The Epidemic Climate

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IN THE 16th century, Fracastoro formulated the idea that communicable diseases were caused by "living agents," a thought that occurred to earlier minds but, except for the scabies mite, without supporting evidence that survived to modern times. Later investigators, such as Snow, Henle, Panum, Budd, Holmes, Semmelweis, and Hirsch, inferred the probable existence of such agents strictly by epidemiological methods. However, it was only after invention of the achromatic microscope that Pasteur, Koch, and their followers, using Henle's principles, demonstrated that microorganisms are the primary cause of certain diseases. This important work put on a firm scientific foundation man's understanding of the pathogenesis of infectious disease.

Since that time many other etiological agents (helminths, protozoans, fungi, bacteria, rickettsiae, and viruses) have been identified with diseases of both man and animals. Principal interest has focused upon the differential disease diagnosis and pathogenesis and the treatment of the patient. In comparison, relatively little attention has been paid to the biological survival mechanisms and mode of transmission of infective agents in a community, particularly during the endemic prevalence or during the interepidemic period. There has also been relatively little investigation of the factors that determine the fluctuations in incidence and distribution of communicable diseases or of those fundamentals that are of importance in determining whether an infection regresses spontaneously or evolves into an overt disease. There is evidence that such factors as climate and season and the nutritional state and hereditary constitution of the host are factors in the natural history of microparasites, but there is little experimental data to indicate just how these determinants influence the spread and survival of the infective agents.

It was recognized early by such investigators as Koch, Pasteur, and Pettenkofer that, while specific agents caused specific illnesses, many other factors were also important in determining whether an individual harboring the infectious agent became diseased. Later workers have expressed similar views—the most recent, Burnet (*1*) and Dubos (*2*). However, although there has been much speculation, science has yet to define the circumstances which determine why in certain infections many individuals become infected but few become diseased.

The importance of the biological approach to epidemiology was fully appreciated by Frost (*3*). In 1934 Theobald Smith, in his Vanuxem lectures on parasitism and disease delivered at Princeton University, formulated concepts which were fundamental to the explanation of these phenomena.

Hamer (*4*), Soper (*5*), Hedrich (*6*), McKendrick (*7*), Wilson and Burke (*8*) and Reed of Johns Hopkins University tried to rationalize the occurrence of epidemics by the use of sta-

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tistical formulas. Given the number of cases of measles, the number of susceptibles, the total population, and assuming an arbitrary value for contact rates in one time period of 14 days, the number of new cases which will arise in the successive time periods of the same length can be calculated. Epidemic theory of this sort yielded some interesting concepts about the spread of contagious disease. However, for infectious diseases, the practical usefulness of this statistical theory is quite limited (*9*). It is impossible with these criteria to take into account and to evaluate the many factors influencing the propagation of an infectious agent in nature.

Another approach to the study of the epidemiology of communicable diseases is the mass serologic survey for which Paul has coined the term "serologic epidemiology." This method has proved of value in such diseases as poliomyelitis and yellow fever. However, in recent years it has become obvious that in certain communicable diseases, such as those caused by certain arthropod-borne viruses, the results of the mass serologic survey technique must be interpreted with the utmost caution. There is an immunological overlapping among the various members of the arthropod-borne viruses, and, too, certain of these viruses appear to require accessory labile human serum factors for the neutralization test. While this latter difficulty can be overcome by adding fresh normal human serum to all neutralization tests, there is no way to reduce the error which arises from the serologic relationships among viruses.

At the writer's laboratory in the Johns Hopkins University School of Hygiene and Public Health, we have approached the study of infectious diseases by attempting to analyze some of the ecological factors in the natural history of certain microparasites. Principally, we have been interested in the part played by the infected human or animal and in such phenomena as the origin of the first infection or case of the disease, the relation between infection and overt disease, the interepidemic reservoir, activation of latent infections, and factors in nature that determine the variation in virulence and antigenic composition of the causative agents. In all this work we have tried to use experimental conditions approximating as closely as possible those that appear to exist in nature. We have used the original isolation of the microparasite whenever possible and infected the experimental host in as natural a way as possible.

Many of our experiments during the past 5 years have been directed at understanding the factors influencing the survival of rickettsiae in nature. More recently, this work has been expanded to include human respiratory and arthropod-borne viruses.

The remaining part of this report is concerned with a general discussion of our results thus far together with the related findings of many other workers. Certainly, the point of view presented is not new, but it is one that has not received as much investigation as it deserves.

Attempts to solve these problems involve long-term studies. For this reason, particularly in our virus investigations, only preliminary data are available. The research program described in this report is a large and varied one. It was specifically organized in this manner in order to train workers in the use of a combined field-laboratory approach to disease problems. We have found that this is best done if the investigator is able to work with different diseases that have different survival mechanisms in nature.

The first experiments deal with the rickettsial diseases, Rocky Mountain spotted fever (RMSF) and epidemic typhus. Subsequent discussion deals with human respiratory diseases and certain arthropod-borne viruses.

Rickettsial Studies

It was established by the classical work of Ricketts (*10*) and Wolbach (*11*) that *Rickettsia rickettsii*, the etiological agent of RMSF, is maintained in nature, first, by transovarial and transtadial passage in various tick vectors, and, second, by infected ticks biting susceptible animals which can then infect uninfected ticks feeding on these animals. Early work by Spencer and Parker (*12*) and more recent studies in our laboratory have further shown that *R. rickettsii* can exist in its arthropod vectors in a phase that is avirulent for animals (*13*). Virulence can be restored by passage through one egg or by keeping the tick at 37°

C. for 24 hours or by a blood meal (*13*). The avirulent phase and its reactivation have been observed in the field, and it, therefore, may be presumed to play a role in the natural history of this agent (*13*).

This work shows that an infective agent may persist in the host's tissues over a long period of time in a form not detectable by laboratory techniques. In view of this possibility, the failure to detect an agent by the usual infectivity tests does not necessarily mean that the parasite is not present. Accordingly, when studying the natural history of a microparasite, it is desirable, when practical, to test for the presence of an agent not only by infectivity tests but also by challenging animals with a known virulent suspension of the organism being investigated or by interference tests (*14*). For example, in studying the natural history of a mosquito-borne virus, the failure of a mosquito suspension to cause disease when injected into a mouse does not necessarily mean that the agent is not present. It is conceivable that the agent is there but is in an avirulent, or masked form. This same mouse, challenged one month after the initial test inoculation with a virulent suspension of the virus, may prove to be immune because antibodies were stimulated by the avirulent phase.

Field observations and laboratory experiments with *Rickettsia prowazeki*, the etiological agent of epidemic typhus, have shown that many persons may harbor the microparasite years after infection (*15–17*). The microparasite may become reactivated and cause Brill's disease, or recrudescent typhus, in the hosts. These persons may then infect human body lice which feed on them (*16*). Man, therefore, may serve as an interepidemic reservoir for this agent, as originally proposed by Zinsser (*15*).

The question of latent infections and what activates them is one of the practical, fundamental problems of infectious diseases, both to host and to the scientist seeking to learn how the microparasite survives in nature. The importance of this problem was pointed out by some of the earliest investigators of infectious diseases, and more recently by Shope (*18*, *19*).

The primary objective of this type of study, of course, is to try to determine the factors that initiate infection. Once the disease occurs, the agent may be carried from host to host in a manner totally unrelated to the activation process. For example, in swine influenza the virus exists in the lung worm which is harbored in the lung of the swine. The virus is in a masked state. Following some provoking experience, the virus is activated; the animal then becomes sick with swine influenza and can spread the agent to other swine by contact (*19*). With typhus, once a louse feeds on a person who has recrudescent typhus, the louse to man to louse cycle can foment an epidemic.

It is still far from clear why specific microparasites vary in incidence and cause epidemics when they do, and why epidemics subside when they do. In our studies on RMSF, for example, seasonal tests on approximately 3,000 ticks for 4 successive years in an area in Maryland showed that the percentage of infected ticks varied only from 0.2 to 0.3 percent each year. Yet during this time there were each year many nonimmune susceptible animals and a countless number of uninfected ticks in the area. The uninfected ticks from this locality could be readily infected in the laboratory by strains isolated in the area. One possible conclusion is that one or more unknown factors in nature contribute to maintaining RMSF in this locality (*20*).

Influenza

Profile of an Epidemic

In our respiratory study, approximately 3,000 persons are under intensive observation; 800 of these are student nurses and medical school students in the Johns Hopkins Medical Institutions.

During the winter of 1954–55 there was an outbreak of influenza B in the 800 students. It began the middle of December, reached a peak the middle of January, and subsided about the second week of February. About 20 percent of the 800 subjects were infected with influenza B as determined serologically by the hemagglutination-inhibition test.

Some interesting data have been accumulated for the 240 student nurses in the group. These nurses are between the ages of 18 and 22. They all live in the same dormitory and eat at the same cafeteria. During the influenza B

outbreak approximately 20 percent of each of the first-, second-, and third-year classes showed serologic evidence of influenza B infection. Of primary importance, however, was the isolation in early January of three A-prime strains of influenza virus from three of the student nurses during the influenza B outbreak. The nurses from whom these viruses were isolated all showed at least a fourfold hemagglutination antibody rise against this virus and were clinically ill with influenza.

None of the other 237 nurses showed any evidence of influenza A-prime infection in serologic tests comparing blood samples taken in October with those taken the end of February and again in April. The hemagglutination-inhibition titers of their serums against these three A-prime isolations were low, 60 percent of them showing titers of less than 1:32. Seventy percent of the nurses showed blood-neutralizing serum titers of less than 1:32 against the A-prime virus. The neutralization antibody titer of their nasal secretions was usually about tenfold lower than the serum titer. These low titers were very similar to those observed when there was an A-prime influenza outbreak in the student nurse population in January 1952. As Francis (*21*), who was the first worker to find influenza antibody in nasal secretions, originally pointed out, the antibody titer of such secretions is very important if the pathogenesis of influenza is considered.

Here we have a situation in which the virus was present in a group that should contain a relatively large number of nonimmune persons. And yet there was no influenza A-prime epidemic, although conditions were favorable for an influenza B epidemic. The possibility that influenza B somehow kept the A-prime epidemic from developing must be considered, but it is difficult on the basis of what is known to believe that this is fact.

We have compared the virulence of the A-prime viruses isolated from the student nurse population during the peak of the epidemic in the winter of 1952 with the virulence of the A-prime viruses isolated from the three student nurses during January 1955. Samples of nasal secretions and throat washings from the latter three nurses and from three nurses clinically ill with influenza at the height of the 1952 epidemic showed no statistical difference in amount of virus. All samples were collected during the first 5 days of the disease and each sample was titrated in human embryonic kidney.

Two isolations from each year were tested. Five volunteers were used for each isolation, 20 in all. The viruses were all in the O phase representing the first amniotic passage. All inoculums contained the same number of particles as determined by titration in tissue culture using human embryonic kidney. The volunteers were between the ages of 19 and 26. They all had serum neutralizing antibody titers of less than 1:32 to the A-prime viruses. Two of them had a very slight neutralizing titer of 1:2 in their nasal secretions.

Eight of the ten volunteers inoculated with the 1952 epidemic strain and 7 of the 10 inoculated with the 1955 A-prime viruses developed clinical influenza. Both of the subjects who had slight neutralizing titers in their nasal secretions developed influenza. Four of the volunteers who did not develop clinical influenza had neutralizing titers in their serums and nasal secretions as high as those who developed influenza. The fifth volunteer who did not develop influenza showed a twofold increase in the neutralizing titer of his serum, but he had no detectable titer in his nasal secretions.

Similar results were obtained when the virus inoculums were diluted thirtyfold and given to another 20 volunteers.

On the basis of these tests there was no difference in the virulence of the strains of A-prime isolated in 1952 and 1955. We, therefore, have no clues as to why the A-prime epidemic occurred in January 1952 but not in 1955 since we have no evidence that antibody levels in the host or virulence of the influenza strains were the important determining factors.

The relation between antibody titer and resistance was further investigated in the 1954–55 influenza B outbreak among the student nurses and medical school students. From table 1 it is obvious that the higher the hemagglutination-inhibition titer of the individual's serum, the less chance he had of showing clinical influenza. The hemagglutination-inhibition titers given in table 1 were observed in October 1954, 2 months before the epidemic started, as measured against the influenza B virus causing the

Table 1. The relation between blood serum hemagglutination-inhibition (HA–I) titer, October 1954, and incidence of influenza B, December 1954–February 1955

Persons tested	HA–I titer	Percent showing clinical and serologic evidence of influenza B infection
50	<32	58. 0
73	32	35. 6
178	64	17. 4
119	128	7. 6
51	256	3. 9
9	512	1. 1
2	1, 024	0

epidemic. Epidemiological and laboratory studies indicated that in the student nurse group about 85 percent of those who developed influenza B had clinical illness, 15 percent of the cases being subclinical.

Many earlier workers have found that many persons with a high blood antibody titer seem to be more resistant to influenza than those with a low antibody titer. However, this statement is not uniformly true. In recorded instances, persons with high antibody titer have developed influenza and those with low antibody titer have escaped infection. In the student nurse group, many nurses with low blood and nasal neutralizing antibody titers escaped infection although they received as much exposure as those who developed influenza. And some of the infected nurses had much higher antibody titers, both in their serums and nasal secretions, against the epidemic strain.

It is of interest to speculate that perhaps genetic differences result in greater resistance or susceptibility, and we are examining this hypothesis.

Seasonal Incidence

Why most influenza epidemics occur in the winter bears on the whole enigma of the seasonal incidence of many infectious diseases. In the Baltimore area, for example, there is no record of an influenza epidemic between the months of May and November.

It is felt that three factors determine the response of the individual to influenza: exposure to the agent; antibody titer; and one or more unknown resistance factors. Proposed as a working hypothesis is the theory that the unknown factor is a virus resistance mechanism that is lowered during the winter months.

Studies made in an adult group over a 2-year period, from May to October of each year, in the Maryland area have shown that the influenza virus was not spread by subclinical infections in the group during summer months. Hemagglutination-inhibition titers of paired serums collected during these two periods failed to reveal one case of influenza in a population of more than 2,000 persons. About 10 percent of this group had influenza during the winter months of 1951, and about 20 percent of the group had influenza in the winter of 1952.

If influenza is being spread in this population during the summer months, it does not result in detectable antibody formation. We checked this point since it may have been argued that there were influenza cases during the summer months but for some reason such cases did not result in clinical symptoms.

Not only was no influenza found in our study group during the summer months, as measured by at least a fourfold rise in hemagglutination-inhibition titer, but the virus could not be recovered from these individuals 2 months before the 1952 influenza A-prime outbreak. Throat washings collected from 800 persons in the group the first 2 weeks of October 1951 failed to yield an A-prime isolate as determined by three amniotic chick embryo passages. In these tests each throat washing was inoculated into the amniotic sacs of three chick embryos. After 72 hours, the amniotic fluid was collected and tested for hemagglutination in the conventional manner, using human type O red blood cells. The three negative amniotic fluids were then combined, and this material was inoculated into three more chick embryos and tested as described. The whole procedure was repeated once more. During the winter epidemic the A-prime virus was readily isolated.

In 1954, during the first 2 weeks of November, we failed to isolate one influenza B virus strain from throat washings collected from 500 nurses in the Johns Hopkins Hospital. The attempted isolations from the throat washings were made by passing each washing three times

amniotically in chick embryo and testing for influenza virus at each egg passage by the conventional hemagglutination technique; 302 of these washings have also been passed in monkey kidney tissue culture without yielding any influenza B isolations. Thus, 6 weeks before the influenza outbreak occurred in the nursing group, no influenza B was isolated, although approximately 20 percent of the nurses contracted the virus infection from the end of December to the middle of February. Virus isolations of influenza B virus were readily made during the epidemic by the same methods that failed to yield virus isolations before the epidemic.

Although these results are admittedly based on small numbers, they do at least suggest that in these two instances neither the A-prime virus nor the influenza B virus had been widely seeded before the outbreak. These results are of interest in view of the hypothesis of Andrews (*22*) that the virus may be seeded in the population before erupting into an epidemic. It is, of course, always possible that the virus may be seeded in some form that cannot be detected by either the chick embryo or tissue culture techniques, or the virus may be in some tissue where it would not be collected by throat washings. Andrews presented his theory, in part, to account for the early summer "flurries" of influenza that have preceded many influenza outbreaks in late fall and winter. In all these instances, after the early summer cases of influenza there were no cases of influenza for several months preceding the epidemic. Although we have no evidence that the virus is seeded during this period, here again we have evidence that with the coming of summer months human cases of influenza stop occurring (*22*). However, in the late fall there is a sudden outbreak of the same influenza strain that had occurred in early summer.

Survival of Human Influenza in Nature

Much has been written about the biological survival mechanism of human influenza in nature. A good summary of the many hypotheses is contained in a recent article by Andrews (*22*). Although it is impossible in this article to go into all of the various aspects of the epidemiology of influenza, several points should be mentioned. First, there is no evidence that a host other than man is concerned with the survival of human influenza in nature. Second, one important survival mechanism of influenza is the spread of the virus from one country to another. But, even if one pictures a yearly swing between the Southern and Northern Hemispheres, influenza A does not break out in Europe every winter (*22*).

Although it is difficult to find influenza in a country between epidemics, the U. S. Army Commission on Respiratory Disease (*23*) reported that during World War II it was able to find influenza in the United States practically every month of the year. This being so, it would appear that influenza could be maintained sporadically throughout the year, with an epidemic when the environmental factors were right.

There are also several reasons for believing that the activation of latent influenza virus may be a factor in its survival, as first suggested by Shope (*24*). This is an extremely difficult problem to investigate because of the widespread nature of this disease and the difficulty of ruling out the possibility of infection from a contact. The fact that the Army commission found sporadic cases of influenza throughout the year does not rule out the possibility that some of the cases represented activation of latent influenza infections, much as Murray's (*25*) studies in Yugoslavia showed that some of the sporadic cases of louseborne typhus fever were really due to activation of latent typhus infections and not to louse bites.

During the summer of 1954, we isolated by tissue culture methods three influenza A-prime viruses from the lungs of patients who had undergone lung operations for various conditions. These strains were not laboratory contaminants since they did not kill mice, whereas the strains of influenza virus used in the laboratory killed mice readily. Since the last big A-prime epidemic was in the winter of 1952, it is felt that these patients had harbored the virus for at least 1½ years. Of course, this assumption would be difficult to prove because of the everpresent possibility of superinfection.

However, it seems to me that some of the persons who have an influenza infection compli-

cated by a bacterial infection might possibly harbor the virus in their lungs. It is of interest that the individuals from whom we isolated the A-prime virus did not have any evidence of clinical infection of influenza, according to their family physicians, for at least a year before their operations. But, here again the possibility of subclinical infection cannot be ruled out.

In support of the reactivation hypothesis, we have found that latent influenza infections in laboratory animals may be activated under certain environmental conditions.

Latent influenza infections have been reactivated in a number of ferrets that had recovered from a previous infection of influenza A-prime and were subjected to cold weather. This phenomenon has been identified by specific serologic tests. The same number of previously noninfected control ferrets showed no evidence of the disease when subjected to the same environmental conditions at the same time. At least two conditions seem essential in demonstrating this reactivation. The ferrets must have recovered from a severe influenza infection, and the influenza antibody titer, as measured by neutralization tests, must be low. It is not clear at present which organ or tissue contains the virus at the time of reactivation. In the only extensive tests made so far, using carefully perfused lungs of ferrets, no active virus was revealed in the lungs. In the tests, 20-percent suspensions of the lungs were passed three times in the amniotic and allantoic sacs of 11-day-old chick embryos. Infectivity was gauged by measuring the hemagglutination titer of the amniotic and allantoic fluids, using chicken and guinea pig red blood cells.

If an activation of a latent influenza infection does occur in nature, the question arises whether the latent virus exists in a fully infective form in some organ such as the lung or whether it exists in a "lysogenic" form. This latter virus phase has been described only for certain bacterial viruses (*26*).

In this phase, the virus exists in a noninfective phase (prophage) which appears to be attached to the bacterial nucleus. All attempts to detect the virus in this phase by splitting open the bacteria and testing for virus infectivity or by serologic tests are completely negative. When the cell divides, each daughter cell contains this incomplete virus. Under certain conditions, the prophage can be activated to form fully infective particles which are liberated from the cell and can then infect all other susceptible bacteria. This represents a model reservoir virus system, and a great deal of work is now going on in our laboratory, as well as in many other laboratories, in an attempt to see if such a situation exists for animal viruses.

It should, perhaps, be pointed out that the fairly rapid decline in antibody titer in humans and laboratory animals after influenza infection does not necessarily mean that the provirus could not be present in the host. Indeed, if a provirus of influenza did exist, it would not be expected to give rise to antibody formation since the provirus of any system that has been studied is not antigenic as tested by any known laboratory procedures.

Immunology and Virulence

Still to be answered is what determines the immunological and virulent properties of influenza virus in nature.

Some epidemiologists have voiced the opinion that the rise and fall of an epidemic is governed by the virulence of the agent. During the early part of the epidemic, they have speculated, the virulence of the agent may be increased by rapid passage from human to human, but as the number of immune individuals increases there is less frequent passage and the virulence of the strain is decreased.

Webster concluded that such changes in virulence play little, if any, role in determining the rise and fall of epidemic waves (*27*). His experiments, however, were carried out under laboratory conditions with particular bacterial systems. In the natural state, parasites encounter ecological situations far more complex than in the laboratory, and such situations conceivably can influence virulence. For example, changes in the micro-organism may occur when such agents infect persons possessing antibody.

There is no doubt that influenza isolations do vary in virulence and antigenic composition in an epidemic. In 1952 in a hospital ward at a home for the aged in Baltimore, we made 13 virus isolations during an outbreak of influenza A-prime. Careful study showed that this out-

break arose from the introduction of an infected person into the ward.

Using a combination of 10 A and A-prime influenza viruses and the absorption technique of Jensen and Francis (*28*), we demonstrated immunological differences among these 13 isolates. For example, an isolate from one patient showed major antigenic components related to the Sweden and Rome prototypes, while another isolate showed major components only to the Sweden and English prototypes. A third isolate was related only to the Rome and Malayan influenza virus prototypes. There was no apparent relation between the antigenic composition of the virus isolated and antibody levels before or after infection.

All viruses isolated were passed five times in chick embryos before being used for the absorption tests. The three differing isolates described were also "purified" by three limiting dilutions in chick embryos, the second chick embryo passage being used for the dilution "purification." This was done in order to work with clones as pure as possible. Viruses prepared in this manner still showed the same immunological differences.

The 13 virus isolates could also be broken up into three groups on the basis of their behavior in chick embryos, ferrets, mice, and by tissue culture. In these tests the isolations also were purified by the limiting dilution technique. Although compared on a quantitative basis at various dilutions, 2 of the strains could not be established in ferrets even after four blind passages; 8 of the strains gave a good reaction in ferrets, and 1 isolation resulted in a mild reaction in the animals.

Some investigators have recently proposed that the recombination phenomenon might be important in determining the virulence and immunological properties of influenza viruses in nature. In this phenomenon, reproduced under laboratory conditions, two strains of influenza virus infect a cell, and some cells yield a virus that is different from the original two infecting viruses (*1*, *29*). However, whether such a phenomenon occurs under natural conditions is open to speculation. Taylor (*30*) has suggested that perhaps the passage of influenza virus through persons having antibodies to various influenza types would have some part in determining the immunological and perhaps the virulent properties of influenza strains that appear in nature. Work by Archetti and Horsfall (*31*) and Gerber and co-workers (*32*) have shown that Taylor's hypothesis can be made to operate in the laboratory.

In further work, we have experimented on the effect of subsequent influenza infection upon the antibody response of laboratory animals previously infected with various influenza viruses. The objective was to obtain a laboratory model to test further the interesting theories of Francis and co-workers that the initial influenza virus infection is important in determining the type of antibody produced by a subsequent influenza infection. They based this idea on a survey of the influenza antibody titers of different age groups. We used 5-week-old Swiss mice and inoculated them intranasally with influenza A-prime virus (FW–1–50), an A-type virus (WS), or swine influenza. Enough virus was inoculated to kill about 15 percent of the animals in all groups. Fifty days after infection the surviving mice in each group were divided into three groups and inoculated intraperitoneally as shown in a typical experiment (table 2). Antibodies were tested by the neutralization test in mice. It is apparent in table 2 that the first infection determined the type of antibody formed when the mice were vaccinated with the different influenza strains.

It was thought of interest to challenge mice intranasally after observing the intraperitoneal effects, since an intranasal test would approximate the conditions in nature. In these tests the mice had a greater tendency to produce antibody to the second virus infection than the first (table 2). It is felt that the mice were truly infected by the intranasal inoculation, whereas they were merely vaccinated by the intraperitoneal route. This may account for the difference in antibody response.

In order to test whether the above phenomenon might occur in humans on immunization, 10 children, 6 to 10 years old, were injected with a swine influenza vaccine. These children had no neutralizing antibodies against the swine influenza virus when their serums were tested in a dilution of 1 : 16. Their neutralizing titers varied between 1 : 64–1 : 128 against the FM1 strain of influenza. Three weeks after

vaccination with swine influenza, 8 of the 10 children showed neutralizing titers to swine influenza between 1:64–1:256 (mean titer 1:128). However, their titers to FM1 varied between 1:512–1:4096 (mean titer 1:2048).

Five adults, ages 40 to 45, were injected with the same amount of swine influenza vaccine. These adults had initial titers to swine influenza between 1:64–1:256. Their initial titers to FM1 varied between 1:32–1:128. Three weeks after the injection of the same amount and the same swine influenza vaccine that the children received, they showed titers to swine influenza virus varying between 1:512–1:2048. Their FM1 neutralizing titer varied from 1:512–1:2048 (mean titer 1:1024). These results appear similar to those reported for the mice, since the adults, according to the work of Francis, would have had early experience with swine influenza while the children would not. This work, therefore, supports the hypothesis of Francis that the initial influenza virus infections orient the antibody response produced by subsequent influenza infections under the experimental conditions employed.

It would appear that this phenomenon is not only of importance in the natural history of influenza, but would also be of great importance in considering how to control this disease.

RI-APC Viruses

Another puzzling problem in respiratory viruses is that concerned with the natural history of the new RI–APC group. Hilleman (*33*) found that 70 to 80 percent of the recruits entering the Army got the APC infection 9 months after they were inducted. Of those recruits inducted during the winter, 70 to 80

Table 2. The effect on antibody response of intraperitoneal and intranasal inoculation of influenza viruses in mice previously infected with different influenza viruses

Primary infection type	Secondary treatment, intraperitioneal or intranasal, 50 days later	Viruses tested against	Mouse neutralization titer X increase after second treatment [1]	
			Intraperitoneal	Intranasal
FW–1–50	Swine	Swine	16	4
		WS	4	2
		FW–1–50	64	64
	FW–1–50	Swine	0	4
		WS	0	0
		FW–1–50	128	64
	WS	Swine	0	16
		WS	4	8
		FW–1–50	128	0
Swine	Swine	Swine	512	64
		WS	2	2
		FW–1–50	0	8
	FW–1–50	Swine	64	0
		WS	0	0
		FW–1–50	4	64
	WS	Swine	128	64
		WS	4	8
		FW–1–50	0	2
WS	Swine	Swine	0	64
		WS	8	32
		FW–1–50	2	4
	FW–1–50	Swine	0	8
		WS	16	4
		FW–1–50	2	4
	WS	Swine	0	8
		WS	128	64
		FW–1–50	0	2

NOTE: The above experiment was repeated 3 times with similar results. Influenza B given as the secondary treatment did not cause any increase in antibodies to WS, FW–1–50, or swine influenza virus.

[1] The serums of 4 mice were pooled for each test.

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percent developed the respiratory infection within 2 or 3 months after joining the Army. RI–APC virus types 4 and 7 appear to be involved.

During a study of 2,015 first-year student nurses, medical school students, and college freshmen in Maryland, Pennsylvania, and New Jersey for a little over 2 years, only 4 percent of them developed APC infections. Methods described by Hilleman (*34*) were used to detect infection. Blood samples were taken every 4 months over a 24-month period. The APC complement fixation (CF) titers of the serums collected at the first interval were compared with the titers of the serums collected at the subsequent intervals. Since the APC antigen reacts with all types of the RI–APC viruses (*35*), failure to detect an increase in titer against the APC antigen would indicate that these students did not develop any type of APC infection which resulted in a titer increase. Since we have found that with an APC infection the complement fixation titer remains at an elevated level for at least 4 months, the interval between tests should have been adequate to detect any antibody rise.

In view of the large number of recruits who came down with APC infections, we feel it surprising that so few of our study group showed the same type of infections since 80 percent of them were of the same sex and age as were the recruits and were subjected to similar, but by no means identical, environment.

In the student nurse group at the Johns Hopkins Hospital, four of the students developed infection with type 3 virus of the RI–APC group. Ninety-one immediate contacts, including roommates of these four nurses, were intensively studied by serologic tests and isolation attempts for 5 weeks (*34*). In spite of the fact that the serums of 71 percent of these contacts showed no complement fixation titer to APC viruses at a dilution of 1:4 or no neutralization titers at a dilution of 1:2 against type 3 virus, not one of the individuals showed any signs of APC infection.

In another study of 1,051 human respiratory illnesses in adults from the outpatient departments of the Johns Hopkins Hospital, Sinai Hospital, and Baltimore City Hospital, which laboratory data showed were not influenza or of bacterial origin, only 4 percent of the illnesses were found to be caused by RI–APC viruses.

A similar low value has so far been found in 1,115 other persons we have been following in the Maryland area. This group is made up of families, adults with chronic disease, and adults between the ages of 60 and 80 who have no chronic physical ailments. Blood samples are taken every 4 months. The RI–APC complement fixation titer of their serums is then compared to their baseline level. Only 4.3 percent have shown rises in their RI–APC CF titer during the 1-year observation period. These rises would include not only clinical infection but subclinical infection with the RI–APC agents.

All these data would seem to indicate that much more investigation is needed before we can be sure just how important the RI–APC viruses are in the civilian population. It is entirely possible that the RI–APC agents may be of clinical importance in children in the civilian population, but this still would not explain why Hilleman found a 70 to 80 percent infection rate in recruits during their first 9 months in the Army.

We have no clues as yet as to why recruits develop such a high incidence of the disease. A combination of emotional strain, physical activity, and hygienic conditions or physical activity and hygienic conditions alone may be the determining factors, since the recruits are subjected to much more strenuous exercise and poorer hygienic conditions than the student group we are observing.

The work of Huebner and co-workers (*35*), who observed that the RI–APC viruses can be found in the adenoids and tonsils of many normal individuals, leads me to wonder whether activation of latent infections may also enter into the survival mechanism of these viruses in nature.

Arthropod-Borne Viruses

Serologic Relationships

Recent work by several investigators has revealed that certain arthropod-borne viruses are more closely related immunologically than had

been thought (*36–39*). Work in this laboratory has been done on West Nile (WN), Japanese B (JB), Murray Valley (MV), and St. Louis (SL) viruses.

We have found that hamsters infected with Japanese B virus and permitted to recover are protected against a subcutaneous challenge of approximately 100 LD_{50} of West Nile or Murray Valley virus. Previous failure to observe the cross-protection between these viruses was due to the fact that in all preceding experiments mice were challenged intracerebrally. In order to see whether this immunological relationship affected the natural history of these viruses, the following experiments were carried out.

Three-day-old chicks were infected at intervals with 100 mouse LD_{50} of JB virus and were later mated. The nestlings of these birds were subjected to further study since work of others indicates that nestling birds have an important part in the epidemiology of these arthropod-borne viruses. The nestling progeny of the infected birds contained antibody to the JB virus which was transferred through the egg from the hen. These nestlings were then infected when 4 days old by subcutaneous inoculation with 1–10 mouse LD_{50} of WN virus or by the bite of *Aedes aegypti* infected with WN virus. Similar results were obtained with both methods of infection. Birds of the same age, species, and not previously infected were used as controls. The control birds showed maximum viremias to WN virus of approximately 10^4 mouse LD_{50}, whereas the progeny of the birds previously infected with JB virus showed a maximum viremia of approximately 10 mouse LD_{50} per 0.03 ml. of blood. Half of the uninfected *A. aegypti* feeding on the control birds became infected with WN virus. The West Nile virus was found in only 4 of the 100 tested uninfected mosquitoes which fed on the birds previously infected with JB virus and then WN virus.

The tests for WN virus were made by incubating the mosquitoes for 14 days, making suspensions of them, and inoculating these suspensions into suckling mice, 4 mice being used for each suspension. In all experiments mosquitoes of the same age and lot were used and were fed at the same time in the same numbers 1 and 2 days before the maximum viremia as well as on the day of the maximum viremia.

In experiments conducted to test the transmission potential of the two lots of mosquitoes in one-half-day-old chicks, 28 percent of the mosquitoes that fed on the control birds were capable of transmission, whereas only 2 of the 100 mosquitoes tested in the group which had fed on the nestlings previously infected with JB virus were capable of transmitting WN virus.

All of the mosquitoes from this latter lot were also tested for WN virus after their transmission tests. They were kept for 5 days at room temperature and ground up. The suspensions were injected into mice. Two mosquitoes showed evidence of WN infection. All mosquitoes were kept for 21 days before virus transmission to chicks was attempted. Similar results were obtained in the above test system when Murray Valley or St. Louis encephalitis viruses were substituted for Japanese B virus.

These experiments approach conditions found in nature. In certain areas where a large majority of animals and humans have been infected with one type of the viruses mentioned, the serologic overlapping may tend to limit the chances that a related arthropod-borne virus will establish a foothold in the area, the result depending upon the viruses involved. It is also apparent that previous infection with one of the viruses will be of obvious importance in determining whether an individual infected with a related arthropod-borne virus develops an overt disease. In this connection, one wonders whether all the arthropod-borne viruses should be classed as neurotropic viruses. It is perfectly true that some cases result in neurotropic symptoms. However, for every host that develops neurotropic symptoms of Japanese B, Murray Valley, or St. Louis encephalitis, there may well be a thousand infected individuals who show no clinical symptoms (*1*). The neurotropic virus may be a rare type in the virus population, most of the viruses that make up the various members of this group being non-neurotropic.

The isolation of these viruses by intracerebral inoculation of mice would favor the isolation of any neurotropic variants. It would be of particular interest to compare the viruses thus iso-

lated with those isolated by chick embryo techniques and various tissue culture procedures.

This problem is important. If the three viruses are not truly neurotropic, the pathogenesis of these diseases would have to be viewed in a different light, and the failure of most individuals to show neurotropic symptoms would not be due primarily to the resistance mechanism of the host but to the virus which infected the host.

This immunological relationship between arthropod-borne viruses may also have practical application in working out vaccination procedure against certain of these arthropod-borne viruses. For example, the killed Japanese B vaccine now in use gives little protection against the virus as measured by its ability to elicit neutralizing antibody. However, we have observed that if the same amount of killed JB vaccine is given to persons who had no previous exposure to JB virus but who had a previous WN infection, a considerable increase in JB neutralizing antibodies is observed (table 3). Serum samples of the 14 subjects were tested before treatment. None of their serums diluted 1:2 neutralized 30 mouse LD_{50} of JB virus. Six weeks after the subjects had been infected with West Nile virus or injected with killed Japanese B vaccine, they were given a subsequent intramuscular injection of the killed JB virus vaccine. All serum dilutions were made in fresh normal human serum. None of this latter serum neutralized 30 mouse LD_{50} of JB virus when diluted 1:2. The values in table 3 give the maximum neutralization titer after the initial treatment and 6 weeks after the subsequent killed JB vaccine injection. Weekly blood samples were taken.

We do not know as yet how long these antibodies will last in such individuals. However, it seems to me that it may be possible by using an attenuated strain of one of the arthropod-borne viruses such as WN, which shows serologic overlapping with many of the other viruses, to immunize the individual in such a manner that he can then be vaccinated much more efficiently with killed vaccines of the more virulent related viruses. It is also possible that if a person had a WN infection and was then vaccinated with JB killed vaccine, he would not only get better

Table 3. The effect of previous infection with West Nile virus on a subsequent injection with killed Japanese B virus [1]

Initial treatment	Neutralization titer to Japanese B virus after initial treatment	Neutralization titer after subsequent injection of Japanese B killed vaccine
West Nile infection	1:10	1:100
	1:5	1:80
	0	1:40
	0	1:60
	0	1:100
Killed Japanese B vaccine	0	0
	0	0
	0	0
	0	1:4
	0	0
	0	0
	0	0
	0	0
	0	0

[1] All titers refer to dilutions of serum which will protect 4 of 8 mice against approximately 30 LD_{50} of Japanese B virus.

protection against JB virus but would have some protection against other related viruses. In other words, WN infection or JB killed virus vaccine by itself would give little if any protection against Russian spring-summer (RSS) virus. But the combination of living WN infection plus killed JB vaccine may result in protection against RSS virus because of the immunological overlapping between WN, JB, and RSS viruses.

Our preliminary data support this hypothesis, and we are now in the process of determining which two viruses will give the best protection against a whole group of serologically related arthropod-borne viruses.

Immunological overlapping may also play a role in the evolution of some of these arthropod-borne viruses. For example, we have shown that if a host has antibodies to Japanese B virus and is infected with West Nile virus the multiplication of West Nile virus may be greatly inhibited.

However, if one infectious dose of WN virus were to contain a few particles that differ in their antigenic composition from the majority of WN particles, that is, if they were less closely related immunologically to Japanese B virus, these particles might multiply in the

above circumstances to the exclusion of the predominating WN particles. This situation would lead to a new antigenic WN virus population. If some of these virus particles were to multiply in another species of mosquito vector than can support the growth and transmission of the WN particles now predominating in nature, this insect vector could act as a further selective medium and give rise to a much different virus than the existing WN virus.

In view of what we know about the biology of viruses, such a speculation must be considered in a discussion of the natural history of arthropod-borne viruses.

Survival Mechanism

The big question that remains to be solved concerning the biological survival mechanisms of the arthropod-borne viruses is how they maintain themselves between epidemics. In spite of the brilliant work of the Rockefeller Foundation, we still cannot answer this question for yellow fever, nor indeed for any arthropod-borne virus. In this country, western equine encephalitis poses a similar problem. No ecological complex has as yet been described which will satisfy all the requirements for an interepidemic reservoir. It is possible that the western equine encephalitis virus is harbored by overwintering mosquitoes. Another possibility is that the activation of latent virus infections in the animal host may play a role in the survival mechanisms of some of the arthropod-borne viruses.

Many experiments in this laboratory carried out with various species of hard ticks as possible reservoirs for western equine encephalitis have been entirely negative. However, we have observed in this laboratory that Japanese B virus loses its infectivity for a time when grown in mosquito tissue culture. The methods used would have detected about 10 Japanese B virus infective particles. When active virus appears, the increase is much greater than could be accounted for on the basis of a few infective particles multiplying and giving rise to more infective virus. It appears that in the mosquito vector this virus goes through an eclipse phase similar to that described for many animal viruses in animal cells as well as for bacterial viruses in bacteria (*1*). Similar results have been reported for Murray Valley virus in *Culex annulirostris* by McLean (*40*). These findings, therefore, together with the fact that the multiplication of these arthropod-borne viruses in their insect vector does not appear to damage their cells, make one consider the possibility that in a few mosquitoes the virus may exist in a provirus-like state (*26*). In this phase the virus would be noninfective and nonantigenic under all the usual experimental conditions, but it could be activated into infective virus under certain conditions.

Conclusion

Although the task of curbing epidemics rarely confronts us in the United States, a major responsibility of public health today consists of anticipating and preventing epidemics. This phase of preventive medicine needs to be supported by studies of the interepidemic history of infectious organisms.

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