Characteristics of a Psittacosis Viral Agent Isolated from a Turkey.* (20277)

K. F. Meyer and B. Eddie.

From the George Williams Hooper Foundation, University of California, San Francisco.

In an attempt to find the source of the recent (1952) outbreaks of clinical psittacosis among employees of poultry-dressing plants (63 cases with 4 deaths), the sera of 126 turkeys from 7 farms were tested by the complement fixation inhibition method (Karrer *et al.*(1)); 25% were positive reactors. Attempts to isolate a viral agent were at first unsuccessful, but when suspensions prepared from the lung and kidney tissues of one turkey (No. 05404) were inoculated subcutaneously and intranasally into mice, a viral agent morphologically indistinguishable from the classical strains of the psittacosis group was isolated. A comparative study conducted according to a standard routine procedure (Meyer and Eddie(2)) identified the strain as a characteristic member of this group.

Morphology. The characteristics of the viral agent isolated from a turkey are as follows: Morphologically the impression preparations made from the peritoneal exudate, from the lung of infected mice or from the yolk sac of inoculated embryonated eggs showed Castaneda-positive elementary bodies, smaller than those formed by other viruses of this group, with the usual developmental stages in the cytoplasm, readily rendered visible by the Macchiavello stain.

Pathogenicity. The infection spectrum of the agent is broad and the pathogenicity is high. *Mice* infected by the intraperitoneal route with a virus dilution of 10^{-8} and occasionally 10^{-9} died in from 4 to 5 days; dilutions of 10^{-4} and 10^{-5} given intranasally were regularly lethal in 6 days; after intracerebral inoculation death occurred on the 5th to 6th day with dilutions of 10^{-7}; on subcutaneous injection with a dilution of 10^{-1} occasionally a few mice succumbed to infection between the 6th and 15th day. All mice that recovered from infection were carriers. Intravenous injection killed in from 6 hours to 15 days, depending on the dilution of the tissue suspensions. Yolk sac viral dilutions of 10^{-8} sometimes killed in 4 to 15 days. *Guinea pigs* receiving by the intraperitoneal route 1 ml of a 20% suspension of infected tissues (liver and spleen or yolk sac) succumbed regularly in 3 to 8 days. *Parakeets* and *ricebirds* on intramuscular injection with a low viral dilution (10^{-1}) presented at autopsy on the 5th or 6th day typical lesions of psittacosis. Three of six 7-day-old *chick embryos* infected by the yolk sac route with 10^{-8} dilution succumbed on the 3rd day, further attesting to the high virulence of this agent. Infected yolk sacs harvested shortly before death, after draining off excess yolk and diluting in phosphate buffered gelatin solution, were highly toxic on intravenous inoculation of mice weighing 10 to 12 g. In dilutions of as high as 1:800, 100% of the animals succumbed, after having had symptoms of lethargy and labored breathing, within 5 days. The ears, paws and tails of those surviving 72 hours were often deep red. The striking capillary dilations in the skin and in part also in the viscera are associated with hemolysis of red cells and are usually observed when highly toxic psittacosis agents are tested by the intravenous route. In rare instances fading of the redness of the ears heralded recovery, but the mice were found to be carriers of the agent when sacrificed several weeks after the infecting injection. The *toxin* of the turkey strain shares all characteristics of the toxins of the psittacosis-lymphogranuloma venereum agents. The death rate following injection of the toxin of the turkey strain is shown in Fig. 1 and 2.

Antigenic relationships. Antisera from infected psittacine birds, pigeons, sheep or man have given complement fixation reactions when the turkey strain was used as antigen. Sera from turkeys or roosters infected with the turkey strain bound complement in the

* Through the courtesy of Dr. I. V. Irons, Texas State Department of Health, Austin, this viral agent was made available to the Hooper Foundation for study.

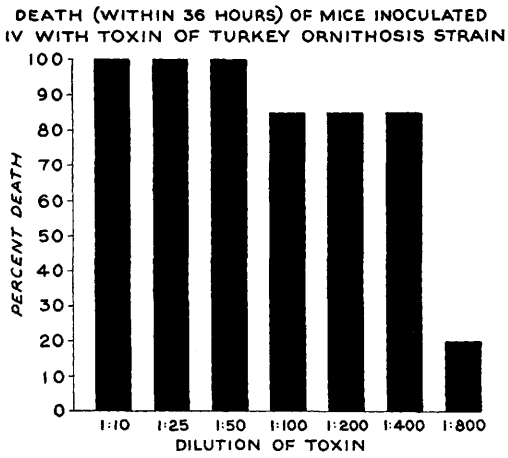

FIG. 1.

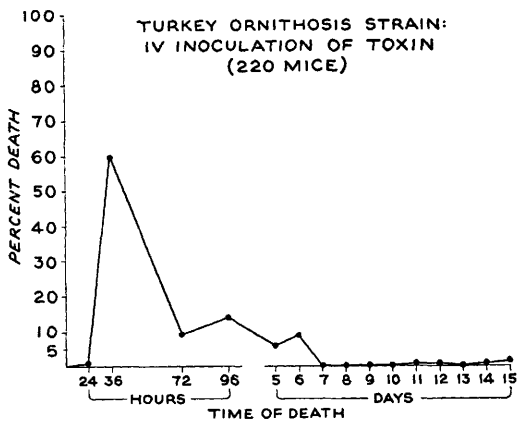

FIG. 2.

presence of Cocto-antigens prepared from avian or mammalian strains of the psittacosis group. Thus the strain gives reciprocal serologic cross reactions.

Guinea pigs surviving infections with an avian strain (egret SE45), as evidenced by complement fixation tests, were completely resistant to intraperitoneal challenge with the turkey strain, which induced death in control animals. On the other hand, depending on the host origin of the mammalian strains employed to immunize guinea pigs, the resistance to re-infection with an avian strain varied from 20 to 60%.

Hyperimmunization of roosters with toxoids prepared from highly toxic yolk sac suspensions (40 ml of a 20% suspension in 25 injections over a period of 2 months) when bled on the 15th day after the last injection (75th day) yielded antisera which reacted in the complement fixation inhibition test in dilutions of 1:32 and even 1:128 (rooster no. 19). Such antisera diluted 1:10 and mixed with 2 LD_{50} doses of toxin in the *in vitro* neutralization test (Rake and Jones(3)) protected all mice against the turkey toxin for at least 48 hours. As Table I shows, the turkey antiserum also neutralized to a less pronounced degree toxins prepared from the human Louisiana (Borg) and egret (SE45) strains. It exerted no protection against the toxins derived from 2 pigeon and 1 bovine encephalomyelitis virus. On the 6th day after injection of the mixture of antiserum and viral agent, when the anti-infectious properties of the turkey serum became active, the apparent relationship of the Louisiana and egret strain had disappeared. In one test 40% of the mice inoculated with turkey serum dilutions and 2 LD_{50} doses of toxin containing 100 million infectious units survived not only intoxication but also infection. At autopsy on the 20th to 30th day the mice were free from latent carrier stages. Thus the *in vitro* contact of the turkey virus with its homologous antiserum proved that not only the toxic, but equally the infectious properties were specifically neutralized. The specific protective action of an anti-psittacosis serum is more definitely demonstrated by intravenous injection than by intranasal instillation or intracerebral or intraperitoneal injection of the mixture (Hilleman(4)). Depending entirely on the potency of the antiserum, even 400 million infectious particles were rendered non-infectious when brought in contact with the antibodies.

To check these results, the turkey toxin was mixed *in vitro* with antisera against other members of the psittacosis-lymphogranuloma venereum group. The data in Table II show that the turkey antiserum again neutralized both the toxin and the infectious elementary bodies and protected 50% of mice from death after the 6th day. An anti-Louisiana or anti-egret strain serum neutralized turkey toxin for up to 36 hours (30 to 45% of the animals survived) but these antisera, except in high concentrations, could not render the infectious particles inactive. The serum prepared with a parakeet strain (N.Y.) or with a cat

TABLE I. Neutralization of Toxin of Turkey Strain by Specific Antisera. Survival rate of mice inoculated intravenously.*

Strains used to prepare antiserum	Total mice	Rate† Hr 24	36	72	96	Days 5	6	7	8	9	10	21‡
Turkey	20	100	90	70	65	55	50	40	40	40	40	40
Egret (SE45)	40	100	60	45	30	15	10	0				
Human (La., Borg)	40	100	72	37.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Parakeet (N. Y.)	40	95	45	22	15	7.5	5	5	5	5	5	5
Duck (Burns)	40	95	55	30	20	15	7.5	7.5	7.5	5	0	
Cat (PP)	40	90.5	40	25	7.5	5	2.5	2.5	2.5	0		
Bovine (BEV)	40	45	0									
Controls	40	70	22.5	0								

* Antiserum diluted 1:10, toxin in 2 LD_{50} doses.
† Calculated on percentage distribution.
‡ Mice surviving until 21st day sacrificed and examined.

TABLE II. Neutralization of Specific Toxins by Antiserum against the Turkey Strain. Survival rate of mice inoculated intravenously.*

Strains used to prepare toxins	Total mice	Rate† Hr 24	36	72	96	Days 5	6	7	8	9	10	21‡
Turkey	20	100	90	70	65	55	50	40	40	40	40	40
Egret (SE45)	40	90	30	20	15	15	0					
Human (La., Borg)	40	90	45	10	5	0						
Pigeon (P207)	40	25	0									
Pigeon (Au46)	40	50	0									

* Turkey strain antiserum diluted 1:10, toxin in 2 LD_{50} doses.
† Calculated on percentage distribution.
‡ Mice surviving until 21st day sacrificed and examined.

strain (PP) were less active with respect to toxin and infection neutralization than those of the highly virulent Louisiana strain. Two pigeon and 1 bovine encephalitis antisera did not neutralize the turkey toxin.

Conclusions. It has been demonstrated that the psittacosis (ornithosis) viral agent isolated from a turkey is antigenically specific. The Louisiana human, the egret and the turkey strain have in common an endotoxic component probably responsible for their high virulence for man and for their broad infection spectrum. Epidemiologic experience has proven such strains to be the cause of serious outbreaks of pneumonitis in inhabitants of the Louisiana bayou country (Olson and Larson (5)), in poultry dressers and in laboratory workers. Until other strains isolated from turkeys have been carefully compared with other psittacosis viral agents from southeastern United States it is premature to conclude that turkeys are the sole hosts of ornithosis agents of virulence greater than that of strains found in pigeons, ducks, chickens and even psittacine birds. Finally, there is no urgency to burden the unfortunate classification of the psittacosis-lymphogranuloma venereum group with a new species when the technic of neutralization of viral activity merely suggests a specific serotype.

1. Karrer, H., Meyer, K. F., and Eddie, B., *J. Infect. Dis.*, 1950, v87, 13.

2. Meyer, K. F., and Eddie, B., *Arch. f. d. gesamte Virusforsch.*, 1952, v4, 579.

3. Rake, Geoffrey and Jones, Helen P., *J. Exp. Med.*, 1944, v79, 463.

4. Hilleman, Maurice R., *J. Infect. Dis.*, 1945, v76, 96.

5. Olson, B. J., and Larson, C. L., *Pub. Health Rep.*, 1945, v60, 1488.

Received April 3, 1953. P.S.E.B.M., 1953, v83.