A Serological Study of Influenzal Antibodies[1]

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The purpose of this preliminary report is to give the data obtained from a survey of a population for antibodies to the influenzal agents A, B, and swine. These studies were initiated in order to ascertain the type of influenza strain that would manifest itself in the anticipated epidemic. That an epidemic of influenza was probable was dependent upon the findings that epidemics usually occur in cycles (*1*). The possibility of this epidemic being due to influenza B appeared remote in view of these cycles and past findings (*2, 3*). The consensus was that if an epidemic occurred, it would be due to influenza A.

MATERIALS AND METHODS

The strains and method of growth of these viruses[2] were the same as those described in a previous report (*3*).

One hundred random specimens of serum were taken from the serological service each week, starting the first week in September 1946. The original blood samples from which these sera were obtained were submitted to the Bureau of Laboratories for routine serological tests for syphilis.

Our procedure for the Hirst test was that suggested by Salk (*4*), but only three dilutions of the serum were tested. The sera were diluted in saline to make initial dilutions of 1:100, 1:500, and 1:1,000. To 0.5 ml. of serum dilution, 0.5 ml. of virus dilution (4 units) was added. To this mixture, 1.0 ml. of chicken erythrocytes (1 per cent) was added, and the test read after 75 minutes at room temperature. The presence or absence of a "button" was used to denote inhibition or agglutination.

RESULTS

Titrations of the sera obtained during September showed a rather high percentage of antibodies for influenza B, especially in the lower dilution. This level gradually decreased until it approximated the influenza A and swine influenza levels. There was little evidence of any antibody changes until the middle of December. A significant change then became apparent, with a steady increase in the number of individuals having antibodies to the swine virus. This increase was first noticed in the 1:400 dilutions of sera. Significant increases in dilutions 1:2,000 and 1:4,000 were not apparent until the middle of January. At this time, every serum tested showed at least a 1:400 antibody titer.

Fig. 1 shows this increase in antibodies among the population. Only that portion of the graph pertinent to the critical period is given here. It appears quite evident that the population in general has responded with antibodies specific for the swine virus. The determination of the cause for this increase in antibodies is difficult. At the time of this antibody increase there was no evidence of clinical epidemic influenza, a condition which still exists. Several volunteers donated blood samples, and the majority of these demonstrated titers above a final serum dilution of 1:2,000. There did not appear to be any relationship between the titers for the swine virus and a history of vaccination with commercial influenza A and B vaccine.

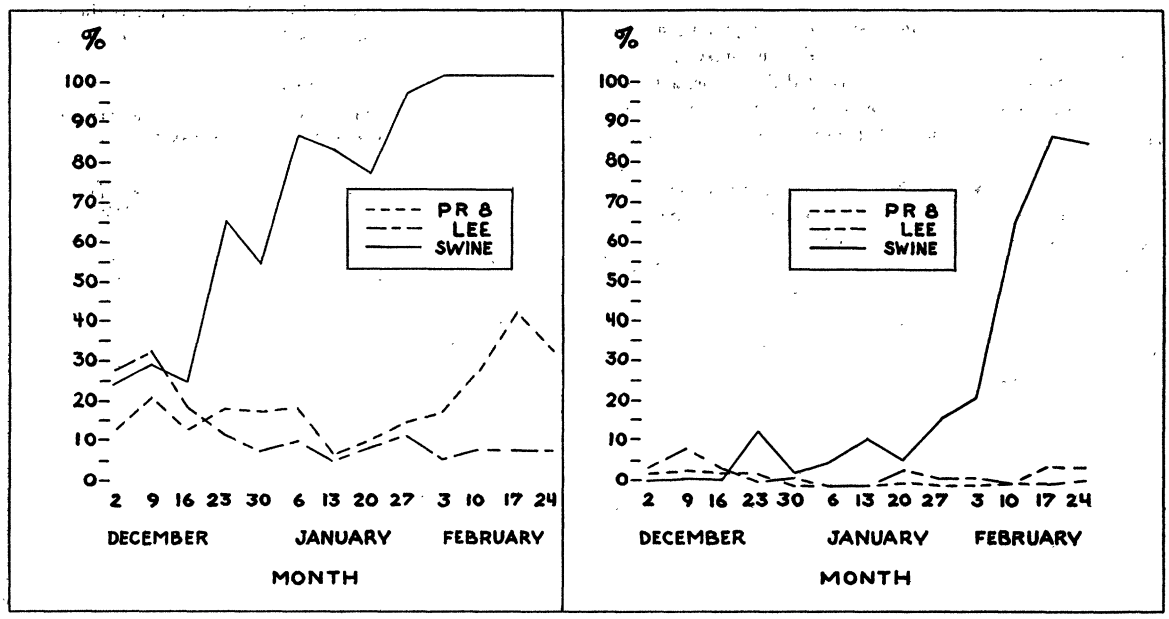

FIG. 1. Percentage sera with titers of 400 and 4,000 (final dilutions).

DISCUSSION

The results were quite unexpected. Studies of the relationship of this swine virus to strains of influenza A and to man are now in progress. Clinically, there was no evidence of epidemic influenza in this area. The only apparent evidence of

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[2] The PR8 and Lee strains were supplied by Dr. Werner Henle; the swine strain, by Dr. Gilbert Dalldorf.

infection in the population, concurrent with the antibody increase, was a marked increase in apparently typical coryza.

It is realized that controls in a study of this sort are difficult. At present we are engaged in studies on sera that had been collected before and after this increase in antibodies became apparent. In addition, throat washings have been obtained from several individuals with this antibody increase, and attempts at virus isolation are being made.

Addendum: Since submission of this report an epidemic of influenza has occurred in this area. Studies are now in progress as to the etiology of this epidemic and its relationship to the above findings.

References

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A Bacterial Disease of the Lobster (*Homarus americanus*)

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During the summer of 1946 lobster dealers in Maine suffered considerable losses due to heavy mortality of lobsters held in pounds and tanks. A spot survey indicated that losses attributable to a new disease varied from 20 to about 50 per cent and that the disease was widely distributed along the coast of Maine.

It was suspected at first that these losses might be caused by the use of DDT in some of the fish canneries. Some of the offal from the canneries, which is used as bait and food for the captive lobsters, might contain toxic amounts of DDT. Microscopic and bacteriologic examination of the diseased lobsters indicated, however, that it was more likely that the disease was caused by a bacterium belonging to the genus *Gaffkya*. Wherever there was a considerable mortality of the lobsters at the time of examination, micrococci of the *Gaffkya* type were found in the blood smears and the blood culture. The blood needed for the microscopic examination and for isolation of bacteriologic media was removed by a puncture from the ventral abdominal sinus with a sharp, pointed, Pasteur pipette following surface disinfection of the thin membrane between the somites of the abdomen.

The diseased lobsters had a pink discoloration of the ventral side of the abdomen; the blood was also pink, less viscid, and usually with much-prolonged clotting time. In advanced cases, in which there was a large number of bacteria in the blood stream, the number of blood corpuscles was sharply reduced.

The severity of the disease appeared to be decreasing with the onset of colder weather. At certain pounds the infected lobsters predominated in September, but in October they could not be found in the same pounds. However, an outbreak of the disease with typical symptoms, high mortality and the presence of *Gaffkya*, was reported in December.

The organism, which was regularly isolated from diseased lobsters in pure culture, could be grown fairly easily on the standard media. Solid and semisolid media prepared with the tryptic digest of casein and yeast extract gave very satisfactory results. On these the growth was reliable, but the amount of growth was always small. Colonies about 1 mm. in diameter, circular, convex, and grayish-white, were produced. In semisolid and liquid media scant granular sediment was produced. The organism neither changed litmus milk nor liquefied gelatin. It produced acid, but no gas, from mannitol, dextrose, sucrose, maltose, and lactose.[1]

In the fresh blood of the infected lobsters the organism was found to be present in tetrads surrounded by a wide pseudocapsule around the group. In media it produced irregular conglomerations without capsules. In stained preparations it was gram positive. It was also nonmotile.

A series of lobsters was inoculated intramuscularly and intravenously with the pure cultures of this microorganism. All inoculated lobsters died within two weeks with the typical symptoms of the disease, and from the blood of these lobsters the organism was reisolated in pure culture.

Two randomized experiments were made on the effect of the inoculation of normal lobsters and on the treatment of infected lobsters with sulfonamides, which have been found effective against some bacterial diseases of fishes (*1*). The lobsters were distributed, two to a tank, in randomized blocks. They were fed ground herring or redfish mixed with gelatin, to which sulfonamides or bacteria were added. Some were fed infected lobster.

The results, compiled in Table 1, indicate that the injection of pure culture of *Gaffkya*, isolated from diseased lobsters, invariably killed the lobsters within two weeks, even if they were treated with sulfonamides. Introduction of bacteria with food was ineffective, and sulfonamides seemed to reduce the mortality only among the lobsters which contracted the disease in a natural way.

TABLE 1

THE EFFECT OF ARTIFICIAL INFECTION OF LOBSTERS AND OF TREATMENT WITH SULFONAMIDES

Treatment	Initial No. of lobsters	No. 2 mortalities
Normal controls	30	2
Diet with bacteria (*Gaffkya*)	30	4
Normal controls		
Sulfamerazine	10	1
Sulfamethazine diet	10	1
Diseased controls	10	5
Diseased		
Sulfamerazine diet	10	1
Sulfamethazine diet	10	1
Normal		
Injected with bacteria	30	29
Injected with bacteria, sulfamerazine diet	10	10
Injected with bacteria, sulfamethazine diet	10	10
Injected with sterile medium	20	2
Fed infected lobster	10	1
Fed normal lobster	10	1

Reference

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[1] Detailed study of the organism is in progress at the Department of Bacteriology, University of Maine; the results will be published elsewhere.