to increased acidity was partly offset by digitoxin. When contractility was depressed by various chemicals digitoxin usually increased the beat provided spontaneous recovery had been incomplete.

Ouabain produced effects comparable to digitoxin with approximately half the concentration. It did not act more promptly or cause relatively greater increases in beat.

16466 P

pH Stability, Response to Antibiotics and Factors Influencing Egg-Culture of Mumps Virus.*

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Like the agents of influenza the mumps virus grows in embryonated eggs,[1-4] reaching high concentrations in the chorio-allantoic fluid. Studies have been undertaken for the purification and examination of the agent by physical and chemical methods.

Preliminary to the development of purification procedures, studies have been made on (a) the conditions influencing the concentration of the virus in the chorio-allantoic fluid; (b) the effects of antibiotics on the virus and on (c) the pH stability of the virus. The results of this work are briefly given here, and the purification and characters of the virus are described in an accompanying report.[5]

Material and Methods. The virus was the Enders chick embryo adapted strain,[2] obtained through Dr. Lalla Iverson. The methods of inoculating the virus into the chorio-allantoic sac and harvesting the chorio-allantoic fluid were similar to those used in studies of influenza virus.[6] Virus infectivity was titrated in 7-day-old embryos. Hemagglutinative capacity[2,7] was measured by the method of Hirst[8] as used with the influenza virus.[9]

To avoid bacterial growth the use of antibiotics seemed desirable. The effects on the virus in chorio-allantoic fluid of penicillin (500 units per cc) and streptomycin (100 μg per cc) separately, and in combination in the same respective concentrations, were studied in *in vitro* experiments. The mixtures were stored at 4°C and titrated after 20 minutes, 7, 14 and 28 days.

The pH stability of virus infectivity was examined in a composite buffer[10] of borate, phosphate and phthalate (0.04 M total concentration) containing penicillin, 500 units per cc and streptomycin, 100 μg per cc. To 16 cc aliquots of the buffer adjusted to pH values at intervals of 1 pH unit over the range of 2.0 to 11.05, 4 cc of virus-containing chorio-allantoic fluid were added. The mixtures were

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kept at 4°C, and infectivity measurements were made at intervals of 1, 8, 15 and 29 days.

Virus growth was studied in embryos of 7, 8 and 9 days incubation at 37.5°C before inoculation; hemagglutination was measured daily after the 4th day of incubation at 35°C until a total incubation of 15 days. Infectivity measurements were made in parallel with the fluid from the eggs 7 days old at inoculation. The influence of concentration of virus in the inoculum was examined with dilutions of chorio-allantoic fluid of 10^{-0}, 10^{-1}, 10^{-2} and 10^{-3}.

Results. There was no evidence of inactivation *in vitro* of the virus by the antibiotics. Penicillin[3] and streptomycin in concentrations of 500 units and 100 μg per cc introduced with the inoculums (0.05 cc) did not hinder virus growth in the egg.

The greatest increase in virus hemagglutinative capacity occurred in 7-day eggs with the hemagglutinative units per cc and total hemagglutinative units increasing to the 8th day after inoculation. Infectivity in 7-day embryos reached a maximum at the 4th to 6th days after inoculation and diminished thereafter. Infectious titers per cc of pooled fluids have varied from $10^{-8.5}$ to $10^{-9.5}$ and the hemagglutination units (2 +) from 45 to 128 per cc. Titers of individual eggs vary widely, from 22 to 360. The concentration of virus in the inoculum was not an influencing factor.

The mumps virus has been very unstable under any conditions thus far studied. Titers in chorio-allantoic fluid at 4°C diminished as much as from $10^{9.0}$ to $10^{7.4}$ infectious units per cc within 2 weeks. The range of optimum pH stability was from about pH 5.65 to 7.9. Maximum stability appeared to be at pH 6.5 to 7.0. Inactivation was very rapid below pH 4 and above pH 9.5. Little inactivation occurred within 8 days in the range of pH 5.65 to 7.9; after 29 days the titers in this range were about 2 10-fold dilutions lower than the initial level.

Summary. Neither penicillin nor streptomycin, separately or combined, caused demonstrable inhibition of the mumps virus *in vitro* or *in vivo*. Maximum virus infectivity occurred in 7-day-old embryos 4-6 days after inoculation; hemagglutinative capacity reached a maximum in like embryos after 8 days of incubation. The concentration of virus containing chorio-allantoic fluid used as inoculum had no apparent effect on growth of the virus in eggs. The range of optimum pH stability was 5.65 to 7.9, but the virus was relatively unstable under any conditions.

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Purification and Sedimentation and Electron Micrographic Characters of the Mumps Virus.*

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In an accompanying paper,[1] there are described the results of experiments on the growth and stability of mumps virus carried out preliminary to studies on the purification of the virus. The purification and the sedimentation and electron micrographic characters of the agent are described here.

Material and Methods. The general methods employed in the work have already been described.[1] The crude chorio-allantoic fluid harvested 5 days after inoculation of 7-day-old chick embryos, and held at 4°C for 1-4 weeks, was spun in an angle centrifuge at 1,700

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