TABLE II.
Antibacterial and Toxic Action of Halogens on Living Chick Embryos.

Bacteria inoculated	Halogen	Conc.,%	No. of eggs under exper.	No. of dead embryos after 48 hr	Death rate, %
$2x10^6$	—	—	23	20	87
$2x10^6$	Br_2	0.2	11	5	45
$2x10^6$	Br_2	0.06	12	6	50
$2x10^6$	I_2	0.08	16	14	87.5
—	I_2	0.08	5	1	20
—	Br_2	0.1	5	0	0
—	Br_2	0.06	5	1	20

In each experiment $2x10^6$ cells of *S. aureus* from 18-24-hours-old broth culture were used. The eggs were placed for 2-3 hours in an incubator at 37°C to allow the bacteria to begin to multiply. The disinfectants were added after 3, 8, and 24 hours. In control experiments saline instead of halogens was added. The embryos were opened after 48 hours. The results obtained with bromine and iodine are given in Table II. These experiments, too, show that bromine in presence of living tissue is a better disinfectant than iodine.

Conclusion. The assertions of Babcock[19] concerning the effectiveness of bromine *in vivo* in treatment of wounds is confirmed by the toxicity tests.

19 Babcock, W. W., *J. A. M. A.*, 1945, **129**, 1094.

The author wishes to state that this investigation owes much to the interest and help of her late teacher, Prof. I. J. Kligler.

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Mumps Meningo-Encephalitis. Isolation in Chick Embryos of Virus from Spinal Fluid of a Patient.*

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Meningo-encephalitis may occur as a complication of parotitis or as a primary manifestation of infection with the virus of mumps. Whereas in the first instance the time relationships tend to permit a diagnosis of mumps meningo-encephalitis, the diagnosis of the primary encephalitic manifestation has been placed on a firm basis only since the development of a complement-fixation technic by Enders and his co-workers.[1] The demonstration of a rise in complement-fixing antibodies in convalescent serum as compared to the antibody titer in the serum specimen obtained in the first days of the disease may be taken as diagnostic evidence of mumps.[2] By this means, a considerable number of cases of meningo-encephalitis without prior or concomitant parotitis can be diagnosed as due to mumps.[3] Differentiation between antibodies to the soluble and to the virus antigens[4] may be of further diagnostic help.

* The work described in this paper has been aided by the Office of Naval Research.

1 Enders, J. F., Kane, L. W., Cohen, S., and Levens, J. H., *J. Exp. Med.*, 1945, **81**, 93.

2 Enders, J. F., Cohen, S., and Kane, L. W., *J. Exp. Med.*, 1945, **81**, 119.

3 Kane, L. W., and Enders, J. F., *J. Exp. Med.*, 1945, **81**, 137.

4 Henle, G., Henle, W., and Harris, S., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 290.

Antibodies to the soluble antigen tend to appear earlier than antibodies to the virus antigen in a high percentage of infections with the mumps virus. When this pattern of serum reactivity occurs, it is only found in the first few days of infection.[5]

Another approach to the diagnosis of mumps meningo-encephalitis has been the attempt to isolate the etiological agent from the spinal fluid. One such attempt has been successful in the monkey[6] but inoculation of chick embryos failed.[6,7] In the case to be reported here a virus was obtained from the spinal fluid of a patient with meningo-encephalitis without parotitis on the second day of illness by the intra-amniotic inoculation of chick embryos. This agent has been identified as mumps virus.

Case history. The 5½-year-old patient A.P. was admitted to the hospital on 17 March 1947, complaining of fever, severe headache, and occasional vomiting of 2 days duration. The child appeared moderately ill. Physical examination revealed mild nuchal rigidity but no other neurological signs. A slight injection of the pharynx and of the orifices of Stenson's ducts was noted but no parotid or other glandular swelling. The temperature on admission was 103°F, the pulse 108 per minute. All other examinations were negative.

In the night following admission the temperature rose to 105°F, but thereafter decreased steadily and returned to normal on the 4th day in the hospital. The headache and nuchal rigidity persisted for 2 days. On the day after admission, 18 March 1947, a slight disturbance in swallowing was reported which was of very short duration, and complaints of moderate abdominal pain were reported. Thereafter the child improved steadily and was released from the hospital after 15 days on 1 April 1947. There was no known exposure of the patient to mumps. However, a sister developed parotitis on 29 March 1947.

Laboratory findings. Spinal fluid was obtained from the patient on the day of admission, *i.e.*, on the second day of illness. The initial pressure was 450 mm H_2O, the final recording 290 mm. The fluid was slightly cloudy and contained 450 cells per cu mm, of which 92% consisted of lymphocytes, 7% of endothelial cells, and 1% of polymorphonuclear leukocytes. No bacteria could be demonstrated in stained smears, nor could any organisms be cultured on bacteriological media. The total protein in the fluid was 10 mg, sugar, 30 mg and chlorides 712 mg/100 ml. Subsequent spinal fluids taken 24 March and 31 March 1947 showed similar, although increasingly milder, changes, the last specimen containing 55 cells per cu mm.

The blood picture on admission was as follows: 4,500,000 erythrocytes, 13 g hemoglobin, 17,000 white cells, of which 60% were granulocytes, 40% lymphocytes, and 4% monocytes. A subsequent study of the blood on 31 March 1947 was essentially normal.

Other studies included tuberculin tests with O.T. 1:10,000 and 1:1,000, which were negative. The Kolmer-Wassermann, Kahn and complement-fixation tests for lymphocytic choriomeningitis with the serum of the patient taken 1 April 1947, likewise, were negative. Attempts to isolate this virus from the spinal fluid drawn 17 March 1947 failed.†

Serological tests for mumps. The preparation of complement-fixation antigens and the technic used for the complement-fixation test have been described.[4] The tests performed with the sera obtained during the early stage of the disease were not revealing in this case.

TABLE I.
Result of Complement Fixation Tests with Sera from Patient A.P.

Serum specimen	Day after onset of disease	Serum titer (initial dilution) vs. Soluble antigen	Virus antigen
3-17-47	2	1: 4*	1: 4
21	6	1: 4	1: 8
25	10	1: 8	1: 32
4- 1	17	1:16	1:128

* Complete fixation of complement in serum dilution 1:4.

† These tests were performed by Dr. M. M. Sigel.

[5] Henle, G., to be published.

[6] Swan, C., and Mawson, J., *Med. J. Austral.*, 1943, **1**, 411.

[7] Beveridge, W. I. B., Lind, P. E., and Anderson, S. G., *Austral. J. Exp. Biol. and Med. Sci.*, 1946, **24**, 15.

As can be seen in Table I, low concentrations of antibodies to the soluble as well as to the virus antigen were found on the 2nd day after onset. However, a definite diagnosis of infection with mumps virus was possible because subsequent specimens of serum showed significant rises in antibodies to both antigens.

Isolation of virus. Spinal fluid, drawn on the second day of illness was kept frozen at −10°C until injection of chick embryos was possible. Eight 8-day-old embryos were inoculated into the amniotic sac with 0.1 ml of undiluted spinal fluid each. After incubation of the eggs at 36 to 37°C for 5 days, the amniotic and allantoic fluids of each embryo were collected separately and tested on a slide for their capacity to agglutinate chicken red cells.[8] A slight degree of hemagglutination was observed with several of the amniotic fluids. The bacteriologically sterile pool of all amniotic fluids was used for further passage. Five out of 16 embryos of the second amniotic passage died, and of the 11 remaining embryos 10 amniotic fluids but none of the allantoic fluids showed positive hemagglutination. On the 4th amniotic passage the virus in the amniotic fluid reached a 50%-infectivity end point of $10^{-6.3}$ and a hemagglutinin titer of 1:1024 as measured by the pattern test.[4] From the 7th amniotic passage on, when the hemagglutinin titer in the amniotic fluid exceeded 1:4096, a slight degree of hemagglutination became discernible in the allantoic fluids. Inoculation of amniotic fluid of the 9th passage into the allantoic sac of 8-day-old chick embryos resulted in some propagation of the agent in this cavity. After 5 days of incubation, the pooled allantoic fluids showed a hemagglutinin titer of 1:128.

The identity of the virus has been established in 3 ways. First, neutralization tests were performed in chick embryos with amniotic fluid of the 4th passage. Rabbit anti-mumps serum in dilution 1:10 neutralized 10,000 ID_{50} of the agent, whereas rabbit anti-influenza sera failed to do so. A human convalescent serum of high complement-fixing activity reacted to an extent comparable to that of the specific rabbit immune serum. Second, complement-fixation tests with the amniotic fluid of the 5th amniotic passage as antigen revealed strong reactions with known mumps convalescent sera but no, or lesser, reactions with sera taken from the corresponding patients during the acute stage of the disease. Finally, the same amniotic fluid (5th passage) was found to produce typical, although mild, parotitis in 2 out of 4 individuals who were judged susceptible to mumps by the results of complement-fixation tests prior to exposure.‡ All 4 cases developed antibodies to both the soluble and virus antigens. Thus, there was no doubt that the agent constituted a strain of mumps virus. This strain differed markedly from the one present in the laboratory at that time in the following respects: The new strain grew in the early passages only in the amniotic sac whereas the laboratory strain propagated equally well in the allantoic and amniotic cavities; also, the new strain gave rise to clinical mumps in exposed human subjects, while the laboratory strain produced only subclinical infections as demonstrable by rises in antibodies beginning on the 13th to 19th day after exposure.‡

Summary. A virus has been isolated from the spinal fluid of a patient with meningo-encephalitis without parotitis by inoculation of chick embryos by the amniotic route. This agent has been identified as mumps virus.

NOTE: Since this paper was written mumps virus has been recovered from the spinal fluid of another patient (T.P.) with meningo-encephalitis without parotitis. Positive red cell agglutination was obtained in second passage amniotic fluid. The finding of circulating complement-fixing antibodies against the soluble antigen (titer 1:16) but not against the virus antigen of the mumps virus established the diagnosis on the second day of illness.

[8] Levens, J. H., and Enders, J. F., *Science*, 1945, **102**, 117.

‡ These data, obtained in collaboration with Drs. Joseph Stokes, Jr., and Harriet Davis, will be published in greater detail separately.