Isolation of a Psittacosis-Like Agent from the Blood of Snowy Egrets.* (19185)

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(Introduced by Morris Schaeffer.)

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During the course of an investigation to determine the animal reservoirs of eastern equine encephalomyelitis, many wild birds inhabiting the swamps and coastal areas of Louisiana were bled, and serum from each was inoculated intracerebrally into six 3-week-old Swiss mice(1). On June 27, 1950, blood was obtained from 24 nestling snowy egrets (*Egretta candidissima candidissima* (Gmelin)) and from several other species of wading birds on an island in Barataria Bay off the Gulf coast of eastern Louisiana. Several mice inoculated with sera from 2 snowy egrets developed ruffled fur and irritability followed by weakness, convulsions, and paralysis, finally resulting in death. No bacterial growth was observed when brains from these mice, and from subsequent passages, were cultured on blood agar plates, in serum or dextrose broths.

Further intracerebral passage in mice established the presence of an infectious agent, or agents, producing death within 2-7 days after inoculation. A great reduction in titer of the agent was observed on filtration through a Seitz EK pad, indicating that all but a few of the infectious particles were retained by this type filter. No evidence of *in vitro* neutralization was obtained when this agent was tested against antisera to eastern equine encephalomyelitis, western equine encephalomyelitis, Venezuelan equine encephalomyelitis, St. Louis encephalitis, lymphocytic choriomeningitis, or encephalomyocarditis viruses.

Sections made from these brains revealed a heavy infiltration of mononuclear cells into the leptomeninges. Many of these cells contained clusters of minute coccoid bodies resembling the elementary bodies of the psittacosis-LGV group of organisms. Macchiavello-stained smears of livers and spleens of intraperitoneally inoculated mice confirmed this impression. Intranasal inoculation into mice produced death, and autopsy revealed widespread pulmonary involvement and the presence of many bodies morphologically and tinctorially like those of psittacosis.

Limited host range studies carried out with material derived from both isolations revealed identical pictures. Material from both isolations was highly fatal for mice by the intracerebral, intraperitoneal, intramuscular, and subcutaneous routes. Guinea pigs and hamsters tested only by intracerebral and intraperitoneal routes of inoculation also succumbed. Inoculation into the yolk sac of 6-day chick embryos was followed by death in 3-5 days. Smears made from the yolk sacs revealed large numbers of elementary bodies. Inoculation into the allantoic cavity of 10-day-old chick embryos resulted in death in 3-7 days. Rabbits inoculated intracerebrally and intraperitoneally exhibited no obvious signs of infection. Temperatures were not taken, however, nor was the serological response studied. Four chickens inoculated intraperitoneally with a 1:10 dilution of freshly harvested yolk sac material showed toxic signs, and 3 died within a few days.

Yolk sac antigens made by the method of Nigg *et al.*(2) fixed complement in the presence of psittacosis antiserum. A rise in complement fixing antibodies against psittacosis and LGV antigens was observed in immunized guinea pigs.

When mice infected by the intraperitoneal route were treated with penicillin, there resulted a significant reduction in mortality rate as compared with untreated controls.

Guinea pigs immunized with material from one of the isolations failed to show evidence of infection when challenged with the other. This fact, combined with the fact that both

* This note constitutes part of an investigation on encephalitis in Louisiana conducted by the Virus and Rickettsia Section, Montgomery, Ala.

agents were isolated from the same species on the same day and with the observation that both agents possessed similar host ranges and histopathological reactions, indicated close resemblance, if not identity, of the two.

The high fatality rate following intraperitoneal inoculation in guinea pigs and intramuscular and subcutaneous inoculation in mice, and the toxicity for chickens inoculated intraperitoneally(3) suggested a relationship to the virus of Louisiana pneumonitis isolated by Olson *et al.*(4-9) during an epidemic of severe pneumonitis among residents of the bayou region of Louisiana in 1943.†

To check on such possible relationship, each of 4 guinea pigs immunized to the newly isolated agent were inoculated intraperitoneally with 1 ml of a 10^{-1} dilution of Louisiana pneumonitis virus in mouse brain. No evidence of infection was observed. The temperature of all 4 control guinea pigs rose above 105° F, and 2 died. The 2 survivors from this control group were kept until they returned to apparently healthy condition and were given an intraperitoneal inoculation of the new agent. They showed no evidence of infection, while 2 control guinea pigs died. The infectivity of material from the two isolations was compared with Louisiana pneumonitis virus and the 6 BC strain‡ of psittacosis by inoculation through various routes into mice, and by intraperitoneal inoculation into guinea pigs. The pattern of infection produced by the new agent was remarkably similar to that of the Louisiana pneumonitis (Borg) virus and was distinct from that produced by the 6 BC strain. The latter was not as highly virulent for guinea pigs by intraperitoneal inoculation, and proved innocuous for mice by the intramuscular and subcutaneous routes.

Although no work had been done in the laboratory with any of the psittacosis viruses for over a year and a half, it was, nevertheless, deemed necessary to rule out the possibility of accidental contamination. The original serum specimens were reinoculated into mice, and the virus again was recovered from one of the specimens. The other specimen produced symptoms in 3 of 6 mice inoculated. One was killed, but its brain was accidentally discarded. The other 2 recovered and no attempt was made to establish the presence of the carrier state.

On the basis of the high mortality produced upon intraperitoneal inoculation into guinea pigs and intramuscular and subcutaneous inoculation into mice and its toxicity for chickens, the agent can be assumed to be similar to the virus of Louisiana pneumonitis.

As yet, there has been no opportunity to perform the type of serum neutralization test described by Hilleman(10), nor to perform the toxin neutralization test(3,11) which might make the specific strain identification more definite.

It is of interest to note that Olson and associates suspected that some animal reservoir may have initiated the epidemic which they investigated in humans, but they found no such reservoir. The first known case in the 1943 epidemic was in the wife of a trapper living in an area with an abundant wild bird population, and could have been contracted through close contact with infected birds. It may also be significant that a potentially dangerous agent has been shown to be active currently among birds. Further work may throw additional light on the significance of these findings in the epidemiology of this type of human respiratory infection.

Summary. A virus has been isolated from 2 snowy egrets. This virus has been identified by histological and serological tests as a member of the psittacosis-lymphogranuloma group. It closely resembles the Louisiana pneumonitis (Borg) virus in host range infectivity studies.

† This virus, sometimes referred to as the Borg strain, was kindly supplied by Dr. Dorland Davis of the National Institutes of Health.

‡ A standard laboratory strain of psittacosis, originally isolated by Dr. K. F. Meyer from a parakeet. This strain was also supplied by Dr. Dorland Davis.

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Metabolism of Fructose by the Liver of Diabetic and Non-Diabetic Subjects.* (19186)

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The metabolic effects of fructose administered intravenously to man have been studied in this laboratory recently(1). During the period of infusion, fructose was removed at the same rapid rate from the peripheral venous blood of both normal subjects and diabetic patients deprived of insulin. The concentration of blood pyruvic acid rose markedly in both groups of subjects. There was an increase in the blood glucose level of most of the normal subjects and of all of the diabetic patients investigated; in the latter group the glucose rise was of greater magnitude than in the normal group. Other investigators have demonstrated the participation of the liver in fructose metabolism in experiments with tissue slices or homogenates and with intact animals(2-8). Accordingly, hepatic vein catheterization studies were performed to evaluate the role of the liver in fructose metabolism in man.

Experimental procedure and methods. The subjects were 3 diabetic and 3 non-diabetic patients. In 2 of the cases, the diabetes mellitus was mild and easily regulated. Subject M.M. had had severe, labile diabetes mellitus for 23 years; she was in diabetic ketosis during the test and her CO_2 combining power was 30 volumes per 100 ml at the end of the test. None of the patients had evidence of hepatic disease. For 3 days prior to the test each patient received B complex capsules which provided at least 20 mg of thiamine daily; these were given to eliminate any possible effect of a sub-clinical thiamine deficiency on pyruvate metabolism. The diabetic patients were given only regular insulin for 3 days before the test and none on the morning of the test; therefore, no insulin effect was present at the time of the procedure. The tests were performed after an overnight fast. Pre-medication consisted of 0.032 g of codeine phosphate and either 0.2 g of phenobarbital or 0.1 g of seconal. A radiopaque catheter was inserted into a superficial arm vein and its tip was guided under fluoroscopic control into a right hepatic vein. Following a control period, 1.0 g of fructose‡ per kg was administered intravenously in a 10% solution in approximately one hour. Specimens of blood were obtained simultaneously from the hepatic vein and a peripheral artery during the control period; at approximately 30, 45, and 60 minutes after the beginning of the fructose infusion; and 30 minutes after the end of the infusion. These specimens were analyzed in duplicate for the following substances: Fructose(9); total hexose (Somogyi's

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