TABLE I.
Permeability of Blood-CNS Barrier to Horse Serum in Poliomyelitis.

History of monkeys	No. of monkeys	Permeability quotients
Normals	5	0*, 0, 0, 0, 0
Preparalytic	3†	100, 120, 200
	4‡	20, 40, 160, 200
Paralytic	4†	160, 240, 340, 760
	4‡	20, 60, 120, 600

* No horse serum detected in spinal fluid.
† Poliomyelitis following nasal instillation of virus.
‡ " " intracerebral inoculation of virus.

tenshaw[9] in human meningococcus meningitis. The high P.Q. values indicate that horse serum penetrated the barrier in small and, from a therapeutic standpoint, probably insignificant amounts. This may be due in part to the fact that horse serum disappears from the bloodstream relatively rapidly and hence fails to maintain the concentration necessary for penetration of the material into the spinal fluid. Whether the same situation obtains with homologous protein is now under study.

Summary. Normal horse serum administered in the quantity and manner described was found to penetrate the blood-CNS barrier of poliomyelitic animals regularly although in small amounts. From a therapeutic standpoint, it would seem that under the stated conditions such concentrations of serum would probably be inadequate.

10660

Comparison of Activity of Viruses of St. Louis and Japanese Encephalitis in the Chick Embryo.

M. G. SMITH AND E. H. LENNETTE. (Introduced by L. A. Julianelle.)

From the Department of Pathology, Washington University School of Medicine, St. Louis.

The close similarity in the clinical and pathologic pictures of the St. Louis and the Japanese Type B encephalitis in both man and the mouse suggested a comparative study of the activity of these viruses in the chick embryo.

[9] Burtenshaw, J. M. L., *Lancet*, 1938, **2**, 1513.

The St. Louis encephalitis virus has been grown[1, 2] and passed for long periods[3] on the chorioallantoic membrane of the developing chick. Haagen and Crodel[4] have recently reported the cultivation and prolonged passage of the Japanese encephalitis virus on this medium. In the present experiments a comparison was made of the anatomical changes produced by the 2 viruses and of the degree of multiplication which took place in the egg in the 2 instances. In each series of experiments the original inoculum of the chorioallantoic membrane consisted of 0.05 cc of the supernatant fluid from a 10% broth suspension of mouse brain infected with the respective virus. Eggs incubated from 10 to 13 days were used. The virus of St. Louis encephalitis was passed in series for a number of months and the virus of Japanese encephalitis* was carried through 7 passages in the egg. With the St. Louis virus, prolonged cultivation did not alter the original pathologic picture seen in the earlier passages.

At 3 and 4 days after inoculation the appearance of the chorioallantoic membranes inoculated with the Japanese encephalitis virus is grossly indistinguishable from that of eggs inoculated with the St. Louis virus. The most conspicuous finding is the edema of the membranes. In both cases the membranes are slightly opaque, and a very fine stippling is apparent. At 5 days the membranes inoculated with either virus are uniformly opaque and the degree of edema is less. A somewhat greater tendency to the formation of necrotic foci is observed with the Japanese encephalitis virus. However, necrotic foci may occur with either virus, most frequently where the membranes have been traumatized. Membranes inoculated with either virus present a similar microscopic picture. The same sort of diffuse proliferation of the ectoderm with focal accentuations of this process occurs in each case. Vacuolization and necrosis of the surface layers of the ectoderm take place especially in areas where the proliferation is accentuated. For the most part the deeper layers of the ectoderm remain intact but at times it becomes completely necrotic leaving denuded surfaces corresponding to the occasional gross ulcerations. Downgrowths of the ectoderm into the mesoderm also occur.

[1] Harrison, R. W., and Moore, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **35** 359; *Am. J. Path.*, 1937, **13**, 361.

[2] Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL AND MED., 1938, **38**, 799.

[3] Smith, Margaret G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 191.

[4] Haagen, E., and Crodel, B., *Zentralbl. f. Bakt.*, 1938, **142**, 269.

* We are indebted to Dr. S. Kasahara of the Kitasato Institute, Tokyo, Japan for several strains of Japanese virus. The Nagayama strain has been used in these experiments.

Microscopically the mesoderm of the membrane is edematous following the inoculation of either virus. There is a moderate proliferation of the fixed mesodermal cells and some infiltration of wandering cells. The latter frequently occur in foci about vessels or just beneath the ectodermal layer of cells. No specific cellular inclusions have been seen in membranes inoculated with either virus.

When the virus of St. Louis encephalitis is used as the inoculum the majority of the chicks, at least in early passages, remain alive until approximately the time of hatching. When the Japanese encephalitis virus is used most of the chicks remain alive at least 5 to 7 days. Little change has been observed in the brains of chicks following inoculation of the chorioallantoic membrane with either virus. In both instances a few foci of mononuclear wandering cells have been observed in the meninges. Chick brains from the first, second and sixth egg passages of the Japanese encephalitis virus have shown no other changes. Following the inoculation of the St. Louis encephalitis virus, in addition to the slight meningeal reaction, small foci of glial proliferation have been observed, but in only a few instances.

Titrations of the virus content of the chorioallantoic membrane and of the brain of chick embryos inoculated with St. Louis encephalitis virus have been carried out on a number of occasions. Even after many passages, the degree of multiplication of the virus in the egg as determined by mouse inoculations of serial dilutions, has varied little. The chorioallantoic membrane is uniformly infectious for mice in broth dilutions of 10^{-2}, irregularly so in dilutions of 10^{-3}. The chick brain is infectious for mice uniformly in dilutions of 10^{-3} and occasionally in dilutions of 10^{-4}.

The chorioallantoic membranes and the brains from the 5th egg passage of the Japanese encephalitis virus have been tested for their virus content by mouse inoculation with results quite comparable to those obtained following inoculation with the St. Louis encephalitis virus. Mice receiving the 10^{-1} and 10^{-2} broth dilutions of the membranes died in 5 to 8 days after inoculation. One of 3 mice receiving the 10^{-3} dilution of the membrane died. Mice receiving the 10^{-1}, 10^{-2}, and 10^{-3} dilutions of chick brain died in 5 to 8 days, while 2 of 3 mice receiving the 10^{-4} dilution survived.

Following inoculation of the chorioallantoic membrane with the St. Louis encephalitis virus, the virus could also be demonstrated in other organs including the lungs, kidneys, spleen, and liver. Following the inoculation of the chorioallantoic membrane with the Japanese virus, only the liver of chick embryos has been tested in addition to the

membrane and brain. A 10% suspension of this organ in broth was infectious for mice.

Conclusion. The Japanese encephalitis virus and the St. Louis encephalitis virus produce the same type of changes in the chorioallantoic membrane and in the brain of the chick embryo. The two viruses multiply in the egg to approximately the same titer as demonstrated by mouse inoculation. Whether these observations imply more than a similarity of action of the two viruses is not clear from data available from this study.

10661 P

Contractions of Frog's Gall Bladder and Its Possible Use as an Assay Method.

LLOYD D. SEAGER. (Introduced by John Auer.)

From the Department of Pharmacology, St. Louis University School of Medicine, St. Louis, Mo.

Contraction of the frog's gall bladder can be readily demonstrated by the intracardiac injection of crude secretin. The frog's gall bladder is usually bluish green, moderately full of bile, pear-shaped, flabby, and is easily observed when the abdomen is opened. After the intracardiac injection of a dilute secretin preparation, there is a latent period of 15 to 100 seconds; the gall bladder then changes to a rounded spherical form, and the organ develops a slight or marked opalescence; the surface may also show a slight or marked puckering; blood vessels over the surface may become tortuous; apparent volume changes may at times be noted. After 2-10 minutes, the surface again becomes smooth, opalescence disappears, and the bladder becomes flabby.

The ease with which the above could be duplicated suggested its use as an assay method for substances contracting the gall bladder.

A number of preparations have been compared using the following procedures as standard. Active dark colored male frogs weighing 25-35 g were selected; the cerebra were crushed, the cords pithed, and the animals pinned out on frog boards; the feet of the frogs were elevated 1-2 inches above the heads and the gall bladders were exposed; the blood flow to the gall bladder was directly observed microscopically and only preparations were used which showed a good circulation.