horse serum, reduces the severity of anaphylactic shock in these animals, there being no deaths in 22 animals as against 9 deaths in 26 controls. As Benadryl has a similar modifying effect upon the shock induced by the injection of histamine, the results of these experiments are consistent with the theory that histamine plays a significant role in anaphylaxis in dogs. Unfortunately, Benadryl merely reduces, but does not obliterate, the vasodepressor effects of histamine and thus the present experiments with Benadryl do not permit conclusions as to whether histamine is or is not the sole vasodepressor factor in anaphylaxis in the dog.

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Corneal Reaction to Viruses of Equine Encephalomyelitis After Intra-Ocular Injection.*

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In a previous publication[1] it was reported that injection of certain viruses into the eyes of suitable animals caused specific reactions in the ocular tissues. Corneal edema and opacity were produced when the viruses of fox encephalitis, influenza, and equine encephalomyelitis were employed. It was demonstrated that virus of fox encephalitis, even in minute dosage, would infect the corneal endothelium and induce the corneal reaction in dogs, foxes and raccoons.[2,3] The influenza virus caused an entirely similar reaction in the eyes of rabbits in spite of a complete absence of infection, by a direct toxic effect.[4] The virus did not grow in the rabbit eye and was effective only in very large doses.

Further studies reported in this paper suggest that the corneal opacity in rabbits caused by the viruses of western and eastern equine encephalomyelitis is analogous to the toxic reaction demonstrated for influenza virus even though these viruses may grow to a limited extent in the ocular tissues.

In our first experiment a 10^{-3} dilution of mouse brain infected with a highly virulent strain of virus of W.E.E. (Western Equine Encephalomyelitis) was injected into the anterior chamber of one eye of each of 3 rabbits. At 48 hours all showed some cloudiness of the cornea and at 3 days the corneas of all injected eyes were opaque.

In subsequent experiments 27 rabbits have been injected with 2 different strains of western virus. One of the western strains was highly virulent and had been maintained by passage in mice for many years by Dr. Carl M. Eklund, who obtained it from Dr. P. K. Olitsky of the Rockefeller Institute for Medical Research. This we call the Rockefeller strain. The second strain of western virus had been isolated from a fatal human case of encephalitis by injection of brain tissue into guinea pigs. Some of the infected guinea pig brain tissue that had been kept frozen for more than a year was used to infect guinea pigs and mice, the brains of which served as the source of virus for injection into eyes. This is called the "low passage" strain of virus.

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TABLE I.

Tests for Virus in Aqueous Humor of Rabbit Eye. All animals were injected with 0.2 ml of a 10% suspension of mouse brain infected with the Rockefeller strain of western equine encephalomyelitis virus.

	Rabbit No.	Eye	Interval*	Fate of mice injected with dilutions: 10^{-0}	10^{-1}	10^{-2}	Cornea
Exp. No. I	K423	Rt.	60 hr	†5 5 s	s s s	s s s	Opaque
		Left	" "	s s s	s s s	s s s	"
	K424	Rt.	" "	s s s	s s s	s s s	Slight cloudiness
		L.	" "	s s s	s s s	s s s	" "
II	K433	Rt.	24 hr	2 2 2	2 2 2	2 2 2	Insufficient time
		L.	" "	2 2 2	2 2 2	2 2 2	" "
	K435	Rt.	3 days	s s s	s s s	s s s	Clear
		L.	" "	s s s	s s s	s s s	"
III	K425	Rt.	5 "	s s s	s s s	—	"
		L.	" "	s s s	s s s	—	"
	K426	Rt.	" "	3 3 3	3 3 3	—	Opaque
		L.	" "	s s s	s s s	—	"
IV	K501	Rt.	3 days	s s s	s s s	s s s	"
		L.	" "	s s s	s s s	s s s	"
	K503	Rt.	2 "	5 5 s	5 s s	s s s	"
		L.	" "	3 3 3	3 3 4	4 4 4	"

* Time between inoculation into ant. chamber and aspiration of aqueous humor.
† Numbers = day of death. S = survived.

Injection of the 12 eyes of 6 rabbits with various dilutions of the low passage strain of virus gave entirely negative results. Five of the eyes were injected with a 10% suspension of infected brain. All eyes, except 2, were injected with an inoculum which killed mice regularly in a dilution of 10^{-7} and sometimes in dilutions of 10^{-8}.

In further studies with the Rockefeller strain, negative results have been obtained in all eyes injected with virus (infected mouse brain) diluted to 1:100 or beyond. When a 10% suspension was employed, only half of the infected eyes showed the characteristic reaction. This was surprising in view of the results of the first experiment in which a 10^{-3} dilution was injected and all 3 injected eyes were positive. Unfortunately, the records showing the results of the titration of the virus used in this first experiment were lost. The later experiments were carried out after an interval of more than one year with virus that killed all mice injected with a 10^{-6} and about half those given 10^{-7} dilution. It was our recollection that the virus used in the earlier experiment killed mice in a dilution of 10^{-8}.

As shown in Table I Experiments I and III, rabbits injected in the same experiment with the same dose of virus might differ in their reactions. Numerous aspirations and cultures of aqueous humor showed an absence of pus cells and bacteria. It was felt that the varying results indicated that the amount of virus injected approximated one 50% minimal reactive dose (analogous to "1 MLD 50").

Tests for virus in aqueous humor were carried out at 24 hours, 48 hours, 60 hours, 3 days, and 5 days with the results shown in Table I. After injection of more than 300,000 mld (for mice) of virus into the anterior chamber, the aqueous humor contained virus in considerable amount at 24 hours but little or none remained at 48 hours and later. No virus was detected in ocular tissues at 3 days in 1 experiment. Of 4 eyes tested at 5 days, one contained a moderate amount of virus. This suggests that some growth of virus may have occurred. Of the 30 rabbits used in these experiments, only 3 showed symptoms indicative of encephalitis.

An eastern strain of equine encephalomyelitis virus obtained from Dr. Carl M. Eklund has been injected into the 10 eyes of 5 rabbits. All 10 eyes developed the

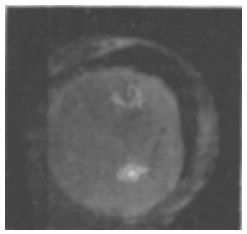

FIG. 1.

Rabbit eye 4 days after injection of the virus of eastern equine encephalomyelitis into the anterior chamber (K513).

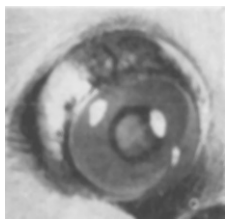

FIG. 2.

Rabbit eye showing minimal cloudiness of cornea with a typical hyperemia of subconjunctival vessels associated with uveitis. K424, 3 days after injection of western equine encephalomyelitis virus.

characteristic corneal opacity, usually of severe degree. The inoculum in each case was a 10% suspension of infected mouse brain. Titrations of the inocula by intracerebral injection in mice have shown that a 10^{-7} dilution was regularly fatal and a 10^{-8} dilution occasionally killed mice.

Tests for virus in the aqueous humor and/or ocular tissues have been made at 2, 4 and 11 days. Results as presented in Table II show that a negligible amount of virus remained. This was true even in an animal suffering from a severe encephalitic infection.

Of the 5 rabbits injected with the eastern virus, 2 were killed at 2 and 4 days and 1 of the remaining 3 developed encephalitis.

Discussion. From the data presented in Tables I and II, it is clear that the corneal opacity observed in rabbits after intraocular injection of the viruses of western equine encephalomyelitis is not the result of the growth of virus in the eye, even though such growth may occur to a limited extent. In control experiments the injection of suspensions of normal brain and of numerous other non-viral preparations into rabbits' eyes has never in our experience caused the type of ocular reaction that characterized the experiments with these viruses or influenza viruses. As in the experiments with influenza virus, large doses of equine virus (in the vicinity of a million mld for mice) must be injected in order to produce the corneal reaction. In most of our experiments, the dosage of western virus was apparently close to the minimum level necessary to produce a reaction inasmuch as frequently some eyes were negative while others were positive. The minimum dose of eastern virus that will cause the reaction was not determined.

In the present paper, attention is centered on the corneal reaction because this is the most prominent and most characteristic change observed. A uveitis is less specific but was even more regularly present as indicated by the occurrence of hyperemia and deep radial folds in the iris and a corona of dilated vessels visible around the cornea and extending back over the bulbar con-

TABLE II.

Tests for Eastern Equine Encephalomyelitis in Rabbit Eyes After Injection of 0.2 ml of 10% Infected Mouse Brain.

Rabbit No.	Eye	Material tested	Interval, days	Fate of mice inj. with dilutions			Cornea
				10^{0}	10^{-1}	10^{-2}	
K510	Rt.	Aq. h.	2	2 2 3	3 4 s	s s s	Opaque
	"	Tissue	"	—	2 2 6	s s s	"
	Left	"	"	2 s s	s s s	s s s	"
K511	Rt.	Aq. h.	4	s s s	s s s	s s s	"
	"	Tissue	"	—	4 s s	s s s	"
	L.	Aq. h.	"	s s s	s s s	s s s	"
K531*	Rt.	"	11	s s s	—	s s s	"
	L.	"	"	s s s	—	s s s	Opaque at 3 days. Cleared

* Severe encephalitis.

junction to a variable extent. Histologically, this uveitis appeared as hyperemia of the iris and ciliary body, usually with very little cellular exudate. The choroid and retina were not visibly affected after injection of either the eastern or western viruses into the anterior chamber.

Conclusion. The corneal edema and opacity resulting from intra-ocular injection of the virus of western equine encephalomyelitis in domestic rabbits occurs only in response to large amounts of virus. It appears to be analogous to the ocular response to influenza virus, which has been attributed to toxic properties of the virus. An entirely similar ocular reaction has been induced by injection of equine encephalomyelitis virus of the eastern type. Limited growth of equine encephalomyelitis virus may occur in the eye but is not a significant factor in the described reaction.

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A Vaccine Against Japanese B Encephalitis Prepared from Infected Chick Embryos.

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(Introduced by J. E. Smadel.)

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Most of the pathogenic viruses affecting man have been cultivated in the developing embryonated egg.[1] In addition the egg has provided a source of vaccine for immunizing human beings against such viruses as yellow fever,[2] influenza,[3] vaccinia,[4] and equine encephalomyelitis.[5] Although the virus of Japanese B encephalitis was shown to multiply in the embryonated egg,[6] growth of this agent in chick embryo tissue was not sufficiently luxuriant to offer promise of providing material for a potent vaccine against this disease. Indeed, the reported experiments regarding such a vaccine indicated that it possessed little immunizing capacity.[7]

The vaccines at present employed against Japanese B encephalitis are prepared from infected brain tissue of mice.[7] These are capable of eliciting neutralizing antibodies against the virus in vaccinated human beings and of inducing resistance to infection in experimental animals. Such vaccines have been prepared commercially and have been authorized for use in the U. S. Army. Although the mouse brain vaccine has desirable immunological properties, it possesses certain potentially objectionable features. For example, it is well known that vaccines containing brain tissue may on occasion produce a demyelinizing encephalopathy. In addition the technical difficulties inherent in the large scale manufacture of such a vaccine are considerable. Because of these disadvantages it seemed worth while to reinvestigate the possibilities of obtaining a potent vaccine from tissues of infected chick embryos.

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