to 2 years. None of these subjects had any skin or allergic disease. The results are shown in Table II.

The elevation of the serum potassium when the eczema has progressed to the point where the papules of the disease coalesce and produce a moist red surface exuding fluid is a most interesting observation. These little patients certainly must have at this stage a marked disarrangement of the normal electrolyte balance. The threshold for the excretion of potassium is raised subjecting the tissue cells including those of the skin to a high potassium content. Water must be drawn into the cells. Swelling takes place with some cell disruption. This may cause the extreme irritability of the skin which is present at the height of the disease. It is in line with the studies of Klauder and Brown,[6] who noted that in an examination of the content of calcium, potassium, magnesium and sodium in rabbit skin in relation to cutaneous irritability as judged by the applications of croton oil, there was an inverse relation between irritability of the skin and calcium content and a direct relation in regard to potassium. The greater the irritability of the skin the greater the likelihood of a low calcium and a high potassium content. Potassium chloride therapy, therefore, does not appear to be indicated in infantile eczema.

13556

Infection of Horses with St. Louis Encephalitis Virus, Experimental and Natural.*

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Within the last year evidence has been obtained indicating a close epidemiological parallel between the viruses of the "equine" encephalitides and that of St. Louis encephalitis. Howitt[1] first called attention to antibodies to both Western equine and St. Louis viruses in the blood of certain patients in California. More recently, Ham-

[6] Klauder, J. V., and Brown, H., *Arch. Dermat. and Syph.*, 1927, **15**, 1.

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[1] Howitt, B. F., *Am. J. Pub. Health*, 1939, **29**, 1083.

mon,[2] and Hammon and Howitt[3] pointed out that human infections apparently due to both viruses were occurring during the same epidemic, and possibly even simultaneously in the same individual. This occurred during an epidemic in the Yakima Valley, Washington. Howitt,[4] over a period of several years, collected a large number of horse sera from California, a high proportion of which neutralized both the Western equine and the St. Louis viruses. Philip, Cox and Mountain[5] noted St. Louis virus antibodies in horses in Colorado, Montana and Washington. The same year Hammon and Howitt[3] made a similar observation in Washington.

In the Yakima Valley, Hammon, Reeves, Brookman, Izumi and Gjullin[6, 7] isolated repeatedly both the St. Louis and the Western equine viruses from the same species of mosquito, *Culex tarsalis,* and from no other species, though all biting arthropods found were tested. Further evidence of this close epidemiological relationship was obtained in an extensive survey of mammals and birds in this area by Hammon, Gray, Evans, Izumi and Lundy.[8, 9] Among approximately 600 sera tested, from domestic and wild species, equal proportions were found positive to the St. Louis and to the Western equine viruses. As was the case with the equine virus, a much higher percentage of horses and mules had antibody to the St. Louis virus than was found in samples of any other species of mammals or birds. It was not surprising, therefore, to learn of experimental confirmation of the susceptibility of horses to the St. Louis virus.

Cox, Philip and Kilpatrick,[10] using a St. Louis strain isolated by Webster, inoculated horses by the intracerebral route. Those without previous serum antibody for this virus developed clinical encephalomyelitis, fatal in some instances. During the terminal phase

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[2] Hammon, W. McD., *J. A. M. A.*, 1941, **117**, 161.

[3] Hammon, W. McD., and Howitt, B. F., *Am. J. Hyg.*, 1942, **35**, 163.

[4] Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 247.

[5] Philip, C. B., Cox, H. R., and Mountain, J. H., *Pub. Health Rep.*, 1941, **56**, 1388.

[6] Hammon, W. McD., Reeves, W. C., Brookman, B., Izumi, E. M., and Gjullin, C. M., *Science*, 1941, **94**, 328.

[7] Hammon, W. McD., Reeves, W. C., Brookman, B., and Izumi, E. M., to be published.

[8] Hammon, W. McD., Gray, J. A., Jr., Evans, F. C., Izumi, E. M., and Lundy, H. W., *Science*, 1941, **94**, 305.

[9] Hammon, W. McD., *Proc. Calif. Mosq. Abatement Soc.*, 1941.

[10] Cox, H. R., Philip, C. B., and Kilpatrick, J. W., *Pub. Health Rep.*, 1941, **56**, 1391.

of the disease they succeeded in isolating the virus from nasal washings of one animal. However, although bleedings were made at frequent and regular intervals, at no time did they isolate virus from the blood of any animal.

It should be pointed out that in these experiments an artificial route of infection was employed, and that the virus used had been thoroughly adapted to brain tissue through repeated serial intracerebral passage in mice. As yet, in no instance has this virus been isolated from the brain of a horse suffering from a naturally acquired encephalitis. Furthermore, large numbers of horses which two of us (W. M. H. and E. M .I., Table I) have tested and also those which Howitt[4] has tested and found to have antibodies have for the most part been horses which have never had recognized encephalitis.

During the fall of 1940 we began a search for a satisfactory group of horses to inoculate. Not till late in the summer of 1941 was a group found available for purchase that did not already possess serological evidence of immunity. In Table I is presented the list of horses tested during the fall of 1940 and summer of 1941 with the results of the tests. This list includes all sera tested, some collected or submitted for various purposes. The sera from Massachusetts furnished the first evidence that neutralization was not a normal characteristic of horse serum. The 4 wild, unbroken colts from an uninhabited mountainous Nevada area were imported for the experiments which are here described.

Method. A St. Louis virus (Culex 103) isolated from *Culex tarsalis*[6] was used in all experiments. It was employed in its first and second mouse brain passage, and suspended in broth containing

TABLE I.
Horse Bloods Tested for Antibody to St. Louis Encephalitis Virus.

Source	Negative	Positive
[1]Berkeley, Calif., Agr. Exp. Sta.	0	1
[2]Davis, Calif., Agri. Exp. Sta.	0	12
[3]Boston, Mass., Biol. Laboratory, State Health Dept.	4	2
[4]Los Angeles, Calif., Stock Yards	1	1
[5]Nevada, Various Ranches	0	3
[5]Reno, Nevada, Agri. Exp. Sta.	3	3
Texas, Southeastern Part	4	4
Pinal County, Arizona	1	0
Yakima, Wash.	3	23
[5]Nevada, Mountainous Area—Wild Colts	4	0
Total	20	49

Note: We wish to acknowledge the kind coöperation of the following persons in collecting and shipping certain of these sera: [1]Dr. C. M. Haring, [2]Dr. Wilson B. Bell, [3]Dr. E. S. Robinson, [4]Dr. L. M. Hurt, and [5]Dr. L. R. Vawter.

5% sheep serum. In the horses, intranasal and intracerebral injections were made under general chloral hydrate anesthesia, given intravenously. Temperatures were taken twice daily. Bleedings were made from the jugular vein, and the blood defibrinated and stored at 5°C until inoculated. Nasal washings were obtained by carefully and thoroughly swabbing each nostril with a cotton ball soaked in saline. The saline washings were centrifuged at 16,000 to 18,000 rpm for 10 minutes before inoculation. All inoculations were made intracerebrally in groups of 5 Swiss mice. In the case of those showing encephalitic symptoms of dying, the brains were removed and if sterile by bacteriological tests, were ground up and passed to a second group of mice. If these all succumbed with typical signs and with the customary incubation period, the identification was considered sufficiently well established. Neutralization tests on sera from recovered horses were made by mouse inoculation according to the method described by Hammon and Izumi.[11]

Experiment 1. A 1-year-old filly was given 3.0 cc of a 10% virus suspension intranasally. Another 1-year-old filly was inoculated subcutaneously (very superficially) with 3.0 cc of a 10^{-4} dilution of the same virus. This represented about 100 to 1,000 fatal intracerebral mouse doses—planned to approximate a possible single mosquito inoculation. Two uninoculated control animals were kept in a separate corral. All were bled for serum neutralization tests just before inoculation and again 47 days later. Temperature taking, bleeding and nasal washing were begun 3 days after inoculation and continued for 21 days. In the inoculated animals, no signs of illness were noted, no elevation of temperature occurred, and no virus was isolated from blood or nasal washings. The preinoculation sera were found free from detectable antibody. The inoculated horses developed a high titer of antibody, but the controls did not.

Experiment 2. Fifty-seven days after the inoculation of the 2 fillies, these and one of the previous controls—a 2-year-old stallion—were each inoculated intracerebrally with 1.0 cc of a 5% suspension of virus. The other previous control—also a 2-year-old stallion—was inoculated subcutaneously (very superficially) with 5.0 cc of a 1% virus suspension. Bleeding was begun 12 hours after inoculation, and each was bled twice daily thereafter for 15 days. Nasal washings were first taken 24 hours after inoculation and made once daily thereafter. No elevations of temperature occurred in any animal except a slight rise during the 2 postoperative days in those

[11] Hammon, W. McD., and Izumi, E. M., *J. Immunol.*, 1942, **43**, 149.

inoculated intracerebrally, and no signs of illness were noted, although these observations were continued for 22 days. No virus was isolated from the nasal washings. However, from the stallion receiving the subcutaneous inoculation, the virus of St. Louis encephalitis was isolated from the blood 26, 36, and 48 hours after inoculation, and from the stallion inoculated intracerebrally virus was isolated 26 hours after inoculation. The blood of both of these horses, drawn just prior to inoculation, failed to neutralize but 24 days later both had developed a high titer of antibody.

Discussion. In the first experiment we obviously erred in waiting till the third day to begin bleeding. In the second experiment it was demonstrated that virus circulates in the blood between 24 and 48 hours after inoculation. It is highly improbable that this represents only a survival of the inoculated virus, for *in vitro,* between the factors of enormous dilution and storage at above 37°C, no detectable amount of virus could be expected to survive. It appears more probable that the virus underwent multiplication in some tissue. The prompt development of antibody from inoculation of a single dose of virus in all inoculated animals is further evidence of infection.

In every instance, whether by subcutaneous, intranasal or intracerebral inoculation, no clinical disease was noted. This, with the finding of circulating virus, is in marked contrast to the findings of Cox, Philip and Kilpatrick, and probably was due to the difference in the tissue adaptation of the two viruses. Our virus, from all evidence at hand, produces in nature through the mosquito vector, just such an inapparent infection in many different animals, including the horse, as we have demonstrated in the horse. This infection is associated with circulating virus to permit infection of other mosquitoes. In this cycle there is no evidence that the virus multiplies in or invades neural tissue. The virus employed by Cox and his associates was definitely adapted to growth in neural tissue, and when inoculated directly into this tissue in horses resulted in encephalomyelitis.

We might reasonably conclude then, from all the data at hand, that the St. Louis virus is as much an "equine" virus as those called Eastern and Western strains, but manifests neurotropic tendencies in the horse much less frequently than these others.

Finding virus in the blood greatly strengthens the case for mosquito transmission of the St. Louis virus.

Summary. Horses from many of the Western States and from Massachusetts have antibodies, in varying proportion, to the St. Louis encephalitis virus. Inoculation in non-immune horses by the

subcutaneous, intranasal or intracerebral routes, of a St. Louis virus freshly isolated from mosquitoes, failed to produce clinical disease. It did produce inapparent infection resulting in the presence of virus in the blood, in 2 instances in which suitable tests were made, and in all 4 inoculated animals induced a high titer of serum antibody. We conclude that in horses, inapparent infection occurs frequently in many widely scattered areas of the United States.

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13557 P

Effect of Estradiol-Benzoate on Serum Lipid of Rats Consuming a High Fat Diet.

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The ability of estrin to induce a lipemia in the male fowl was demonstrated by Lorenz, Chaikoff, and Entenman.[1] This was confirmed by Zondek and Marx,[2] who not only showed a marked lipemia but also the accumulation of fat in the internal organs, particularly the liver. Very large doses were employed of the order of 40,000 I.U. (4 mg estradiol-benzoate) given on 2 successive days. Their results with mammals were negative. They could not induce lipemia in rabbits, rats, and man with large doses of estrin. The work reported below is part of a larger experiment and is presented, primarily, to show that an increase of serum lipid in rats is possible with estradiol-benzoate.

Methods. Rats were taken at weaning time and placed on low fat diets for 4 weeks. During the subsequent 8 weeks which comprised the experimental period the special diets and estrogenic hormone were administered. One group received the fat-free, high carbohydrate diet 550-B;[3] a second group received the balanced ration containing 20% lard, diet 560-B;[3] and the third group was

* Rockefeller Foundation Fellow.

1 Lorenz, F. W., Chaikoff, I. L., and Entenman, C., *J. Biol. Chem.*, 1938, **126**, 763.

2 Zondek, B., and Marx, L., *Arch. Int. Pharmacodyn.*, 1939, **61**, 77.

3 Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.