eration to the next, it does not follow that the positive pools were infected by feeding on Hosts 21 and 22. The infection may have originated in the larvae of a previous generation feeding on another host species.

The evidence presented above establishes the recovery of *R. orientalis* from *Trombicula fletcheri* and indicates that this species of trombiculid mite serves as a vector of tsutsugamushi disease in northeast New Guinea.

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AGGLUTINATION OF STAPHYLOCOCCUS AUREUS IN THE PRESENCE OF CHORIO-ALLANTOIC FLUID FROM HEN'S EGGS[1, 2]

CERTAIN strains of *Staphylococcus aureus* will agglutinate when grown in the presence of normal[3] or influenza (PR8) virus-infected chorio-allantoic fluid. However, egg fluids vary in their ability to manifest this phenomenon, as is shown by the fact that a clumping of bacteria will occur in a dilution of 1 to 64 with some fluids and only 1 to 4 with others. Moreover, various strains of staphylococci, when cultured in the same chorio-allantoic fluid, may differ in their ability to show agglutination. Therefore it appears that there are two principal variables involved in the manifestation of this reaction, one associated with the organism, determining whether it will or will not be refractory, the other present in the fluid, determining whether or not it will exhibit the effect.

The phenomenon may be demonstrated by adding a drop of a six- to eight-hour nutrient broth culture of susceptible *Staphylococcus aureus* to varying dilutions, in extract broth, of normal or virus-infected chorio-allantoic fluid. These cultures are then incubated over-night at 37° C. and observed the following morning after being held at room temperature for an hour. Readings are made on a rough quantitative scale in a manner similar to that used in the conventional agglutination test; assigning values ranging from 1+ to 4+.

A wide variety of bacteria, including Gram-positive and negative cocci and rods, have been tested, but of those investigated only certain strains of *Staphylococcus aureus* and possibly *Staphylococcus albus* definitely participate in the reaction. *Staphylococcus citreus,* tested a few times, has been consistently refractory. The phenomenon may be elicited by using heat-killed staphylococci or living bacteria. While the agglutination takes place in a higher dilution of egg fluid when killed organisms are used, the clumps formed are usually smaller and the extent of reaction from tube to tube may vary irregularly. All the present observations were made with living bacteria.

Of the 31 cultures of *Staphylococcus aureus* studied, including 26 freshly isolated strains from human sources, two of animal origin and three laboratory strains, 10 of the human pathogens and one of the laboratory cultures were clumped when grown in egg fluids. While most of the agglutinable strains give an occasional negative reaction, those bacteria which are refractory are consistently so. The most outstanding example of the latter is the Wood 46 strain. On the other hand, a freshly isolated strain of staphylococcus, designated as "Craig," has always agglutinated in the presence of egg fluids infected with influenza virus and practically always with normal fluids. These two strains, therefore, have been used to evaluate the relative potencies of various chorio-allantoic fluids. Of 106 tests run with the Craig strain against normal fluid, only 11 failed to yield clumping. Of the 48 tests carried out with Craig and the virus-infected fluids, all showed agglutination. The Wood 46 strain has never shown agglutination with either normal (43 tests) or virus-infected (81 tests) egg fluids.

The agglutination of susceptible bacteria by chorio-allantoic fluid is not limited to any one medium in which the organisms may grow. All the media tried—extract broth, infusion broth, "Savita" and peptone broth—support the phenomenon. Everything else being equal, the extent of agglutination in these various media appears to depend upon the degree of growth or the number of the organisms present. However, there is one exception to this generalization, in that for some reason yet to be determined, susceptible staphylococci growing in a medium of vitamin-free casein digest, nicotinic acid, thiamin, biotin and salts are completely refractory to the effect of all chorio-allantoic fluids, normal and virus-infected.

It does not matter so far as the qualitative aspects of the test are concerned whether the staphylococcus-egg-fluid mixtures are incubated at 37° C. or at room temperature. If the growth is too heavy, the test may prove difficult to interpret. Most of the tests have been run at an original pH of 7.3, which may be reduced to around 6.8 after 18 hours' incubation. However, further studies have shown that the reaction

[1] Preliminary studies.

[2] This investigation is aided by grants from the Fluid Research Funds, Yale University School of Medicine, and the International Cancer Research Foundation.

[3] The term "normal" as used in this paper with reference to chorio-allantoic fluid and mouse lung filtrates is in contrast to the influenza-infected materials. It does not imply that these "normal" substances may not possess other anomalous agents which may support this agglutination phenomenon.

is favored by more alkaline pH. Changes in surface tension of the media[4] do not alter the manifestation of agglutination by susceptible bacteria so long as growth is possible.

No correlation has been found between agglutinability of susceptible organisms and their capacity to produce coagulase, to ferment mannitol and to produce rabbit-cell hemolysin. Agglutinable staphylococci have been tested with other materials from the egg, such as albumin, yolk and an emulsion of chick embryo, but none has given the phenomenon as described. A limited number of observations suggest that amniotic fluid probably possesses, in common with the chorio-allantoic fluid, the ability to exhibit the reaction. The effects of normal horse serum as well as peptone have been tested with negative results. Further, there is no apparent relation between the present agglutination reaction and the known property of some staphylococci to show clumping when grown in media containing human red blood cells. Experiments have ruled out the possibility that this reaction is associated with any colony change due to bacterial dissociation. Preliminary observations suggest that bacteriophage[5] does not play a role in this phenomenon, since both the Craig and the Wood 46 strains have been shown to carry bacteriophage.

This property of the egg fluid, whether in normal or virus-infected material, disappears very rapidly (in 1 to 2 min.) at 56° C. If, however, the titer is unusually high, exposure for 5 minutes at this temperature may be required for inactivation. The agglutinating property of the fluid will remain active at ice-box temperature for a month or more.

When this agglutination phenomenon was first observed, it was in association with influenza virus-infected chorio-allantoic fluid. However, some normal fluids were soon found to manifest the same effect with susceptible bacteria. In these early experiments filtrates[6] of mouse lung infected with influenza virus were tested as well as filtrates from normal mouse lungs. Similar results were obtained using these reagents as have been described for the chorio-allantoic fluids. Wood 46 strain showed no agglutination with virus-infected or normal lung filtrates in the tests made, 14 and 12, respectively. The Craig strain was agglutinated by all 14 virus-infected filtrates, but in only 4 of the 12 tests with normal lung material did the reaction take place. This aspect of the work while interesting has not been stressed, for when filtrate-broth mixtures stand for 18 hours at 37° C. or at room temperature, the precipitation of denatured proteins makes reading of the test difficult. While most samples from normal lungs tested against various strains of staphylococci gave negative reactions (27 tests), occasionally positive results were obtained (4 tests). Agglutinins for staphylococci were lacking in the blood serum from these mice.

Just what part, if any, the influenza virus plays in this agglutination is not understood at present. The fact that most normal chorio-allantoic fluids give the reaction would tend to minimize the importance of this infecting agent. Also, recent experiments show that if one adsorbs the influenza virus from infected fluids with chicken red blood cells and elutes the virus in buffer,[7] the fluid which no longer gives the agglutination of red blood cells agglutinates susceptible staphylococci to the same titer as before adsorption. On the other hand, the buffer into which the virus is eluted does not possess the ability to agglutinate susceptible bacteria even though the virus has been concentrated, as shown by the chicken red blood cell agglutination test.

Summary: Certain strains of *Staphylococcus aureus* will agglutinate when grown in the presence of normal or influenza virus-infected chorio-allantoic fluid. Fluids are rendered inactive after heating at 56° C. for one or two minutes. Surface tension and temperature are not important factors in the manifestation of the phenomenon so long as they are within the limits compatible with growth. The reaction is favored by an alkaline pH. Bacterial dissociation is not a feature of this phenomenon and the relationship of this reaction to bacteriophagy is probably not significant but is being investigated further. Similar reactions as described for the egg fluids may be seen when Berkefeld filtrates of influenza virus-infected and normal mouse lungs are used. A more detailed account of all the data will appear at a later date.

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AN ANTIGEN-ANTIBODY REACTION WITH TETRAHYMENA WHICH RESULTS IN DYSTOMY[1]

AN interesting reaction of the free-living, holotrichous ciliate, Tetrahymena[2] (Colpidium-Glaucoma group), has been observed in experiments dealing with several strains of the organism and immune rabbit sera. These ciliates show the agglutinative, paralytic and sheath-formation reactions described by Robertson[3] when they are incubated for an hour or

[4] W. E. Larson, W. F. Cantwell and T. B. Hartzell, *Jour. Infect. Dis.*, 25: 41–46, 1919.

[5] F. d'Herelle, "The Bacteriophage and Its Behavior," p. 73. Baltimore: Williams and Wilkins, 1926.

[6] Mouse lungs, both normal as well as virus-infected, were ground and filtered through a Berkefeld "N" filter before use in the test.

[7] W. M. Stanley, *Jour. Exp. Med.*, 79: 255–65, 1944.

[1] This work was aided by a grant from the Rockefeller Foundation.

[2] We are very grateful to Professor George W. Kidder for the cultures used in this work.