(9) The Eastern, Western and Argentine viruses injected intracerebrally may produce in goats a transitory or a rapidly fatal encephalitis.

These observations indicate a very close relationship between the Western and Argentine virus not only with respect to infectivity but also with regard to cross protection. By contrast the Eastern virus of 1933 exhibits a greater virulence and thus may break the immunity established against the Western virus. On the other hand, the Eastern virus apparently protects against the Western virus. Further, these experiments lend considerable support to the conception of an insect transmission of the encephalitis virus as demonstrated by Kelser. The Eastern virus infects readily by the cutaneous route and the infective agent circulates for many hours in the blood of the horse.

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Propagation of Virus of Equine Encephalomyelitis after Intranasal Instillation in the Guinea Pig.

B. F. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco, California.

In an attempt to determine whether or not the virus of equine encephalomyelitis reported by Meyer, Haring and Howitt[1] spreads by way of an initial blood stream invasion and secondary penetration of the meningo-choroid plexus or by axonal propagation as in poliomyelitis,[2–5] the distribution of the infective agent in the tissues of guinea pigs following the intranasal instillation of the virus was studied.

Several series of small guinea pigs were each given 2 cc. of a 20% saline suspension of California virus dropped into the nares. Three animals were killed by bleeding from the heart at each of the different periods of time as shown in Table I. No blood was removed at the twelfth hour, however. The tissues were removed

[1] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.

[2] Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

[3] Flexner, S., *Science*, 1933, **77**, 413.

[4] Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235.

[5] Schulze, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728.

TABLE I.
Chronological Appearance of the Virus of Equine Encephalomyelitis in the following tissues:*

Hrs.	Serum	Filtered Nasal Mucosa	Cervical Glands	Olfactory Bulbs	Maxillary and Sub-maxillary Glands	Pons and Medulla	Cerebellum	Corpora Quadrigemina	Thalamus	Cerebral Hemispheres	Hind Brain	Midbrain	Anterior end of Forebrain
4	0	1st Series 0 2nd Series +	0	0	0							0	
8	+	+	0	0	0						0	0	0
12		+	0	0	0							0	
15	+	0	+	0	0						0	0	0
18	+	+	+	+	0						0	0	0
19	+	+	+	0	+						0	0	0
20	+	+	+	+	0						+	0	0
21	+	+	+	+	+	+	+	0	0	+			0
22	+	+	+	+	+	+	+	+	+	+			+
23	+	+	+	+	0						+	0	+
24	+	+	+	+	+	+	+	+	0	0			1st series 0 2nd series slt. +
36	+	0	+	+	+						+	+	+
48	+	+	+	+	+						+	+	+
96	+		+	+	+	+	+	+	+	+	+	+	+
120	0	+	+	+	0						+	+	+

* + = virus present; 0 = no virus recovered.

aseptically, pooled from the 3 animals, ground with saline and injected intracranially into guinea pigs. The nasal mucosa was extracted in saline and passed through a Seitz filter before inoculation. The serum was given both into the brain and subcutaneously. Several guinea pigs were allowed to live until prostration as controls on the viability of the virus. They all succumbed to the disease.

The results as given in Table I show an immediate and earlier invasion of the blood stream for this experiment than that previously reported[6] for guinea pigs. The virus was constantly present in the serum after the eighth hour and was almost always recovered from the nasal mucosa. Its constant presence in this tissue, however, may have been due to the amount of blood necessarily obtained from this region. It was then recovered from the cervical and salivary glands, the olfactory bulbs and subsequently from the cerebral nerve tissues beginning with the hind brain. After the 36th hour, coincident with the rise in temperature, the virus was constantly present in all of the tissues examined, except for the disappearance from the blood after defervescence and the subsequent prostration of the animal.

To further corroborate the evidence just given of the primary invasion of the blood stream rather than the nerve tissues, an experiment similar to that described by Brodie[4] and by Schulze and Gebhardt[5] for poliomyelitis in monkeys was performed on guinea pigs. The olfactory bulbs were removed surgically from 4 guinea pigs under anesthesia. After one week they, together with 4 normal animals were given 2 cc. of virus intranasally. All 8 guinea pigs succumbed to the disease with typical symptoms. To show that the virus had not been absorbed through the digestive tract, 2 more animals were fed 2 cc. each of the same virus by catheter into the stomach. Neither of them developed the disease.

From the evidence obtained it would seem that the virus of equine encephalomyelitis when given intranasally, gains entrance primarily into the blood stream, presumably through the vascular nasal mucosa and that there is a systemic septicemic invasion before localization in the nerve tissue. A more detailed report of this study will be given later.

[6] Howitt, B. F., *J. Infect. Dis.*, 1932, **51**, 493.