absorbed from the intestinal tract than Vitamin D_2, produced from this pro-vitamin by irradiation.

Summary. 1. 7-hydroxy-cholesterol when given subcutaneously to rats, that are subsequently exposed to ultra-violet irradiation has a higher anti-rachitic effect than 7-dehydro-cholesterol injected into rats under identical conditions. 2. 7-keto-cholesterol when given subcutaneously to rats that are subsequently exposed to ultra-violet irradiation has equal or higher-anti-rachitic effect than 7-dehydro-cholesterol. 3. The *per os* introduction of 7-keto-cholesterol, 7-hydroxy-cholesterol and 7-dehydro-cholesterol into rats subsequently irradiated produces a lesser anti-rachitic effect than the subcutaneous injection of similar amounts of the sterols under otherwise identical conditions. Activated 7-dehydro-cholesterol forming an exception as shown in a previous report.

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A Sensitive Test for Fox Encephalitis Virus.

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Work with the virus of fox encephalitis has been hampered by the failure of this virus to cause visible illness or death in all infected animals. In a small series of from 5 to 10 foxes or dogs, evidence of infection may be observable in from 0 to 100% of animals after the intramuscular or intracerebral injection of adequate doses of living virus. Such inconstant results make accurate titrations of virus or antiserum, or the test of the efficacy of a vaccine, so costly as to be impracticable.

The following experiments have been conducted in developing a sensitive test for fox encephalitis virus. It was believed that foxes that failed to show symptoms of infection after the injection of virus underwent inapparent infections.[1] Inasmuch as cells infected with the fox encephalitis virus develop characteristic, large, intranuclear inclusions, an attempt was made to place the virus in contact with susceptible cells that could be subsequently examined histologically. It is known that this virus attacks vascular endothelium, reticulo-endothelium of the liver, spleen, and lymph nodes, histiocytes, and certain specialized cells such as ependymal and hepatic cord cells.

In our laboratory, fox encephalitis virus is routinely preserved as a 20% suspension of homogenized fox brains in 50% neutral glycerin. One such suspension, Lot 94, mixed with an equal volume of a similar suspension of liver from infected foxes was used in the first experiment. Just before injection, the tissue suspensions were diluted with 9 volumes of isotonic NaCl solution.

A 3-fold approach to the problem was made by searching for inclusion bodies in (1) tissues at the site of subcutaneous and intramuscular injections, (2) lymph nodes draining such regions, and (3) cells of the eye after intra-ocular injection. Virus was injected into each of the 4 legs and into the floor of the mouth of 5 foxes. Tissue from the sites of subcutaneous and intramuscular injections (1 or 2 cc of 2% virus suspension) was removed at intervals of from 1 to 5 days and examined for inclusion bodies. The results were consistently negative. It is probable that inclusions develop in such areas but are too hard to find to be of value in detecting small amounts of virus.

From the same animals, lymph nodes draining the regions in which the inoculations had been made were removed at intervals of from 1 to 5 days. Grossly, a few of the lymph nodes were enlarged, edematous and hyperemic; others were normal. As shown

[1] Green, R. G., Katter, M. S., Shillinger, J. E., and Hanson, K. B., *Am. J. Hyg.*, 1933, **18**, 462.

TABLE I. Development of Fox Encephalitis Inclusion Bodies in Lymph Nodes and Eyes of Foxes.

Days after injection	Fox No.		Lymph nodes: Popliteal L	Popliteal R	Inguinal L	Inguinal R	Iliac L	Iliac R	Axillary L	Axillary R	Supraclavicular Submaxillary L	Supraclavicular Submaxillary R	Eyes L	Eyes R
1	30678	Biopsy		0		0				0				++
	30682	"			0				0					++
2	30678	Killed	0		0		++++	++	0		0		++	
	30679	Biopsy												++
	30682	Killed		0		0	0*	0		0		0	++	
3	30679	Biopsy		0		0				++		0	+++	
	30680	"	++		0				+++		0			+++
	30681	"												++
4	30679	Killed	+		+++		++++	+++	++		0		++	
	30680	Biopsy												+++
	30681	"	0		0				+++		0		+	+++
5	30680	Died		++		0*	++++	++++		+++		+++		+
	30681	Killed		+++		++	+++	+++		++		+		0

*Inflammation but no inclusion bodies. Ciphers indicate that no inclusion bodies were seen. Plus signs indicate relative abundance of inclusion bodies.

in Table I, inclusions were found regularly in lymph nodes from the axillary and iliac regions. It is probable that the lesser degree of success in finding inclusion bodies in lymph nodes from other areas could be ascribed principally to our failure to remove the correct node. The results indicated that examination of regional lymph nodes after injection of virus would prove a successful method of detecting fox encephalitis virus. This method was not studied further only because intra-ocular injection was found to be even more satisfactory. However, it seems probable that in the case of some other viruses in which silent infections are an obstacle to investigation, utilization of lymph nodes in this manner will be of value.

All 5 foxes used in the preceding studies were also inoculated in the eye. Under ether anesthesia a hypodermic needle was inserted through the sclera near the corneal margin and was passed forward into the anterior chamber. About 0.5 cc of aqueous humor was removed and an equal volume of virus suspension was injected. Eyes were removed at 24-hour intervals up to 4 days. Microscopic study of sections showed that all 10 eyes contained cells bearing inclusion bodies.

At 24 hours the inclusion bodies were few and were identified only in cells in the region of the pectinate ligament. At 48 hours inclusion bodies were common both in the angle of the anterior chamber and in endothelial cells lining the inner surface of the cornea. The number of inclusion bodies subsequently increased and cells containing these structures were abundant in eyes removed on the fourth day. At this time the cornea was grossly opaque and of a light blue-gray color. In view of the fact that every injected eye showed conclusive evidence of infection, this method of detecting virus was chosen for further investigation. Inoculations of the eye are now made by inserting a needle through the conjunctiva and passing it forward in the subconjunctival space and through the cornea close to its junction with the sclera. This route reduces the incidence of hemorrhage.

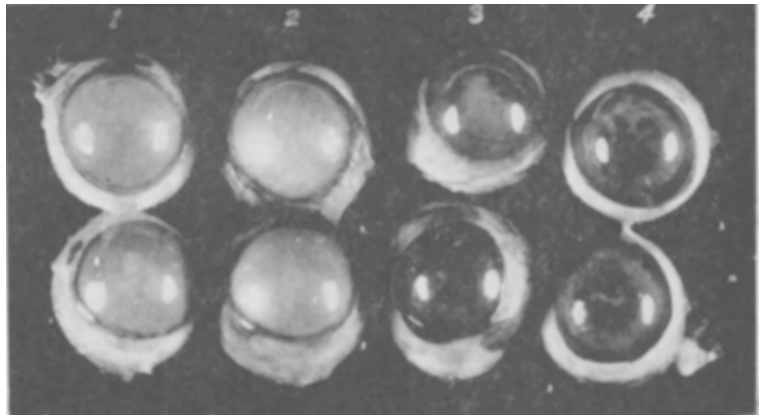

FIG. 1.

Eyes of foxes removed 5 days after intra-ocular inoculation. Inoculum in eyes 1 and 2 was active fox encephalitis virus. In eyes 3 and 4 an equal amount of virus that had been inactivated by incubation with hyperimmune serum was injected. The opacity in eyes 1 and 2 is due only to changes in the cornea. The aqueous humor remains clear. There is very little conjunctival exudate. The fibrin clot present in the anterior chambers in eyes 3 and 4 and dimly visible in some of the other eyes is a nonspecific reaction.

Several years ago we devised a technic for demonstrating quickly the inclusion bodies of canine distemper in animals that had died of that disease, by staining smears of bladder epithelium.[2] This technic has now been adapted to the demonstration of inclusion bodies in certain tissues of the eye. In fox encephalitis we ordinarily use the endothelium of the cornea and stain the smear with a modified Shorr's stain.[3] Other stains are also satisfactory and other tissues of the eye can be studied by this method. All eyes that have shown gross evidence of fox encephalitis have been found positive when suitable smears of the corneal endothelium were examined. (See Fig. 2.)

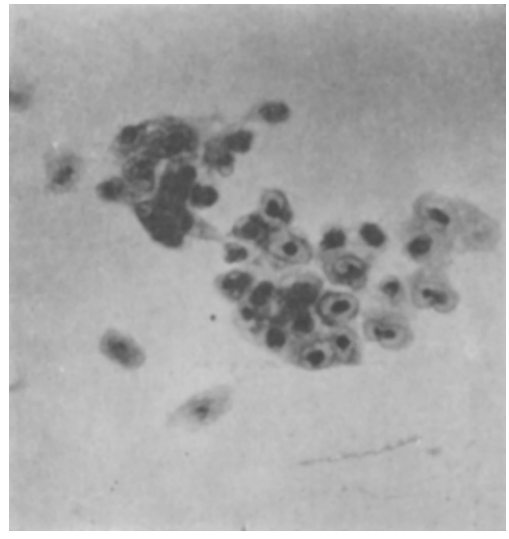

FIG. 2.

Intranuclear inclusion bodies of fox encephalitis in a smear of corneal endothelium stained by a modification of Shorr's stain.

Titrations of fox encephalitis virus have been performed by the use of eye inoculation. Three different lots of brain virus (Lot 77, Lot 93, and a recently isolated strain of virus) were used. Serial, ten-fold dilutions were prepared in Ringer's solution and the virus was injected into the anterior chamber of eyes of foxes. In each instance the virus was found fully active in a dilution of 10^{-5}. Subsequent further titration of Lot 93 showed that no infection occurred at dilutions of 10^{-6} or greater. The results of all such titrations are evident grossly, and are readily confirmed by smears at 5 days. Positive results have not been obtained at a later time in eyes that were negative at 5 days.

The smallest dose of virus that will cause an infection in the eye that is demonstrable

[2] Green, R. G., and Evans, C. A., *Cornell Vet.*, 1939, **29**, 35.

[3] Page, W. G., and Green, R. G., *Cornell Vet.*, 1942, **32**, 265.

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 antiserums. 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. Reëxamination 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	Dosage %*	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.