Comparative Study of Fowl Plague Virus and a Virus Isolated from Man

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FOWL PLAGUE is caused by an infectious filtrable virus and is characterized by high mortality among chickens. Although the disease has not occurred in the United States since 1929, it has been considered enzootic chiefly in eastern Europe, the Mediterranean basin, Egypt, and Asia. The disease has often been confused with that caused by the virulent form of Newcastle disease virus.

In 1959, slightly more than a month after he returned from a 2-month trip abroad, a 46-year-old white man with clinically diagnosed infectious hepatitis was hospitalized. He had been generally healthy during visits to Japan, Thailand, Ceylon, Egypt, Israel, Greece, Yugoslavia, Italy, Switzerland, and Spain. After his return, he complained of lassitude, headaches, and occasional chills during the first 2 weeks and bloating and indigestion during the next 2 weeks. On the 31st day he had a slight fever, and on the following day he was noticeably bloated and had indigestion, dark urine, constipation, and loss of appetite. Four days later his sclera showed a yellowish tinge. At this time, his physician clinically diagnosed his illness as infectious hepatitis and sent him to the hospital. Blood samples were collected the following day.

Initial studies were conducted at the National Communicable Disease Center, Public Health Service, Atlanta, Ga. These studies included inoculation of developing chicken embryos with blood clot material from the patient. This material was lethal for the embryos, and chickens inoculated with allantoic fluid from the embryos had clinical signs which resembled those of fowl plague. The results of these preliminary inoculations, and the fact that the patient had visited Egypt where fowl plague had been reported in 1959 (*1*), prompted the decision to submit the material to the Plum Island Animal Disease Laboratory for further study.

Material and Methods

Viruses. Blood from the patient was inoculated into primary rhesus monkey kidney tissue culture, HeLa tissue culture, suckling mice, 2-week-old mice, and developing chicken embryos.

Texas GB strain of Newcastle disease virus, the Brescia strain of fowl plague virus, and "N" virus (a fowl plague isolate) were used in serologic or challenge inoculation tests.

Diluents. Tryptose phosphate broth (Difco), physiological saline solution, and Alsever's solution (*2*) were used as diluents.

Animals. The monkeys (cynomolgus) were approximately 1 year old. The chickens, White

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Leghorns obtained from a breeding flock, were 6 to 12 months old. The ducklings (Pekin) were 2 to 3 weeks old. The developing chicken embryos were from a flock of White Leghorns. The chickens had not been vaccinated against Newcastle disease or exposed to infection with Newcastle disease virus.

Challenge inoculation. The chickens were inoculated intramuscularly with 1.0 ml. of Brescia strain of fowl plague virus containing 10^5 chicken lethal doses.

Virus neutralization. Lyophilized serums of chickens immunized against Newcastle disease virus and Brescia and Alexandrien strains of fowl plague virus and "N" virus were reconstituted with distilled water. The serums had been prepared at the Plum Island Animal Disease Laboratory in 1957. Fowl plague-like virus was diluted from 10^{-1} to 10^{-9} in tryptose phosphate broth, and 0.5 ml. of each virus dilution was added to a 0.5 ml. volume of reconstituted antiserum. The serum-virus mixtures were held for 30 minutes at room temperature, and then 0.1 ml. quantities of the mixtures were injected into the allantoic cavity of 10-day-old developing chicken embryos. Controls included diluent and normal chicken serum inactivated at 56° C.

Hemagglutination-inhibition. Hemagglutination-inhibition tests were conducted using fowl plague-like virus versus anti-fowl-plague serum (Brescia) and Brescia fowl plague virus versus fowl plague-like acute-phase and convalescent-phase serum samples from the patient. In these tests, virus dilutions of 1:640 were prepared in saline, serums were diluted 1:5, and 0.5 percent washed rooster red blood cells were prepared in Alsever's solution. Each tube contained 0.25 ml. each of diluted serum, virus dilution, and rooster red blood cell suspension. Hemagglutination controls employing inactivated normal chicken serum were included. The preparations were incubated 2 hours at room temperature.

Results

On first passage of blood clot material, one of five developing chicken embryos died 48 hours after inoculation. Guinea pig and chicken erythrocytes were agglutinated with suspensions of allantoic fluid from the embryo. In subsequent passages all the embryos died following inoculation, and allantoic fluid from the developing chicken embryos was infectious for chickens.

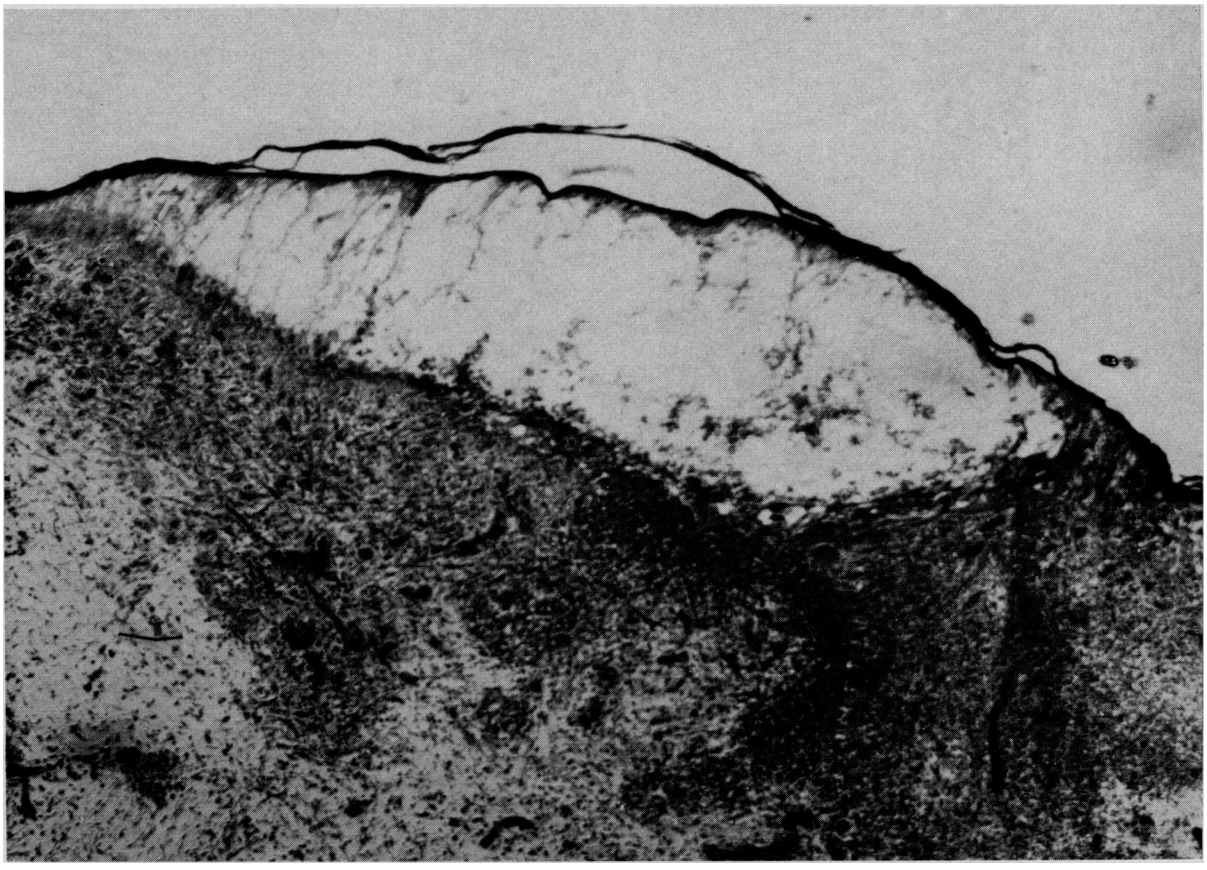
Ether treatment of the egg material containing infected allantoic fluid reduced infectivity from 10^{-6} to 10^{-3}. Attempts to pass the agent in primary monkey kidney tissue culture, suckling mice, and weanling hamsters were unsuccessful. The infected allantoic fluid was re-isolated in eggs from the original blood clot about 6 months after the first isolation.

Eighty-four embryos, inoculated with allantoic fluid of the second passage in developing chicken embryos, died within 48 hours.

Infection in chickens. Nine chickens were inoculated with allantoic fluid from the second passage in developing chicken embryos. Five birds were inoculated intramuscularly with 0.5 ml., two birds were inoculated with 0.5 ml. both intramuscularly and intravenously, and each of two birds received 0.5 ml. intravenously. All four birds inoculated intravenously were dead or moribund at 6 days postinoculation. Only one of five birds inoculated intramuscularly died.

Moderate signs of infection were observed in the survivors, which were reinfected with fowl plague-like virus passaged from embryo to chicken to embryo. Eight chickens were inoculated with fowl plague-like virus passaged once in embryo and once in chickens. Eight chickens were inoculated with 0.5 ml. of spleen suspension, two intramuscularly and six intravenously. Signs of infection developed in both birds inoculated intramuscularly, but one recovered. Signs of infection were seen in all six birds inoculated intravenously, and all died within 7 days postinoculation. The same inoculum tested for viability after storage for 8 months at −30° C. was found to be infective—four of seven chickens inoculated intravenously with 0.5 ml. died, and marked signs of infection were observed in all seven birds.

Clinical signs. Roughened feathers, depression, temperature elevation, cyanosis—particularly of the comb and wattles—and conjunctivitis occurred in nearly all birds infected with fowl plague-like virus. Some birds had diarrhea, and vesicles were seen on the comb of one bird (see photograph). Survivors had a thermal response at 4 to 6 days postinoculation, cyanosis, and depression followed by recovery within 10



Vesicles in the comb of a chicken inoculated with fowl plague-like virus (magnified approximately 35 times)

days postinoculation. Thermal reaction in survivors did not exceed 108° F.; chickens that died had thermal reactions to at least 110° F. Cloacal temperatures of birds inoculated with recently passaged fowl plague-like virus reached 109° F. at 72 hours after inoculation. Virulence appeared to decrease following 8 to 10 months' storage at −30° C.; however, virulence was restored following one passage in developing chicken embryos. All birds inoculated with freshly passaged material died 7 days following inoculation: 10 to 60 percent of the birds in other groups survived inoculation with fowl plague-like virus that had been stored for 10 months with no intermediate passage in embryos.

Necropsy. The lesions seen most frequently were catarrhal enteritis and hemorrhages (1 mm. to 3 mm. in diameter) in the proventriculus and in the submucosa of the small intestine. The hemorrhages were not visible from the serous side of the intestine. Lesion areas in the intestines were examined and found to be free of coccidial oocysts. Hemorrhages were observed occasionally on the mucous membrane in the upper two-thirds of the trachea.

Petechial hemorrhages were observed throughout the skeletal muscle, and vesicles developed on the comb and wattles. No bacterial pathogens were found in liver and spleen tissue suspensions.

Histopatholgy. Many villi in the mucosa of the small intestine were detached and there were marked vascular changes. Round cell infiltration was observed in the subcapsular area of the gizzard. Extensive degenerative changes were found in the liver cells.

Inoculation of experimental animals. Three cynomolgus monkeys were inoculated intramuscularly with 10^5 chicken embryo lethal doses of fowl plague-like virus. The monkeys did not show clincial signs, and they were killed at 15

days postinoculation. A slight and transitory thermal response occurred at 4 days postinoculation (table 1). Blood samples collected from monkeys 10 days after inoculation were not infective for chickens.

Ducklings have been reported to be susceptible to fowl plague but not Newcastle disease (*3*). Fifteen ducklings inoculated by the intramuscular route with 10^3 chicken lethal doses of fowl plague-like virus had no clinical signs. Fowl plague-like virus also failed to elicit signs in rabbits, guinea pigs, ferrets, and weanling hamsters.

Serology. Virus neutralization tests in developing chicken embryos were conducted with anti-fowl-plague serum and allantoic fluid from embryos infected with fowl plague-like virus (table 2). Unequivocal evidence of at least a one-way serologic cross between anti-fowl-plague serum and fowl plague-like virus was shown (table 2). Similarly, evidence of a reciprocal cross-reaction was shown (table 3) by neutralization of fowl plague virus with serums from chickens recovered from infection with fowl plague-like virus. The chickens were initially inoculated intramuscularly with fowl plague-like virus from embryos. Ten days after initial inoculation, four survivors were reinfected with fowl plague-like virus passaged from embryo to chicken to embryo. Virus neutralization tests with fowl plague virus were conducted with serum from blood samples taken 12 days after reinfection with fowl plague-like virus. Approximately 10^3 chicken lethal doses of fowl plague virus (Brescia) were neutralized by serum from chickens which had survived inoculation with fowl plague-like virus. Antibodies of fowl plague virus were not detected in serum from the patient's blood samples collected during either the acute or the convalescent stage (table 4).

Table 1. Temperatures of 3 monkeys after intramuscular inoculation with 10^5 chicken embryo lethal doses of fowl plague-like virus

Days postinoculation	Temperature of monkeys		
	No. 6	No. 16	No. 30
1	101. 6	100. 0	101. 0
2	101. 8	100. 4	101. 0
3	101. 0	99. 6	100. 8
4	[1] 101. 8	[1] 102. 0	[1] 102. 6
5	101. 0	100. 4	100. 8
6	101. 8	100. 4	100. 8
7	101. 0	101. 0	101. 4
8	101. 2	101. 4	101. 0
9	101. 0	101. 4	102. 2
10	101. 0	101. 2	102. 0
11	101. 6	101. 2	102. 4
12	101. 6	100. 8	102. 0
13	101. 4	101. 2	101. 9
14	101. 4	101. 6	101. 8
15	101. 6	101. 4	101. 6

[1] Blood taken for viremia test.

Hemagglutination and hemagglutination-inhibition tests. Fowl plague-like virus agglutinated chicken red blood cells in dilutions through 1:80. Anti-fowl-plague serum (Brescia) diluted 1:10 reduced the hemagglutination titer of fowl plague-like virus from 1:80 to 1:5. Serum from a blood sample collected from the human host 4 months after his illness did not reduce the hemagglutination activity of fowl plague virus.

Cross-protection. The Brescia strain of fowl

Table 2. Results of neutralization tests in developing chicken embryos using fowl plague-like virus, fowl plague, and Newcastle disease antiserums

Antiserums	Fowl plague-like virus [1] dilutions						
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
None	+	+	+	+	+	+	−
Fowl plague (Brescia)	−	−	−	−	−	−	−
Fowl plague (Alexandrien)	+	+	−	−	−	−	−
"N" virus (a fowl plague isolate)	−	−	−	−	−	−	−
Newcastle disease	+	+	+	+	+	−	−

[1] Infected allantoic fluid passed once at Plum Island Animal Disease Laboratory in developing chicken embryos.

NOTE: Plus signs indicate embryos died in 48 hours; minus signs indicate embryos survived.

Table 3. Neutralization of fowl plague virus (Brescia) with serum from chickens after inoculation with fowl plague-like virus

Serum	Fowl plague virus dilutions								
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Normal chicken	(1)	(1)	(1)	(1)	+	+	+	−	−
Fowl plague-like virus antiserum (chicken)	+	−	−	−	−	−	−	−	−

[1] Test not run.

NOTE: Plus signs indicate embryos died; minus signs indicate embryos survived.

Table 4. Assay for fowl plague virus neutralizing antibodies in serums of a patient from whom fowl plague-like virus was recovered

Serum	Fowl plague virus dilutions								
	0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Normal chicken	(1)	(1)	(1)	(1)	+	+	+	+	−
Acute	+	+	+	+	+	+	+	+	−
Convalescent, 5 months after patient's illness	+	+	+	+	+	+	+	+	−
Convalescent, 20 months after patient's illness	+	+	+	+	+	+	+	+	−

[1] Test not run.

NOTE: Plus signs indicate embryos died; minus signs indicate embryos survived.

plague virus was injected into four chickens that had survived two inoculations with 1,000 chicken lethal doses of fowl plague-like virus (table 5). One of the chickens died 4 days post-inoculation, a transitory increase in body temperature was noted in two chickens, and one had no clinical signs. The mild reactions of these three birds and their subsequent survival indicated a significant degree of resistance to fowl plague-like virus. Six control chickens, inoculated with 1,000 chicken lethal doses of virus, died with signs and lesions of fowl plague.

Relation to viruses other than fowl plague. After further passage in eggs, the isolate was tested by hemagglutination-inhibition techniques (*4–6*) against the following serums: influenza A Asian, Denver, PR 8, FM1, and swine; influenza B Great Lakes; parainfluenza types 1, 2, and 3; Newcastle disease virus; mumps virus, reovirus type 1, and acute-phase and convalescent-phase serums from the patient. No inhibition of agglutination was noted with any of these serums. The isolate was tested by the diagnostic complement fixation technique (*7*) against the following serums: Eastern equine encephalitis, Western equine encephalitis, vaccinia, herpes simplex, adenovirus 4, and acute-phase and convalescent-phase serums from the patient. No fixation was noted with any of the serums.

Discussion

Although fowl plague-like virus did not prompt an immune response in man, it did elicit antibody response in chickens. This antigenic behavior of fowl plague-like virus agrees with other properties of the virus, indicating that man is an alien host and the chicken is the natural host. Further evidence of host specificity was shown by failure to infect either monkeys, ducklings, rabbits, guinea pigs, ferrets, or weanling hamsters.

The comparative studies between fowl plague-like virus and fowl plague included observations other than serologic, yet there has been no need to reconcile clinical discrepancies with a serologic likeness. The characteristics of the fowl plague-like virus were in such close agreement with those of fowl plague that a diagnosis of fowl plague would have been un-

Table 5. Response of 4 chickens recovered from fowl plague-like virus to intramuscular inoculation with 10^5 chicken lethal doses of fowl plague virus

Days post-inoculation	Temperature of chickens			
	No. 9326	No. 9329	No. 9342	No. 9349
1	108. 4	107. 2	106. 4	108. 2
2	108. 2	109. 0	107. 4	109. 0
3	109. 0	107. 8	106. 8	110. 0
4	(1)	Dead	(1)	(1)
5	106. 4		106. 2	107. 4
6	105. 2		106. 2	105. 8
7	104. 6		105. 8	104. 2
8	104. 0		105. 6	104. 4

[1] Not taken.

questioned had the isolation been made from chickens. Although definite evidence of the patient's exposure to fowl plague virus is lacking, his subsequent recovery may be explained on the premise that the fowl plague-like virus had remained latent until he was infected by a second agent.

Conjecture concerning possible sources of the virus other than blood from the patient prompts a question concerning the initial developing chicken embryos used for inoculation as a possible source. This possibility cannot definitely be excluded, although some embryos from a second group inoculated with the blood died and hemagglutination positive tests were obtained with the allantoic fluid. Furthermore, it did seem unlikely that evidence of infection in chickens would not be detected if infection were present in developing chicken embryos of commercial flocks.

Summary

Fowl plague-like virus isolated from a man was infective for chickens. Clinical signs resembling those of fowl plague developed in the chickens when they were inoculated with blood clot material from the man, who had become ill after a trip abroad. The trip had included visits to areas from which fowl plague had been reported.

Fowl plague-like virus and fowl plague virus elicited neutralizing antibodies for the heterologous as well as the homologous virus. A serologic relationship between the two was also shown by hemagglutination-inhibition tests.

The course of fowl plague-like virus infection in chickens and its clinical signs and lesions were indistinguishable from those seen in chickens infected with fowl plague virus. Chickens that recovered from infection with fowl plague-like virus were refractory to fowl plague virus.

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