept demands further attention. Again, EEE, to be referred to later, may be cited as an example.

The importance of determining the pathogenicity of these viruses particularly in man is self-evident and when a new viral agent is isolated, especially from arthropods and lower vertebrates, the questions that immediately arise and should be investigated are: Is it infectious to man? What is the symptomatology? How frequent and under what conditions does infection take place?

Methodology should be given a major rating because success in solving the above-listed problems will depend in large measure upon the discovery of new methods and the refinement of the old.

Our studies here at Yale are concerned to some degree with all these problems. They consist of:

The study of the ecology of eastern equine encephalomyelitis (EEE) in the Connecticut area. In New England EEE has in the past produced costly epidemics in horses and pheasants and occasionally has been the cause of human encephalitis, always severe and frequently fatal. It remains a constant threat.

This virus has for many years received the attention of other investigators and a great deal has been learned about its natural history, in which birds

FIG. 1. The shading on the map represents the principal areas where Eastern equine encephalomyelitis (EEE) has been reported. The following sources were used. For distribution in U.S.A.: Report on surveillance of arthropod-borne encephalitis in the United States, June 16, 1958, Communicable Disease Center, Atlanta, Georgia. For Latin America: Plate 13, Explored areas of arthropod-borne viral infections, American Geographical Society; Downs, W. G. *et al., Amer. J. trop. Med.,* 1956, *5,* 626; Causey, O. R. and Theiler, M., *Amer. J. trop. Med.,* 1958, *7,* 36; Alice, F. J., *Rev. bras. Biol.,* 1951, *11,* 125 (including references to other Brazilian publications). Some liberty has been taken in outlining infected zones in South America by including areas of similar biological environment contiguous with places where the presence of the virus has been recognized and reported.

and mosquitoes seem to be mainly involved. Its geographical distribution, so far as is known, is illustrated in the accompanying map. As will be seen from the map, infection by this virus has been recognized mainly along the east and southern border of the United States, the east coast of Mexico, Panama, Cuba, the Dominican Republic, and the northern coast of South America and Brazil. Recent studies indicate that it is endemic in the Amazon rain forests. Those familiar with the flyways of migrating birds will immediately recognize similarities in the pattern of the distribution of this virus and the main eastern flyways of certain migratory birds. However, there is still a good deal to be learned to understand completely the behavior of this virus.

Our studies involve obtaining further information on the life cycle of the virus, the vectors and vertebrate hosts involved, the determining factors in epidemics, and the question of the virus 'overwintering' or being implanted each season by migrating birds. Fortunately for the pheasant farmers but unfortunately for our studies, there has been little evidence of the activity of the virus in this area since the studies were begun in the summer of 1956.

However, some evidence has been obtained which suggests that the virus does not overwinter in hibernating mosquitoes, and if the virus does overwinter in this area, we believe some other mechanism must be involved. We are now investigating the possibility of a hitherto unsuspected cycle in non-blood-sucking insects as a mechanism of survival of the virus and for explaining the sporadic infections which seemingly occur during the nonepidemic years. It has been found that larvae of insects common in this region (such as the beetle *Tenebrio molitor* which in the larval form is frequently referred to as the yellow meal worm) are susceptible to infection by parenteral inoculation and through food, and that nestling birds may be infected by consuming these infected larvae. It has also been found that the infection in the larvae may be carried from the larval through the pupal to the adult form of the insect. The question of the virus being passed from one generation of this beetle (*T. molitor*) to the next is now under study.

Isolation of a "new" virus. As a by-product of the EEE study, at least one virus has been isolated from a nestling bird which, according to investigations so far completed, may represent a new or rather a previously unrecognized virus. It has been tentatively designated as *Hilo* virus. The ecology of this virus, including search for evidence of human infection, is now under study and promises to be of considerable interest.

The antigenic classification and behavior in tissue culture of virus strains isolated in Egypt and the Sudan. This study, now nearing completion, involves 20-odd *Phlebotomus* (sandfly) fever strains and about an equal number of strains that are harbored and transmitted by ticks. Among these latter there appear to be 4 and probably 5 hitherto undescribed viruses distinguishable by their antigenic characteristics, arthropod and vertebrate host range and to a degree by their behavior in tissue culture. Two of these tick viruses are transmitted by *Argas* ticks, a variety that normally parasitizes birds. Birds seem to be the main vertebrate hosts of these viruses. Incidentally, the principal bird host of one of these viruses in Egypt, the cattle egret or *Bubulcus ibis ibis,* has in recent years appeared on this continent and has now become numerous in southern Florida. One wonders if the virus was also introduced. It is worthy of investigation since the virus which infects these birds in Egypt is also infectious to man.

The application of tissue culture (TC) to the study of the arthropod-borne category of viruses. These studies have been under the supervision of Dr. Henderson,[1] and in our opinion the results have been most encouraging. It has been possible by the use of various types of primary cell lines to cultivate virtually all of the prototype strains representing the major sero-groups of the arthropod-borne viruses, and, in addition, many ungrouped viruses including sandfly fever strains which had not hitherto been grown in tissue culture. The overlay plaque technique has been found most useful in detecting growth and for virus titrations. We are hopeful, therefore, that tissue culture may prove to be a valuable adjunct in:

1. *Isolating arthropod-borne viruses.* By selecting the proper cell line and utilizing the overlay plaque technique for revealing growth, it has been found that most of the viruses studied can be detected in as minute amounts as with the infant mouse method. It is also quite possible that, as with the enteric and respiratory virus families, tissue culture may uncover types of arthropod-borne viruses which have heretofore escaped detection. Indeed, the "new" virus (*Hilo*) encountered during the EEE studies was isolated in tissue culture, and could not be isolated from the original material by inoculation of infant mice.

2. *Performing neutralization tests.* The tissue culture method has proven useful in performing neutralization tests with several of the recognized arthropod-borne viruses, particularly those belonging to sero-group A. We are currently using it in our EEE investigations and in studies on Hilo virus. And we believe that the choice of suitable cell lines and the use of the overlay plaque technique will lead to a much broader application of tissue culture for neutralization tests among the arthropod-borne viruses.

3. *Classifying the arthropod-borne viruses.* The following tabulation of the behavior of 23 virus strains belonging to three different sero-groups in

chick embryo, Pekin duck, and rhesus monkey kidney tissue culture illustrates how tissue culture may be employed for virus classification. It is obvious that viruses of sero-groups A have the widest growth spectrum in that members of this group may grow on all of the three cell lines used, while those of group B are limited largely to growth on Pekin duck kidney and will not grow on chick embryo; group C, the most exacting of all, grow only on monkey kidney cells. The tabulation also illustrates the superiority of the overlay plaque method for detecting virus growth, especially in B and C sero-groups.

Virus sero-group	*No. virus strains examined*	*No. strains forming plaques and CPE*					
		CE		*PDK*		*RMK*	
		CPE	*PL*	*CPE*	*PL*	*CPE*	*PL*
A	10	7	8	4	10	7	10
B	8	0	0	3	8	0	1
C	5	0	0	0	0	0	5

CE—Whole decapitated chick embryo tissue culture.
PDK—Pekin duck kidney tissue culture.
RMK—Rhesus monkey kidney tissue culture.
CPE—Cytopathogenic effect observed in monolayer fluid phase cultures.
PL—Plaque formation in monolayer agar overlay cultures.

In conclusion the question might be asked why we at Yale should bother with these arthropod-borne viruses since for the most part they involve insects, birds, and lower mammals, and human infection with most of them is accidental. Admittedly, infection of man by these viruses, particularly in temperate zones, is much less frequent than by those viruses which require no intermediate host and circulate within the human population by direct transfer. However, we believe that these arthropod-borne viruses deserve attention for the following reasons: diagnosed, and probably a much larger number of undiagnosed, infections associated with severe and sometimes fatal encephalitis occur each year in North America; several epidemic outbreaks of St. Louis and Eastern and Western equine encephalomyelitis have occurred in the past and unless prevented may be anticipated in the future; arthropod-borne virus infections are much more frequent in the humid tropics and hitherto unrecognized members of this category are being constantly discovered, and the danger of these "new" viruses being implanted in temperate zones should be anticipated, and, if possible, prevented or controlled; two of the long recognized arthropod-borne viruses—yellow fever and dengue—are endemic in South America, and there is a continuous threat of their introduction into our southern states. Here we have an excellent example of the fruits of research, for if it were not for the knowledge gained by research on methods of controlling these two infections if introduced, they would indeed constitute a very grave health hazard. Finally, these arthropod-borne viruses with their frequently complicated life cycles and their divergent vagaries offer the inquisitive investigator, with an ecological bent, many fascinating and intriguing problems to exercise his talents.

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