virus becomes so highly virulent that inoculation of chickens with minute doses of it causes death in 4 to 6 days, and hemorrhagic lesions, particularly of the proventriculus and small intestines, are present in a majority of those which succumb. An interesting, and as yet unexplained, phenomenon of the artificially induced disease is that the infected chickens do not have the respiratory symptoms which are predominant in the natural disease. This applies to chickens infected by inoculation with material from field cases as well as to those inoculated with cultured virus.

Because of the highly virulent nature of the cultured pneumoencephalitis virus, it seemed of interest to determine if it might be related to the viruses of Newcastle disease or fowl plague, two highly fatal diseases of chickens which were not present in the United States. Through the cooperation of the Bureau of Animal Industry of the U. S. Department of Agriculture, a small quantity of anti-serum for each virus was received from England in February, 1943, and was used for *in vitro* neutralization tests with pneumoencephalitis virus. In these tests, mixtures of equal parts of serum, undiluted or diluted with saline, and of embryo-cultured virus were prepared and used for the intramuscular inoculation of chickens, 61 days old, in doses of 0.1 cc.

Chickens were not infected by inoculation with 1,000 infective doses of pneumoencephalitis virus when it was mixed with an equal quantity of undiluted or 1:10 or 1:100 dilutions of Newcastle disease immune serum. The virus was not affected, however, by mixing it with the fowl plague immune serum. These results indicate that the virus of pneumoencephalitis is immunologically identical with the virus of Newcastle disease.

It is hoped that the further studies of avian pneumoencephalitis which are in progress may yield an explanation of marked difference between the characteristics of the natural and artificially induced disease.

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THE ISOLATION OF THE ST. LOUIS ENCEPHALITIS VIRUS FROM CHICKEN MITES (DERMANYSSUS GALLINAE) IN NATURE*

EPIDEMICS of St. Louis encephalitis have occurred in St. Louis and in St. Louis County in 1933 and 1937. Since the epidemic of 1937 sporadic cases of the disease have been identified[1, 2] in St. Louis County. Although few in number the occurrence of these sporadic cases indicates that an endemic focus exists. The St. Louis area seemed to offer the opportunity for investigating the problem of inapparent viral infection in a community during a non-epidemic period. Therefore, a survey for the presence of type specific antibody to the St. Louis encephalitis virus in the human and animal population of the St. Louis area was undertaken by one of us to determine to what extent the population is being immunized to the virus of St. Louis encephalitis. In this study the data[3] obtained up to this time indicate that few individuals who have come into St. Louis County since 1937 show specific antibody to the St. Louis virus. On the other hand, a significant number of chickens, approximately one year of age, has shown a low titer of neutralizing antibody for the virus.

The work of Hammon *et al.*[4] has shown the natural occurrence of the St. Louis encephalitis virus in the mosquito *Culex tarsalis Coquillet* during epidemic periods. The experimental transmission of the St. Louis virus in chickens and pigeons by 9 species of mosquitoes from 3 genera has been reported.[5] These findings, together with other epidemiological studies by Hammon and his colleagues,[6] appear to indicate beyond reasonable doubt that the mosquito is a vector concerned in human epidemics of St. Louis encephalitis. However, the demonstration of neutralizing antibody in a significant number of one-year-old chickens in certain flocks in an area where the human population does not appear to be developing antibody suggested the possibility that some blood-sucking vector which does not bite man was transmitting the disease to fowl.

The common chicken mite, *Dermanyssus gallinae*, frequently infests fowl in this area. The chicken mite belongs to the same order of Arachnida as does the tick and the life cycles of the two are comparable in so far as the mite requires a blood meal before the moulting of the nymphs and before the oviposition by the adult females.[7] Therefore, the chicken mite seemed a possible vector in light of the experiments which have shown that ticks, under experimental conditions, are capable of becoming infected with two neurotropic viruses, the *Dermacentor andersoni* with the western equine encephalomyelitis virus[8] and the *Dermacentor variabilis* with the St.

* From the Department of Pathology and the Department of Pediatrics, Washington University School of Medicine. Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] R. J. Blattner and J. V. Cooke, *Jour. Inf. Dis.*, 70: 226, 1942.

[2] R. J. Blattner and F. M. Heys, to be published.

[3] M. G. Smith, to be published.

[4] W. McD. Hammon, W. C. Reeves, B. Brookman and E. M. Izumi, *Jour. Inf. Dis.*, 70: 263, 1942.

[5] W. McD. Hammon and W. C. Reeves, *Jour. Exp. Med.*, 78: 241, 1943.

[6] W. McD. Hammon, W. C. Reeves, B. Brookman and C. M. Gjullin, *Jour. Inf. Dis.*, 70: 278, 1942.

[7] H. P. Wood, *U. S. Department of Agriculture Bull.* No. 553, 1917.

Louis encephalitis virus.[9] Furthermore, in both instances the ticks are capable of transmitting the infectious agents to susceptible animals by bite and of the hereditary transmision of the virus to their offspring.

Mites (*Dermanyssus gallinae*) were collected from a coop in which there were chickens whose sera had been shown to have neutralizing antibody for the St. Louis encephalitis virus. Of 6 chickens, 2 were definitely positive and 2 questionably so. The mites were kept in test-tubes for 7 days without feeding. At the end of that time 60 mites were triturated in tryptose broth in an agate mortar and .1 cc of the supernatant fluid was inoculated intraperitoneally into each of 6 nineteen-day-old Swiss mice. Young mice were used because they are more readily infected than adults by the intraperitoneal inoculation of the St. Louis encephalitis virus.[10] In 8 days 2 of the 6 mice showed signs of illness with slight twitching. When moribund these 2 mice were killed. Necropsy showed no gross pathological lesions and bacteriological cultures of the brains, spleens and lungs were sterile. The brains were emulsified and diluted 1:10 with tryptose broth. After centrifugation .03 cc of the supernatant fluid was inoculated intracerebrally into each of 8 mice and also on the chorio-allantoic membrane of the developing chick egg. Three days later all 8 mice appeared sick and developed convulsions. Five of these died before the close of the third day. The 3 others were killed when moribund. The latter 3 brains were emulsified and diluted 1:10 with broth. After centrifugation the supernatant fluid was inoculated intracerebrally in .03 cc amounts into 6 mice. On the third day these mice developed convulsions.

The egg membranes inoculated with the brain material of the 19-day-old mice appeared slightly thick and opaque when examined 3 days following inoculation. The embryos were alive and the allantoic fluid clear. The ground-up membranes were passed to mice by intracerebral inoculation and the mice developed convulsions on the third day following inoculation. All the mouse brains and the egg membranes have been bacteriologically sterile. The infective agent appears to have been established in mice and in the chick egg.

Filtration experiments with the infectious agent in a broth medium have shown that it passes readily through a Berkefeld N Filter.

Microscopic sections of one of the mouse brains from the second intracerebral passage show an encephalitic process which is apparently indistinguishable from the pathological picture of St. Louis encephalitis in the mouse.

TABLE 1

MOUSE PROTECTION TEST—A COMPARISON OF THE NEUTRALIZATION OF THE ST. LOUIS ENCEPHALITIS VIRUS AND OF THE VIRUS ISOLATED FROM MITES WITH ST. LOUIS ENCEPHALITIS IMMUNE SERUM

Dilution of virus mixture added to equal part of serum	Serum: Rabbit immune St. Louis serum — St. Louis virus	Rabbit immune St. Louis serum — Virus from mites	Rabbit Normal Serum — St. Louis virus	Rabbit Normal Serum — Virus from mites
10^{-1}	.....	6* 7 8 8	.....	.....
10^{-2}	9 10 S† S	9 9 S S	.....	4 4 4 4
10^{-3}	10 S S S	8 S S S	.....	4 4 4 4
10^{-4}	S S S S	S S S S	.....	5 5 5 5
10^{-5}	7‡ S S S	.....	4 5 6 6	5 6 6 S
10^{-6}	S S S S	.....	5 5 5 6	9 S S S
10^{-7}	.....	.....	6 8 S S	S S S S
10^{-8}	.....	.....	7 S S S	.....

* Numbers = day of death of 1 mouse.
† S = survival of 1 mouse.
‡ = death from unknown cause.

Identification of the newly isolated virus was attempted after 2 serial mouse brain passages. The brains of mice infected with the newly isolated virus are infective to a dilution of 10^{-6}, as compared with 10^{-8} for brains of mice infected with the strain of St. Louis encephalitis (Hubbard strain) used in this laboratory. With two different lots of serum of rabbits immunized to a known strain of the virus of St. Louis encephalitis, the newly isolated virus is neutralized to approximately the same extent as is the known strain of St. Louis encephalitis virus (Hubbard strain), Table 1. It is not neutralized by serum of a guinea pig immunized to the western strain of virus of equine encephalomyelitis.

A second isolation of the virus was attempted with mites from the original collection after they had been kept in test-tubes without feeding for four weeks. Thirty mites, predominantly nymphs, were triturated in broth as in the first isolation of the virus. The supernatant fluid was inoculated in .1 cc amounts intraperitoneally into each of 3 Swiss mice, 11 days of age. On the ninth day following inoculation 2 mice developed convulsions. Each of the 2 brains was passed to 6 adult Swiss mice by intracerebral injection. The inoculum was bacteriologically sterile. Two and a half to three days following inoculation these mice developed convulsions.

Conclusion: The St. Louis encephalitis virus has been isolated from chicken mites (*Dermanyssus gallinae*) in nature in the St. Louis area during a non-epidemic period.

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