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Susceptibility of the "Gopher," *Citellus Richardsonii* (Sabine), to Equine Encephalomyelitis.

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Epizootic equine encephalomyelitis, the specific causative virus of which was first recovered from affected horses by Meyer, Haring and Howitt,[1] is a disease with a definite seasonal occurrence and an advancing geographical distribution in North America.[2] Two distinct types of the virus have been recognized, Western and Eastern[3, 4], of which the latter is restricted to the Atlantic coast states, whereas the former is spreading over an increasingly large area.[2] The disease is limited in nature, insofar as present knowledge is concerned, to members of the equine family, although evidence somewhat suggestive of its transmission to man has been presented by Meyer.[5] Experimentally, the susceptibility of a wide variety of hosts has been demonstrated.[1,2,4,6,7,8,9] (The host range is more limited for the Western than for the Eastern type.) In spite of this fact, however, no possible residual host has as yet been demonstrated among the wild animals native to North America. The discovery of such a host would be noteworthy, especially if the new host were representative of a large number of wild animals with natural habitats in close proximity to equines during the limited time of year at which the disease occurs. Evidence that such a host has been found is offered in the present communication, which reports the susceptibility of the "gopher," *Citellus richardsonii* (Sabine),* to the virus

1 Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227; *J. Am. Vet. Med. Assn.*, 1931, **79** (N.S. **32**), 376.

2 Giltner, L. T., and Shahan, M. S., *J. Am. Vet Med. Assn.*, 1936, **88** (N.S. **41**), 363.

3 Ten Broeck, C., and Merrill, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 217.

4 Giltner, L. T., and Shahan, M. S., *Science*, 1933, **78**, 63.

5 Meyer, K. F., *Ann. Int. Med.*, 1932, **6**, 645.

6 Syverton, J. T., Cox, H. R., and Olitsky, P. K., *Science*, 1933, **78**, 216.

7 Olitsky, P. K., Cox, H. R., and Syverton, J. T., *J. Exp. Med.*, 1934, **59**, 159.

8 Hurst, E. W., *J. Exp. Med.*, 1934, **59**, 529.

9 Remlinger, P., and Bailly, J., *Bull. Acad. Vet. France*, 1935, **8**, 420.

* We wish to thank Mr. George G. Goodwin of the American Museum of Natural History, New York City, for specific allocation of the "gophers" used.

of equine encephalomyelitis, Western type.† This type was used because the geographical distribution of the disease in equines embraces that of the newly demonstrated host. Furthermore, the lower virulence of this type of virus lends greater significance to the successful serial passage by intranasal instillation of virus.

The Richardson ground squirrel, *Citellus richardsonii* (Sabine), is native to Saskatchewan, Alberta, Montana and the Dakotas. It is known locally by the name "gopher." Probably the most common wild animal in this district, it represents one of a large number of closely related species which are distributed throughout much of the United States and Canada. Its natural habitat is pastures and fields where grain, roots and other vegetation provide ample food supply. The adult animal, gray-brown in color, measures from 7 to 9 inches over all and is characterized, as are the related species, by the possession of long claws and powerful limbs with which to burrow beneath the surface of the ground. Several litters of from 2 to 8 offspring each are born during the summer months. This explains the great increase in numbers as well as the marked activity of the animals during this period.

The brain tissue of a guinea pig *in extremis* following the intranasal instillation of the Western type of equine encephalomyelitis virus was used as source virus. This material was found to be active in the guinea pig brain in a dilution of 10^6. The tissue was ground without an abrasive and diluted to make a 10% suspension in nutrient broth. Following centrifugation at 1500 r.p.m. for 5 minutes, the supernatant fluid was introduced into the nares of a "gopher" under light ether anesthesia. Introduction of the fluid was accomplished by means of a tuberculin syringe fitted with a blunt needle, care being taken to prevent the needle from touching the tissues. Using a similar procedure, the inoculum consisting of brain tissue of the generation preceding, the infectious agent was carried through 12 successive passages in "gophers." The clinical syndrome, which terminates fatally in most instances, is characterized by an incubation period of from 2 to 4 days, followed by general weakness, ataxia, marked tremors, grinding of the teeth, with death on the third to sixth days. Thus it is similar to that observed in domesticated laboratory rodents. It differs from that of the guinea pig and rabbit in that less than 50% show a febrile response.

Following 12 successive serial passages in "gophers" by intra-

† Samples of the Eastern and Western types of equine encephalomyelitis virus were sent to us through the kindness of Doctors L. T. Giltner and W. S. Gochenour of the United States Bureau of Animal Industry, Washington.

nasal inoculation of virus-brain tissue suspension derived from the preceding passage host, the virus was found to be lethal in a dilution of 10^4. The titer was determined by the intracerebral inoculation of guinea pigs, using two animals for each decimal dilution. This titer does not differ materially from that in guinea pigs following serial passage by the intranasal technique.

Following the "gopher" passage, the specificity of the passaged virus was verified by its specific neutralization with rabbit anti-equine encephalomyelitis serum of the Western type.‡ Briefly, the technique consisted of mixing equal parts of a 2% broth suspension of "gopher" brain virus with an equal volume of anti-equine encephalomyelitis rabbit serum and normal rabbit serum for control. The serum-virus mixtures were incubated at 37°C. for 2 hours and used in 0.15 cc. amounts for the intracerebral inoculation of guinea pigs under light ether anesthesia. Two animals were used for each decimal dilution of a series extending from 10^1 through 10^7. The antiserum protected all of the animals with the exception of one receiving the 10^2 mixture. All of the control animals died up to and including the pair which received 10^4.

It is noteworthy that one "gopher" was found to be refractory to the disease. This suggested that this animal may have contracted the disease in nature. With this possibility in mind, cage contact was attempted. To date none of the small number of contact animals tested has contracted the disease. Work is under way to determine the susceptibility of other native wild rodents as well as the possible rôle of arachnid vectors in the dissemination of this disease.

Summary.—Evidence is presented of the susceptibility of the "gopher" or Richardson ground squirrel, *Citellus richardsonii* (Sabine), to the virus of equine encephalomyelitis, Western type. Twelve successive passages through "gophers" have been effected by intranasal instillation of virus-brain tissue suspensions derived from the preceding passage. The virus was recovered following the final passage. Its pathogenicity for guinea pigs and its specificity were demonstrated. It is suggested that the "gopher" and other wild rodents may possibly act as reservoir hosts for the virus in nature.

‡ We are indebted to Doctor H. R. Cox of the Rockefeller Institute for Medical Research, New York City, for supplying us with rabbit anti-serum against both the Eastern and Western types of equine encephalomyelitis virus. This enabled us to establish the serological specificity of the gopher-passaged virus with anti-sera prepared against different lots of virus than that used for initiating the present studies.