STUDIES ON ENCEPHALITIS

by

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REVIEW OF THE LITERATURE

History

For many years, equine encephalomyelitis has been a disease of great economic importance among horses and mules. One of the earliest reports of this disease was made by Large in 1867. In 1897 Williams (104) described cases of cerebrospinal meningitis in horses. This epizootic disease had occurred in Idaho and from the symptomstology described, it is now thought to have been equine encephalomyelitis. In 1912, a severe outbreak of "horse plague" occurred in Kansas and Nebraska (98) which is now thought to have been a virus encephalomyelitis. This epizootic was studied at that time by Mohler (69), Udall (101), Stangle (88), and others. More recently in 1938, some 79,606 cases were reported by Shanhan, Giltner, and Schoening (86). A report made by Mohler (70) of the incidence of the disease in 1940 shows that there were 16,941 cases of the disease in United States which resulted in 4,187 deaths, which is about a 25 per cent mortality. The disease was present in 38 states with more than 96 per cent of the cases west of the Mississippi River. The indicence of the disease was 2.58 cases per 1000 in affected areas. One of the most important discoveries in this field was the demonstration of the filterability of the virus from the brain tissue

of affected animals by Meyer, Haring, and Howitt (65) in 1930. The source of this material was obtained from an outbreak of the disease in the San Joaquin Valley of California. In 1933, Howitt (33) and Howitt and Meyer (43) differentiated this virus from that of poliomyelitis and Borns disease by means of the serum neutralization.

Later, encephalomyelitis in a more highly fatal form, became quite prevalent along the eastern seaboard of United States. Studies of this outbreak by TenBroeck and Merrill (97), and by Shanhan and Giltner (85) proved that the disease, in this region, was caused by a virus which was immunologically different from that isolated by Howitt. The geographical distribution of these viruses at this time was limited by the Appalachian Mountain range. In more recent reports, however, there has been some spread of both viruses. The western virus has been reported in 15 states, namely: Alabama, California, Colorado, Idaho, Illinois, Iowa, Kansas, Kentucky, Minnesota, Montana, Nevada, North Dakota, South Dakota, Texas, and Utah. The eastern virus has been reported in 11 states: Alabama, Delaware, Florida, Georgia, Maryland, Massachusetts, New Jersey, North Carolina, South Carolina, Texas, and Virginia. The eastern virus seems to be closely associated with the eastern tidewater regions as the virus has not been found any great distance from this region. The western virus, however, has spread from California to Kentucky and Alabama, and is now more prevalent in the Mississippi

watershed. At this time the viruses are still geographically separated except in Alabama and Texas. But there are indications that the eastern virus may be found farther west in the near future.

These two viruses are the only causative agents of the disease so far encountered in this country. The virus isolated in the Argentine has been shown to be the same serologically as the western strain by Howitt (30). Beck and Wyckoff (3) in 1938 found the Venezuellan virus to be different than either the eastern or western viruses. The Moscow 2 or Russian virus is likewise serologically different from the American types as shown by Howitt (41). This leaves five distinct viruses of equine encephalomyelitis, namely: Borna, Moscow 2, Venezuellan, American eastern and western. Cox, Philip and Kilpatrick (11) in 1941 reported that horses were susceptible to a human type of encephalitis virus, the St. Louis virus. This virus caused a severe outbreak of human encephalitis in St. Louis, Missouri (75). It occurred first in Paris, Illinois, in 1932 but assumed a much larger proportion in St. Louis in 1933. A total of 1,097 human cases was reported. The St. Louis type of encephalitis in horses is believed to be subclinical in field cases as the natural host of this virus is man. The disease, however, does present a problem in that the horse may become a carrier of the virus without showing any apparent symptoms. The Yakima Valley (58) outbreak in the state of Washington showed that this epidemic was due to both western equine and St. Louis viruses. Serum

from 50 human patients tested by neutralization showed 86 per cent positive for western virus, 72 per cent positive for St. Louis virus and 56 per cent positive to both. This clearly shows that some patients may have had both infections. However, there must be further work done on this phase of the problem in order to establish the correct role of the horse with the St. Louis virus.

Properties of the Virus

The filterable virus of equine encephalomyelitis is easily destroyed in an acid reaction. It is easily destroyed by a physiological salt solution with a pH of 6.0 or lower. The increase of lactic acid in brain tissue after death destroys the virus in a short time. For this reason isolation of the birus must be attempted before the tissues become too acid. In most cases, the animal has been destroyed at too late a stage of the disease for virus isolation. However, once recovered the virus remains potent for long periods of time when stored at low temperatures at a pH of 7.4 to 7.5 (21).

Pathogenicity

The virus of equine encephalomyelitis is rather unique with respect to its pathogenicity. The virus is capable of

producing the disease in a large number of animals and birds. However, most of the susceptibilities have been demonstrated by intracranial inoculation. Meyer (63), in 1933, showed that guinea pigs, monkeys, rabbits, rats and mice were readily susceptible but dogs and cats were resistant. A great number of birds have been proven susceptible including almost all domestic varieties and many wild birds.

Means of Transmission

The epidemiology of the disease seems to point most clearly to an insect carrier. A great deal of work has been done to check the ability of various insects to transmit the virus under laboratory conditions. Eleven varieties of mosquitoes were proven capable of transmitting the disease by Kelser (45) and by Merrill, Lancaillade and TenBroeck (59). These species are Aedes aegypti (Linn.), A. sollicitans (Walker), A. taeniorhynchus (Wiedemann), A. vexans (Meig), A. albopictus (Skuse), A. nigromaculis (Ludlow), A. dorsalis (Meigen), A. cantator (Coq), A. atropalpus Coquillett, A. triseriatus (Say) and Culex Tarsalis Coquillett. Many of these varieties were of the salt marsh type. Later, Merrill and TenBroeck (60) showed that the virus multiplied in the body of the mosquito, Aedes aegypti. Syverton and Berry (92) found that the tick Dermacentor andersoni was capable of transmitting the virus. Several isolations of the virus have

been made from insects from the field. This was done by Kitselman and Grundmann (51) who isolated western virus from the assassin bug (Triatoma sanguisuga Le Conte). The first isolation was the Triatoma 1 virus which was used in the experiments described in this paper. Another virus, the Triatoma 4 was isolated by Kitselman and Grundmann from another collection of the assassin bug. The typing of this virus will be described in another phase of this paper. Several other isolations of the virus were made by Kitselman and Grundmann but these viruses were lost before they were typed. The Triatoma sanguisuga was found to be able to transmit the virus of the western strain of equine encephalomyelitis from guinea pig to guinea pig by Grundmann, Kitselman, Roderick, and Smith (28).

Human Infection

As early as 1932, Meyer (62) suggested the occurrence of the disease in man, but it was not until 1938 that the first human proven case due to eastern virus was reported in southeastern Massachusetts by Fothergill, Dingle, Farber and Connerley (23). After this first case was established, more human cases were found which paralleled the epidemic of horses in that region. Although the incidence of human infection paralleled that of horses, in most cases the human patients had no history of contact with horses. The final

report of the outbreak by Getting (25) showed that nine cases were diagnosed by isolation of the virus and ten by the demonstration of neutralizing substances in the convalescent sera of human patients. Eklund and Blumstein (14) first reported the presence of antiviral bodies in the serum of patients recovering from this disease. Since that time the serum neutralization test has been used as a means of checking the type of virus to which the patient has been exposed. About the same time, the western virus was isolated from a human case by Howitt (39) in 1938. Later Howitt (42) showed that sera, from human cases in the San Joaquin Valley outbreak in 1937-1938, contained neutralizing antibodies for both western and St. Louis virus. Two isolations of western equine virus were made from the brain and blood respectively of two human cases. Leake (54) reported an epidemic of the disease in horses and man occurred simultaneously in North Dakota, Minnesota, South Dakota, and Manitoba in 1940-1941. There were 1,080 human cases reported with 96 deaths in North Dakota, 815 cases in Minnesota, and 180 in South Dakota. In the Canadian region of Manitoba, 434 cases occurred in the human. Sera from convalescent patients tested by neutralization showed the presence of antibodies for the western virus. From these reports, it is evident that these cases were due to western equine encephalomyelitis virus, St. Louis virus or both. Thus it is evident that humans may be infected with eastern, western, and St. Louis viruses with the production of

the specific neutralizing substances in the blood serum.

EXPERIMENTAL WORK

Serum Neutralization Tests of Sera From Human Encephalitis Patients in Kansas

Since the demonstration of human cases of equine encephalomyelitis, a large quantity of work has been done regarding the incidence of human infection. The first case in the human, which occurred in Massachusetts, was reported in 1938 by Fothergill, Dingle, Farber, and Connerley (23). This outbreak, as summarized by Getting (25), included some 34 cases of encephalomyelitis due to the virus of eastern equine encephalomyelitis. Of the 34 cases reported, nine were diagnosed by isolation of the virus from brain material and ten cases by the demonstration of antibodies in the blood sera. The latter method of diagnosis was used by Howitt (42) in the study of in vitro neutralization tests of western equine encephalomyelitis in California. Howitt (36), in her report, set forth certain arbitrary standards for this test which were followed in this report.

Due to the incidence in Kansas of encephalitis in the human, it was suggested that this test should be applied to the sera of some suspected cases. Previous to this report, no serological differentiation of encephalitis in the human had been attempted in Kansas. The collection of these

samples was largely due to the efforts of Dr. J. A. Wheeler of Newton and the Kansas State Board of Health.

The viruses used in this work were two strains of western equine encephalomyelitis isolated from the assassin bug, (Triatoma sanguisuga) by Kitselman and Grundmann (51). These two strains are designated as Tr. 1 and Tr. 4. The potency of these viruses was maintained by frequent passage through guinea pigs at least once a month as suggested by Kolmer and Rule (52). The brain tissue was placed in small vials containing a 50 per cent glycerol buffer solution and was stored in the refrigerator until the next passage. The buffer solution used was prepared as follows:

Sodium acid phosphate	3.8 g
Potassium phosphate	0.9 g
Sodium chloride	3.8 g
Water	1000.0 cc

After the salts were completely dissolved in water the solution was adjusted to a pH of 7.6. This mixture was then sterilized at 15 pounds of pressure for one hour. The final titre was then adjusted to pH 7.4. For the preservation of the brain tissue an equal quantity of glycerol was added to this mixture. The buffer solution without the addition of blycerol was used as a diluent in making the brain tissue virus emulsion for inoculation.

The inoculum for a regular guinea pig passage was made by placing a small amount of brain material in a sterile mortar and grinding to a fine paste. This material was slowly mixed and diluted with buffer solution until the final

quantity equaled ten cubic centimeters. This suspension was then centrifugalized for five to seven minutes in order to remove the larger particles. The supernatant fluid was then used as the inoculum. When the work was first started, the supernatant fluid was filtered through a Jenkins filter and the filtrate used as the inoculating material but later this step was omitted as it appeared to be unnecessary to filter the material after careful handling and storage in 50 per cent glycerol solution.

The guinea pigs used were approximately 250-300 g in weight. Due to the delicacy of the intracranial injection the guinea pigs were inoculated under ether anesthesia. The syringe used was a 1 cc tuberculin syringe with a 20 gauge needle cut down to one-half inch in length. In all intracranial inoculations 0.2 cc of inoculum was introduced into the cranial cavity.

After inoculation, daily rectal temperatures were recorded until the termination of the experiment. The brain tissue was removed aseptically from animals immediately after death or from animals which had just been destroyed in the last stage of the disease. The tissues saved for the virus were placed in the 50 per cent glycerol buffer solution and held under refrigeration. The course of the disease in these cases of western virus infection was usually from four to five days. A typical temperature chart of a regular virus passage is shown below in Table 1.

Table 1. Regular passage of triatomal 1 virus (laboratory number 1359).

Guinea pigs	Days 1	2	3	4
Brown	104.1	105.6	105.3*	dead
Brown ear	105.2	106.1	dead	
Black and white	104.2	105.3	106.5	dead

* paralysis

In many cases only one-half of the brain tissue was harvested for the virus and the other half was used for a microscopic study of the diseased areas. The procedure and handling of the tissues for this work and the results found will be discussed in another phase of this report.

This technique followed in the serum neutralization test was essentially the same as described by Howitt. Thirty-six human sera were tested. These sera were received at the laboratory either as clear serum or as whole blood samples. The whole blood samples were centrifugalized as soon as received and the clear sera decanted. These samples of sera were stored at below freezing temperatures until used.

It was first necessary to inject a series of guinea pigs with varying quantities of the virus in order to find the minimal infective dose. The brain virus suspension was prepared as described previously in the discussion of a

regular virus passage. This initial solution was made to approximately a 1-100 dilution. From this suspension of the virus, various ascending dilutions were made up to 1-1,000,000. A series of three dilutions were usually employed for inoculation into guinea pigs, these were 1-1,000,000; 1-100,000, and 1-10,000. Most titrations showed that a 1-1,000,000 dilution was either just above or just below the minimal infective dose. From these data, the final strength of the serum virus mixture to be used in the serum virus neutralization test was established. Typical titration temperatures are shown in Table 2.

Table 2. Typical titration temperatures of triatoma virus (laboratory number 1694).

Guinea pigs:	Dilutions	Days 1	2	3	4	5
Brown and white	1-1,000,000	103.9	105.0	104.9	99.8	dead
Brown	1-100,000	103.9	106.1	105.0	dead	
Tan	1-10,000	105.1	105.6	101.9*	dead	

* paralysis

It is shown in Table 2 that a dilution of 1-1,000,000 contains more than the minimum infective dose. Therefore, the final dilutions of virus used in the serum neutralization test were 1-1,000,000 and 1-100,000.

In mixing the serum virus dilutions, two strengths of

virus were used; one with a virus dilution just slightly stronger than the minimal infective dose and another dilution at least ten times this strength. For example, serum 1712 was tested for antiviral properties in the following manner: The final virus dilution to be used was established before as indicated in Table 2. The 1-100 dilution of virus was diluted with buffer solution to 1-25,000 and 1-500,000. Then equal quantities (usually 0.5 cc of each) of the serum to be tested and each respective virus dilution were mixed in a small serile vial. Thus the two final dilutions of virus would be 1-1,000,000 and 1-50,000. These mixtures were placed in the refrigerator at approximately 37° F. for 24 hours. The inoculations were made into guinea pigs in duplicate. The guinea pigs were injected intracranially under ether anesthesia with 0.2 cc of the mixture. Daily rectal temperatures were recorded until the termination of the experiment.

A typical positive reaction will be found in Table 3 while Table 4 shows the reaction of a serum which gave no protection against the virus. Table 3 gives the daily rectal temperatures of guinea pigs receiving the serum of laboratory number 1718. This serum must have contained antibodies of western equine encephalomyelitis virus as the guinea pigs failed to show any symptoms of the disease. These antibodies have, during the incubation of the mixture for 24 hours, combined with the virus and destroyed its pathogenicity. Thus the guinea pigs remained healthy and did not show

a typical temperature rise as seen in Table 4. The presence of protective substances in the blood of patient number 1718 indicates that they had developed as the result of an attack of western equine encephalomyelitis.

Table 3. Serum neutralization of human sera number 1718[1].

Guinea pigs:	Dilution	Days: 1	2	3	4	5
Black	1-50,000	102.2	102.6	101.1	102.7	102.1
White	1-50,000	103.5	104.0	103.9	104.6	104.3
Grey	1-1,000,000	103.8	104.0	102.8	103.7	102.6
Brown	1-1,000,000	103.8	102.0	101.4	103.0	102.8

[1] Titration of virus laboratory number 1720, Tr. 4.

The serum neutralization test of laboratory number 1712, however, shows a marked rise in temperature in most cases. All guinea pigs were dead within five days after showing typical symptoms of salivation and progressive paralysis. The marked contrast of the results in these two seras definitely show the difference between a serum containing antiviral substances and one which does not. The absence of protective substances in the blood of patient number 1712 indicates that no infection with western equine virus has occurred.

Table 4. Serum neutralization of human sera number 1712[1].

Guinea pigs:	Dilution	Days: 1	2	3	4	5
White	1-50,000	104.1	105.2	105.6	dead	
Black	1-50,000	102.9	105.1	101.1	dead	
Grey	1-1,000,000	102.4	104.6	99.9*	99.0*	dead
Brown and black	1-1,000,000	103.6	104.4	101.0*	dead	

[1] Titration of virus laboratory number 1720, Tr. 4.
* Paralysis.

A total of 36 human samples were tested by this method. Fifteen of these or 41 per cent gave protection against western equine encephalomyelitis virus when injected into guinea pigs. Table 5 shows a summary of these tests. These results seemed to show that there has occurred in Kansas an infection of humans with the virus of western equine encephalomyelitis.

These serum neutralization tests were made only with western virus because the eastern virus has not been recovered in Kansas.

The serum neutralization test is used as a method of diagnosing encephalitis in humans. Howitt (35) has shown that antiviral bodies are present in the blood sera about the seventh to tenth day following infection with the virus. Since the viruses of encephalitis are serologically dif-

Table 5. Summary of sera tested by neutralization test.

Laboratory number	Date	Virus Tr. no.	Titration	Results: Strong	Results: Weak	Checked
1641	9/ 3-41	1	1651	x*	x**	1673
1642	9/ 3-41	1	1651	x	x	
1643	9/ 3-41	1	1651	0	x	
1646	9/13-41	4	1673	0	0	
1650	9/13-41	4	1673	0	0	
1656	9/13-41	4	1673	0	0	
1667	9/31-41	4	1673	0	0	1694
1669	9/31-41	4	1673	x	x	1694
1675	9/31-41	4	1673	0	0	
1677	10/18-41	1	1694	0	0	
1691	10/18-41	1	1694	0	x	
1706	11/ 7-41	4	1720	0	0	
1712	11/ 7-41	4	1720	0	0	
1718	11/ 7-41	4	1720	x	x	
1724	11/ 7-41	4	1720	0	0	
1725	11/ 7-41	4	1720	0	0	
1726	11/12-41	4	1720	0	0	
1728	11/12-41	4	1720	0	0	
1729	11/12-41	4	1720	0	0	
1732	11/12-41	4	1720	0	0	
1735	11/12-41	4	1720	0	0	
1748	11/28-41	1	1758	0	0	
1749	11/28-41	1	1758	0	0	
1753	12/23-41	1	1792	x	x	2023
1759	12/23-41	1	1792	0	0	
1763	12/23-41	1	1792	0	0	
1770	12/31-41	1	1792	0	0	
1778	1/22-42	1	1792	x	x	
1780	1/22-42	1	1792	x	x	2023
1829	12/12-41	1	1855	0	x	
1839	12/17-41	1	1855	0	x	
1853	2/ 5-42	1	1855	x	x	
1986	7/28-42	4	2023	x	x	
2083	10/ 6-42	4	2090	x	x	
2092	10/ 6-42	4	2090	0	0	
2101	10/ 8-42	4	2090	x	x	

Totals 15 positive, 41 per cent
21 negative, 59 per cent

* protected
** no protection

ferent, the serum virus neutralization test can be used to show what virus, if any, is responsible for the disease. Eklund and Blumstein (14) were the first to report the use of this test for diagnosis.

At various intervals sera from horses recovering from an attack of the disease were tested at the same time with the same virus as used with the human sera in order to check results. Temperature (Tables 6, 7, and 8) show typical positive and negative human sera reactions and positive equine reactions. In this test, three dilutions of virus and serum were used and one guinea pig injected with each instead of in duplicate.

Table 6. Serum neutralization of human serum number 2101[1].

Guinea pigs:	Dilution	Days 1	2	3	4
Brown and white	1-20,000	102.9	103.2	104.1	dead
Three color	1-100,000	103.0	101.0	100.9	100.9
Black and white	1-500,000	102.3	101.5	101.3	100.7

[1] Titration of virus laboratory number 2090, Tr. 4.

Table 7. Serum neutralization of human serum number 2092[1].

Guinea pigs	Dilution	Days 1	2	3	4	5	6
White	1-20,000	104.0	105.9	100.9	dead		
Black and White	1-100,000	103.2	105.1	104.6	103.0*	98.2	dead
Brown and white	1-500,000	103.1	106.0	105.4	103.9	96.1	dead

[1] Titration of virus laboratory number 2090, Tr. 4.
* Paralysis.

Table 8. Serum neutralization of horse serum number 2091[1].

Guinea pigs	Dilution	Days 1	2	3	4	5	6	7
Two color	1-20,000	102.5	105.4	105.3	100.1*	99.1	dead	
Grey	1-100,000	101.9	103.4	101.8	101.5	102.0	101.0	
White	1-500,000	102.4	103.5	101.4	102.0	101.5	101.1	101.3

[1] Titration of virus laboratory number 2090, Tr. 4.
* Paralysis.

Table 6 shows the reaction of the serum laboratory number 2101. This serum from a human patient has protected in a dilution of 1-100,000 of the virus. This protection constitutes a positive reaction showing that the patient

had contacted western equine encephalomyelitis virus. However, the serum laboratory number 2092 has failed to protect against any virus dilution. Therefore, the patient probably had not contacted the western virus and consequently no antiviral substances were present. Table 8 shows the results of a serum from a clinical case of equine encephalomyelitis which was treated at the Veterinary Clinic at Kansas State College. This serum shows the presence of antiviral substances by its protective action in the guinea pigs and that constitutes a positive reaction. The amount of virus in a dilution of 1-20,000 used in this series of tests was many times the minimal infective dose and neither the horse serum nor the human serum was able to protect the guinea pigs against that strength of virus.

Serum Neutralization Tests of Pack Rat Sera

Western equine encephalomyelitis virus has been isolated from the assassin bug by Kitselman and Grundmann (51) and later by Grundmann, Kitselman, Roderick, and Smith (28). These bugs are often found in rodent burrows and frequently attack animals. Experiments were arranged, therefore, to throw some light on the role which these wild rats may play in serving as the reservoir of the virus.

The wood rat and the cotton rat were shown to be susceptible to intracranial inoculation of eastern and western equine encephalomyelitis virus by Grundmann, Kitselman, Roderick, and Smith (28). An attempt was made to immunize the wood rat against the western virus. Five wood rats were given 0.2 cc of western chick embryo vaccine (Fort Dodge) intramuscularly. This initial dose was followed in seven days with a second dose of the same amount. The immunity was challenged seven days after the last injection by 0.2 cc intracranial inoculation of a 1-500 dilution of Triatoma 4 virus. None of the wood rats showed any reaction to this virus. A week later a 0.2 cc of a 1-500 dilution of Triatoma 1 virus was injected intracranially. All rats were solidly immune for no reaction occurred. These viruses were checked for potency by intracranial injection into guinea pigs in the same dilutions. All guinea pigs so injected died in four to five days showing typical temperatures and symptoms. These five wood rats were destroyed a week later by intracranial injections of eastern virus. It has been shown, therefore, that the wood rat is not only susceptible to both eastern and western equine encephalomyelitis virus but the animal likewise responds to an immunizing procedure similar to the guinea pig.

After establishing these facts, it was decided that tests should be made to determine if the wood rats may have

been exposed to western equine encephalomyelitis virus in the field. The criterion of this test to be the finding of antiviral substances in the blood serum of wood rats. Seven wood rats were obtained from the surrounding countryside. Blood samples were obtained from each rat and the clear serum saved for serum virus neutralization tests. The tests were conducted in the same manner as described for the human serum virus tests. None of these sera showed any evidence of containing antibodies. The tests were checked by the addition of a horse serum to this series of tests. The horse serum protected the guinea pigs against the virus. A typical temperature reaction of pack rat serum is shown in Table 9 and that of the horse serum in Table 10. The titration of the virus is shown in Table 11.

Table 9. Serum neutralization of wood rat serum number 2046[1].

Guinea pigs:	Dilution	Days: 1	2	3	4
White	1-100,000	103.1	104.9	106.0	dead
Tri-color	1-100,000	102.0	104.3	105.4	dead
Brown	1-400,000	104.3	dead		

[1] Titration of virus laboratory number 2057, Tr. 4.

Table 10. Serum neutralization of horse serum number 2062[1].

Guinea pigs	Dilution	Days 1	2	3	4	5
White	1-100,000	101.8	102.0	102.2	102.1	102.7
Tri-color	1-100,000	104.0	103.5	103.2	102.8	102.9
Tan	1-400,000	102.9	104.0	103.9	103.4	103.0

[1] Titration of virus laboratory number 2057, Tr. 4.

Table 11. Titration of Tr. 4 virus.

Guinea pigs	Dilution	Days 1	2	3	4	5
White	1-10,000	106.1	106.5	94.6*	dead	
Tri-color	1-1,000,000	104.5	106.7	105.3	97.1*	dead
Tan	1-100,000	105.5	105.5	100.4	dead	

* Paralysis.

The titration of the virus as seen in Table 11 shows that a dosage of virus in a dilution of 1-1,000,000 is greater than the minimal infective dose. Therefore, the final virus dilutions used in the neutralization test could have been as high as 1-1,000,000 but it was decided to use stronger dilutions. Dilutions of 1-100,000 and 1-400,000 were used for these sera. Serum from the wood rat laboratory number 2046 showed no evidence of containing protective

bodies as all guinea pigs showed a typical rise in temperature and died within four days. The horse serum, which was taken from a clinical case of the disease, did protect all guinea pigs. This horse serum served as a check on the test.

Since none of the wood rat serums gave any evidence of containing antibodies it can be assumed that these wood rats had not been exposed to the virus in the field.

Microscopic Study of Brain Sections From Animals Affected With Western Equine Encephalomyelitis

As previously stated in this report, many of the brains harvested for the western encephalomyelitis virus were also sectioned for microscopic study. Most of these brain tissue sections showed the typical pathology of equine encephalomyelitis as described by Neal (72) and by Hurst (44).

Preparation for Sectioning

The brain tissue was prepared for sectioning by fixing in Zenker's solution. After 24 hours of fixation the material was washed in running tap water for 24 hours. Following this step, the brain material was dehydrated by passing it through a series of alcohol solutions and then cleared in oil of cedar for ten to 12 hours. The brain material was then imbedded in paraffin andlater cut at five to seven microns.

Staining

These sections were stained with the slum hematoxylin alcoholic eosin method. The directions used for staining by this method are as follows:

1. Xylol - 5 minutes
2. Alcohol - 100 per cent 3 to 5 minutes
3. Iodized alcohol - 5 to 10 minutes
4. Alcohol - 95 per cent 3 minutes
5. Sodium thiosulfate - 5 per cent aqueous until yellow color disappeared
6. Tap water rinse
7. Tap water rinse 3 to 5 minutes
8. Harris or Delafield's hematoxylin - 4 minutes
9. Tap water rinse
10. Acid alcohol - until properly differentiated
11. Tap water rinse
12. Alkaline tap water rinse until sections are blue
13. Tap water rinse
14. Tap water rinse 2 to 5 minutes
15. Saturated solution of alcohol 95 per cent eosin 2 minutes
16. Alcohol - 95 per cent a few seconds
17. Alcohol - 100 per cent a few seconds
18. Neutral xylol - 2 to 5 minutes
19. Neutral xylol - until clear
20. Mount in Canada balsam

Pathology

The majority of the sections studied were sagittal sections of the cerebrum and cerebellum. The most significant pathological changes in these sections were perivascular infiltration of the sheaths of the blood vessels and scattered areas of leukocytic infiltration. The infiltrating cells were lymphocytes, polymorphonuclear leukocytes and monocytes. The perivascular cuffs extended for some distance

along the blood vessels. Most sections showed considerable edema.

Many of the larger nerve cells showed intranuclear inclusion bodies much like those described in Borna disease. These bodies were rounded or oval in shape. The nerve cells were found in various stages of degeneration. Frequently phagocytic cells were found within or bordering partially degenerate nerve cells.

In the guinea pig, then, the western equine encephalomyelitis virus produces an acute encephalomyelitis. This is characterized by perivascular cuffing and acute degeneration of the nerve cells.

SUMMARY

1. The purpose of this experimental work was to determine the incidence of human infection with western equine encephalomyelitis virus in Kansas.

2. Thirty-six sera from clinical human encephalitis cases were tested by serum neutralization and 41 per cent of these sera showed the presence of neutralizing substances against this virus.

3. It appears that an infection with the western strain of equine encephalomyelitis was involved in these cases.

4. The remainder of these cases were therefore due to some other cause possibly St. Louis or poliomyelitis virus.

5. Seven wood rats tested by serum neutralization showed that they had not been exposed in the field and therefore they were not carriers of the virus.

6. Brain sections from guinea pigs inoculated with triatoma (bug) viruses showed, on microscopic study, typical pathological changes.

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