TABLE I.

Fibrinolytic titration of *S. hemolyticus.*

The table records the highest dilution of the 24-hour Chamberland filtrate giving distinct fibrinolysis.

Strain No.	Fibrinolytic titer with normal plasma-clots			Immune plasma-clots	
	Rach.	Rei.	Van.	Con.	Sim.
135	16	8	2	0	0
10	8	4	4	1	0
28	8	4	4	0	0
95	8	4	1	0	0
93	4	2	1	0	0
46	4	4	0	0	0
168	1	0	0	0	0
175	1	0	0	0	0
41	0	0	0	0	0
Average titer	5.5	3	1.3	0.1	0
Relative susceptibility	4	2.3	(1)	0.1	0

Strain No.	Fibrinolytic titer with normal plasma-clots				Immune plasma-clot
	Muel.	Van.	Mad.	Till.	Sim.
30	16*	16*	16*	16	0
3	16*	16*	16	4	0
91	8	2	1	1	0
22	8	2	1	0	0
4	4	4	1	1	0
61	4	2	1	1	0
94	1	0	0	0	0
15	0	0	0	0	0
Average titer	7	5	4.5	3	0
Relative susceptibility	1.4	(1)	0.9	0.6	0

*Dilutions above 1:16 not tested.

sistant to all strains. Within the limits of the experimental error (one dilution, plus or minus) all normal plasmas are consistently susceptible.

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Intranuclear Inclusions in Brain of Chick Embryo after Inoculation of Egg with Virus of Equine Encephalomyelitis.

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Recently intranuclear inclusions have been described by Hurst[1] in the nerve cells of animals suffering from equine encephalomyelitis.

[1] Hurst, E. W., *J. Exp. Med.*, 1934, **59**, 529.

These bear a close resemblance to those occurring in Borna disease and in poliomyelitis. It becomes of interest to report the finding of inclusions in the brain of the chick embryo after inoculation of the developing egg with the virus of equine encephalomyelitis.

The procedure of inoculation of the developing chicks and membranes has been adequately described by Higbie and Howitt[2] as well as a study of the propagation and neutralization of the virus *in vivo*. Material obtained from this experimental work was fixed either in Zenker's fluid or in saturated corrosive sublimate containing 5% glacial acetic acid. The 2 to 5 micra sections of the membranes and embryos were stained with Giemsa or phloxine-methylene blue. Two series of sectioned embryos and membranes representing 3-hour intervals over a period of 24 hours were available for study. The New Jersey strain of virus and the California strain being represented in each of one series.

A noticeable edema of the chorio-allantoic membrane appeared in 3 to 6 hours following inoculation of the 10 to 12-day incubated egg. Normal horse serum was found to provoke a similar reaction but to less degree while physiological saline failed to produce an edema of the membranes. The swelling and gelatinous appearance of the membranes onto which the virus had previously been dropped rapidly increased and by 18 to 21 hours there was a collapse of many of the vessels and slight patchy streaking of the membranes. No definitely circumscribed areas of infection such as occur when herpes simplex is cultivated on the chorio-allantoic membrane (Dawson[3]) are seen.

Microscopically the membranes first show a thickening of the mesothelial layer and this subsequently becomes more prominent. There is no increase in the numbers of cells in this layer but the intercellular spaces are greater. In 9 hours there is a tendency towards ectodermal proliferation and slight inflammatory reaction. This process is more or less generalized, different areas revealing these reactions in varying degrees. In later stages a necrosis of the ectodermal layer with breaking up of the nuclear chromatin into irregular shaped blocks, ballooning of nuclei and necrosis of cytoplasm results. No intranuclear inclusion bodies are to be found in the cells of the membranes.

The brain of the embryonic chick is markedly softened, edematous and congested with prolonged cultivation of the virus. The New Jersey strain of the virus caused these changes in a shorter period of

[2] Higbie, E., and Howitt, B. F., in manuscript.

[3] Dawson, J. R., *Am. J. Path.*, 1933, **9**, 1.

time than did the California strain. Inclusion bodies in the embryonic nerve cells were more numerous in the various regions of the brain with the former virus. No definite regions of the brain contained greater numbers of inclusions except larger nerve cells were more disposed to their formation. No intranuclear inclusion bodies were found in the embryonic nerve cells of the spinal cord. They made their first appearance after the ninth hour and were abundant in 18 to 24 hours. It is of interest in this regard to note that Higbie and Howitt found the virus to be present in the brain of the chick embryo in 9 hours and thereafter.

The inclusions are rounded acidophilic masses usually located nearer the periphery of the nucleus. They are variable in size, ranging from tiny bodies surrounded by an halo and occupying only a small part of the nucleus, to those which appear to make up one-fourth to one-third of the nuclear volume. Usually there is one inclusion body to a nucleus but more than one is by no means rare; they seldom exceed 2 in a single nucleus. There are in addition to inclusion bodies certain necrotic nerve cells which stain deeply with phloxine and bear a resemblance to nerve cells found in acute poliomyelitis. The nucleus of such a cell is shrunken, the marginated chromatin no longer stains blue and intranuclear inclusions may be present.

To determine if similar bodies appeared after inoculation of certain other neurotropic viruses into the 10-day incubated egg Borna disease and poliomyelitis viruses were utilized. Embryos and membranes were taken from a series of eggs every 6 hours over a period of 12-72 hours following inoculation of the eggs with the virus. In no instance were intranuclear inclusion bodies of the type found in the nerve cells of embryos infected with equine encephalomyelitis apparent in the brains of the embryos infected with Borna disease or poliomyelitis. The inclusions described by Hurst are similar in appearance to those in the embryonic nerve cells of the developing chick. The most obvious difference between the two is the relatively larger size of the inclusions in the embryonic nerve cells.