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Isolation of Mumps Virus from the Blood of a Patient.

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The isolation of mumps virus from the blood of a patient is not known to have been reported. One case is described, the methods of isolation and identification being essentially those employed by others for saliva[1] and for spinal fluid.[2]

Case History. B.M., a boy aged 3 years, 9 months, was admitted to the Children's Hospital of Boston* May 9, 1948, for treatment of cellulitis of the left chest wall incident to a fall. He appeared acutely ill. The temperature was 103°F, an area over the left chest wall was tender and red, and a scarlitiniform rash, thought to be a drug eruption, covered the entire body. Sulfadiazine and penicillin had been administered. Three days later, May 12, the left parotid was firm, swollen and tender. A clinical diagnosis of mumps was made, the temperature at that time being 101°F, pulse 120, and respirations 25. The total leucocyte count on May 11, was 13,800, with 92% granulocytes and 8% lymphocytes; on May 13, it was 10,400, with 52% granulocytes, 38% lymphocytes, and 10% monocytes. Spinal fluid taken May 12 had a trace of globulin, sugar content within normal limits, and no white blood cells. A tuberculin test was positive in 1:1000 dilution of O.T. A marked right parotid swelling appeared on May 16. No signs or symptoms of meningoencephalitis were noted. Recovery was rapid and the patient returned home May 22.

Isolation of Mumps Virus. Specimens examined for mumps virus were introduced into the amniotic sac of 7-day-old embryonated eggs and amniotic membranes and fluids harvested separately after 6 days incubation at 35°C. Fluid from each egg was examined for virus by macroscopic hemagglutination of hen erythrocytes. Cultures in thioglycollate broth of pooled amniotic fluids from each egg passage showed no bacterial contaminants. The amniotic membranes of egg passages giving no hemagglutination were ground with alundum, pooled with their fluids and passed in 0.2 cc amounts intra-amniotically. Only positive fluids were pooled and passed when hemagglutination appeared in subsequent passages. Positive amniotic fluids had a bluish opalescence, those showing no hemagglutination were usually clear.

Blood was drawn from patient B.M. on May 12, the day parotitis was first observed and 5 cc treated with sodium citrate to give a final concentration of 0.5%. Because whole citrated blood had resulted in a high mortality in previous egg inoculations, the plasma was separated from the red blood cells after natural sedimentation, and the two fractions stored in carbon dioxide ice. Two weeks later 12 eggs were inoculated intraamniotically with 0.1 cc of the plasma, and 5 survived for harvesting. The red blood cell fraction was diluted 1:2 in infusion broth, and the mixture in 0.2 cc amounts inoculated into 12 eggs intra-amniotically. Only 3 embryos survived. Since the amount of harvested material was small, it was added to the amniotic membrane and fluid pool of the plasma passage. In the third egg passage, virus was detected in 4 of 8 amniotic fluids, and in almost all eggs of 2 subsequent passages.

The cerebro-spinal fluid of May 12, inoculated into eggs in 0.2 cc amounts, yielded no virus in 3 passages; a not unexpected result since the patient had no symptoms suggesting meningo-encephalitis and the spinal fluid contained no leucocytes. Isolation of virus from the saliva was not attempted because of the age and lack of cooperation of the patient.

[1] Leymaster, G. R., and Ward, T. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 346.

[2] Henle, G., and McDougall, C. L., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 209.

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Attempts to isolate mumps virus from the blood of 7 other patients in the first 5 days of the disease were unsuccessful, all but one of the specimens being drawn after the first day of illness. Whole defibrinated blood is less lethal to chick embryo than citrated blood.

Serologic Studies and Identification of Virus. The inhibition of hemagglutination and the complement fixation tests used in identification of mumps virus have been well-described by others.[3] Paired serums from a patient, taken in the acute and convalescent phases of mumps and known to demonstrate an increasing titer of inhibiting antibodies, were kindly furnished by Dr. J. F. Enders. Strain B.M. mumps virus, freshly isolated from the blood and used as an antigen with the standard acute serum gave inhibition of hemagglutination in a titer of 1:16; with the convalescent serum in a titer of 1:256, a 16-fold rise, titers being expressed in terms of initial dilution. The clinical diagnosis of mumps for patient B. M. was confirmed by a similar test, using the Enders strain of mumps virus as antigen against paired serums of the patient.† Serum of May 12 had a titer of 1:32 and that of May 22, 1:256, an 8-fold rise in titer.

Identification of the virus as that of mumps was further substantiated by test for fixation of complement with pooled amniotic fluids of the sixth egg passage as antigen. Paired sera from a patient with mumps, and with demonstrated increasing complement fixing antibodies (Diagnostic Laboratory, Massachusetts Department of Public Health) were used as a standard. The acute phase serum had a titer of 1:16 and the convalescent serum 1:256.

A complement fixation test of serums from patient B. M., using the Enders strain of mumps virus as antigen, gave a titer of 1:32 with acute phase serum and a four-fold rise to 1:128 with convalescent serum.

Isolations from Spinal Fluid. Successful isolation of mumps virus has been accomplished in 5 or 7 cases of meningo-encephalitis, all of which demonstrated a rise in complement fixing antibodies during convalescence, where spinal fluids were examined within 5 days of onset. An 8-fold or greater rise of titer in the inhibition of hemagglutination test was used in identification of the viruses isolated.

Discussion. A virus was isolated from the blood of a patient on the first day of a parotid swelling conforming to that of mumps. The clinical diagnosis was confirmed by a significant rise in titer of mumps complement-fixing and inhibition of hemagglutination antibodies in the course of convalescence. Two methods, inhibition of hemagglutination and complement fixation, identified the virus as that of mumps.

That mumps virus invades the blood stream is not surprising, in view of the considerable amount of virus appparently present in an infected parotid gland. The regularity of such invasion and its significance in the pathogenesis of the disease, awaits the result of studies now in progress.

Summary. The virus of mumps was isolated from the blood of a patient with bilateral parotid swelling through culture in embryonated eggs. Inhibition of hemagglutination and complement fixation tests confirmed the clinical diagnosis and identified the agent as the mumps virus.

[3] Diag. Procedures for Virus and Rickettsial Diseases. Am. Pub. Health Assn., 1948, p. 139.

† Test was kindly performed by the Research Division of Infectious Diseases, Children's Hospital, Boston.