

by microscopic study of sections and smears is sufficient to cause the typical opacity of the cornea and not uncommonly will result in death of the animal.

Similar titrations have been made to determine the potency of antisera. A specimen of serum from a group of 5 foxes that had been given a long series of hyperimmunizing injections of virus was found to neutralize completely an equal volume of a 10^{-1} dilution of virus. (See Fig. 1.)

When virus is injected into one eye, it does not produce visible infection in the other eye. Even if the second eye is traumatized by the injection of some noninfectious preparation, there has been no crossing-over of the infection in our experience. However, it is possible that in the future such cross-infections will be observed occasionally.

Summary. The difficulty involved in the

study of fox encephalitis because of the highly variable susceptibility of experimental animals to fatal infection has been overcome by the use of methods that produce demonstrable, although not always fatal, infection. Instillation of the virus in the anterior chamber of the eye leads to infection of the eye while intramuscular or subcutaneous injection leads to infection in the regional lymph nodes. Extensive studies on the intra-ocular method have revealed that it is so uniformly sensitive that accurate virus titrations and neutralization tests can be made. A positive result is manifested by the development of opacity in the cornea, which can be observed grossly and is readily confirmed by the simple technic of demonstrating characteristic inclusion bodies in smears prepared with scrapings from the inner surface of the cornea.

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Susceptibility of the Raccoon to Fox Encephalitis.

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The virus of fox encephalitis has been shown to be characterized by a limited zoologic adaptation. In the past, only members of the family *Canidae* have been found susceptible.¹

In a series of 5 experiments carried on over a period of 4 years, we used a total of 28 raccoons in attempts to transmit fox encephalitis to animals of this species. Only

one raccoon died and inasmuch as no inclusion bodies were found on microscopic study, the results of all experiments were considered negative. Reexamination of the sections from this animal has recently shown that inclusion bodies were present in one area of meningitis. Using the technic of intra-ocular injection,^{2,3} we have now succeeded in infecting raccoons regularly. The earlier attempts

TABLE I.

Experiment No.	No. of raccoons	Dosage cc %*	Site of inoculation	Results
209T	5	1.5 10	Intracerebral	1 died 5 days after injection
209Ta	10	1.0 5	Subcutaneous	Remained well
209Tb	4	2.0 10	Cisterna magna	" "
		2.0 10	Intramuscular	
471	9	0.5 10	Intracerebral	" "

* Percentage of tissue in the inoculum.

¹ Green, R. G., Ziegler, N. R., Carlson, W. E., Shillinger, J. E., Tyler, S. H., and Dewey, E. T., *Am. J. Hyg.*, 1934, **19**, 343.

² Evans, C. A., Yanamura, H. Y., and Green,

R. G., *Science*, in press.

³ Evans, C. A., Yanamura, H. Y., and Green, R. G., *Proc. Soc. Exp. Biol. and Med.*, 1943, **53**, 183.

to infect raccoons were conducted as shown in Table I. The inoculum in each instance was a suspension of brain from foxes that died of fox encephalitis.

Upon development of the technic of intra-ocular injection as a sensitive test for the fox encephalitis virus, it was decided to reëxplore the range of zoologic adaptation of this virus.

Five normal adult raccoons were inoculated with fox encephalitis virus in the anterior chamber of the left eye (0.2 cc of a 1% pooled brain suspension, Lots 77 and 93, plus a recently isolated strain). At 48 hours, the first evidence of infection was visible as a clouding of the cornea.

At 72 hours, an inoculated eye was removed from one of the raccoons. Aqueous humor was inoculated into veal infusion broth

and remained sterile. A smear of the corneal endothelium showed numerous typical inclusion bodies. The inoculated eyes of the other 4 raccoons all developed complete opacity of the cornea (Fig. 1). Two were removed on the fifth day and 2 on the sixth day. All showed numerous characteristic intranuclear inclusion bodies in the corneal smear. Culture of the aqueous humor in each instance was negative. On the fifth and sixth days, one raccoon showed hyper-irritability and had convulsions, typical symptoms of fox encephalitis. Retransmission was made of virus from this animal. Virus was shown to be present in the spleen by inoculation of the eyes of 2 raccoons, both of which were grossly and microscopically positive. Further transmission from the eye of one of these animals to the eye of another raccoon was also successful.

Two of the raccoons in the original group of 5 inoculated on March 10 were subjected to surgical removal of the inoculated left eye at 3 and 6 days respectively. Both eyes were found to contain typical inclusion bodies of encephalitis. Thirty-six days after inoculation, these animals were given a second injection of encephalitis virus as a test for immunity. Two-tenths of a cc of a 10^{-4} dilution of virus (pooled fox brain, Lot 93) was injected into the anterior chamber of the right eye. This dose constitutes about 10 minimal infective doses for the eye of a fox. Both animals were killed 5 days later. At this time the cornea was clear and no inclusions could be found in smears of the corneal endothelium.

Conclusion. Although raccoons are relatively resistant to fox encephalitis, they are susceptible to invasion by the virus, as shown by a positive intra-ocular test, by demonstration of the virus in the spleen of 1 animal showing typical symptoms, and by the development of immunity as evidenced by negative intra-ocular tests 36 days after the original inoculation.



FIG. 1.

Appearance of the eye of a raccoon 6 days after inoculation of fox encephalitis virus into the anterior chamber. The uninoculated eye remains normal.