

Equine Encephalomyelitis

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Equine encephalomyelitis has been recognized as a virus disease of horses since 1931. In that year, Meyer, Haring, and Howitt (1), investigating an outbreak of cerebro-spinal disease among California horses, established the affliction as an infectious disease of virus origin. Previous to this time, many outbreaks, characterized by the same symptoms but of unknown etiology, had been reported. These early cases were referred to by a number of different names, some of the most common terms being Borna disease, forage poisoning, botulism, staggers, and cerebro-spinal meningitis. In 1912, 35,000 horses died as a result of an epizootic of a disease called "staggers," and, in 1919, Colorado alone lost almost 1,800 head to forage poisoning (2). It is now generally suspected that both of these epizootics were caused by the virus of encephalomyelitis. Forage poisoning, a disease caused by the consumption of moldy corn, does affect horses, but, unfortunately, the name has not been restricted to cases where toxic food is definitely involved. It is also true that botulism may affect horses, but these cases are rare and need not be confused with encephalomyelitis. Other bacterial and toxic infections of the brain and spinal cord would naturally lead to paralytic symptoms, somewhat resembling those of encephalomyelitis, but should not confuse one familiar with the latter disease. Virus diseases closely related to the American encephalomyelitis have been reported in Germany, France, and Russia, but all of these diseases are immunologically distinct.

In 1933 a disease very similar to that noted in California was reported as affecting horses in Virginia, Maryland, and Delaware (3). Except for a slight difference in incubation period and virulence, the outbreak was indistinguishable from that in the west. Records and Vawter (4, 5) found that in guinea pigs no cross immunity between the two could be demonstrated. Guinea pigs, injected with intranasally collected virus from horses suffering from the eastern disease, upon contracting the disease and surviving, were immune to a second injection of the same virus but succumbed to the western virus. In further experiments by these authors (4, 5), six out of eight horses immune to the western type also resisted the eastern type. These authors, therefore, decided that the chief difference in the virus of the two epizootics was one of virulence. However, Shahan and Giltner (3) and TenBroeck and Merrill (6), in 1933, published evidence that the eastern and western viruses were immunologically distinct. It has since been shown repeatedly that animals immune to the western virus are susceptible to infection by the eastern virus and that the reverse is also true. The disease is now known as eastern or western type encephalomyelitis in recognition of the two immunological types.

Typing tests which have been made by the Bureau of Animal Industries (7) and by investigators in private laboratories have shown

that the Appalachian Mountains form the dividing line between the eastern and western types of the disease. Until the summer of 1939, no case of infection with eastern type virus had been found west of this range, and no western type had been found east of the line. However, this summer Scofield reported a case of eastern type encephalomyelitis in Ontario, and the Bureau of Animal Industries reported another eastern type infection on an Alabama farm.

The causative virus of encephalomyelitis localizes its damage to the higher nervous centers of the brain and spinal cord. The symptoms of the disease are obviously the results of pathological changes in the brain and cord. The infection leaves practically no gross lesions, and only examination of nervous tissue yields evidence of specific pathological change. Hurst (8) made a study of the histopathology in horses and laboratory animals. The most striking microscopic lesions reported by him are: acute primary degeneration of nerve cells, nuclear inclusions in neurons, polymorphonuclear infiltration, especially in grey matter, and perivascular cuffing with mono- and polymorphonuclear cells. Eastern and western viruses cause the same changes, but the extent of damage is less in the case of western infection. In certain stages, the histopathology of the two types may be indistinguishable. A very intensive study of the histopathology of the nervous system in encephalomyelitis-infected guinea pigs has also been made by King (9). Histological changes and the histogenesis of the disease processes are described in detail.

The course of infection in encephalomyelitis is usually divided into three stages. During the first stage there is a rise in temperature, but other effects are so slight that this phase usually escapes detection in all but experimental cases. The second stage brings on the objective symptoms that result in the recognition of field cases. Infected animals show clearly the signs of nervous involvement with paralysis and incoordination apparent. The specific manner in which the disease manifests itself varies somewhat with individual horses, but typical symptoms are as follows: Normal skin irritability may give way to hypersensitiveness, or, at the other extreme, to complete lack of sensation. Partial or complete inappetence is often noted and grinding of the teeth, yawning, and twitching of various body muscles are common. In many cases there is a tendency to walk in circles. Later the animal becomes drowsy; this is so typical that the disease is widely known among the laity as "sleeping sickness." As paralysis and incoordination develop, the animal stands with feet spread wide or leans for support against stall partitions or fences. Occasionally, at the end of this second stage the animal manifests great excitement and irritability.

If the disease runs a fatal course, the third stage is that of complete incoordination in which animals are unable to stand but lie in a state of complete paralysis or thrash violently with running movements of the legs. This stage usually terminates quickly in death, but occasionally horses remain comatose for several days.

In small laboratory animals the usual symptoms are those of advancing paralysis, leading to coma and death. Mice and less often guinea pigs may suffer a convulsive spell just before the stage of pros-

tration. Since the intracerebral method of infection is the usual one in experimental cases, symptoms progress more rapidly and regularly than in field cases.

Diagnosis of field cases is made on the basis of the above clinical picture and the seasonal and epizootic character of the disease. Laboratory diagnosis, while an indispensable aid to research, is too slow for application to individual field cases. However, in investigating the possibility of spread into new areas or suspected out-of-season cases or in establishing the identity of the disease early in an epizootic, laboratory diagnosis is extremely useful. The slowest but surest means of diagnosing the disease is the inoculation of laboratory animals with brain tissue specimens from the suspected case, using normal animals and animals immune to both types of the virus. When such a group of animals is inoculated intracerebrally with a suspension of properly handled encephalomyelitis tissue, the normal animals and one group of immune animals will succumb. Such an immunological test leaves little question as to the identity of the disease and its type. A characteristic histopathology furnishes another means of laboratory diagnosis, but specimens for this test must be carefully handled and preserved, and the comparatively complicated and expensive procedure of tissue sectioning is necessary for reliable results. In recovered or suspected subclinical cases, neutralization tests with patients' serum and known virus often result in a reliable diagnosis.

The method by which the virus enters its natural host in the field is not definitely established. Kelsner (10) has presented evidence that mosquitoes may carry the virus, and Merrill and TenBroeck (11) in 1935 published proof that *Aedes aegypti* can harbor the virus for a period of at least two months, provided it is fed a high concentration of the virus. Madsen and Knowlton (12) reported that several other species of the genus *Aedes* may carry the disease. However, there is no transmission of the virus between the male and female mosquitoes; nor are eggs and larvae from infected females or those grown in infected media dangerous as adults. The possibility of transmission by mosquitoes has been made more plausible by the demonstration of the virus in the blood stream of infected animals. The invasion of the blood stream by the virus is confined to a short period during the first temperature rise and just before the onset of objective symptoms. However, workers engaged in research for the United States Bureau of Animal Industries (7) have failed to detect virus in mosquitoes collected from epizootic areas. The obstacles in the way of anyone undertaking such experiments are great, and only positive results would be of great significance. There are still a great many who cling to the idea that an insect vector will some day be incriminated in the spread of encephalomyelitis. Those who believe in other methods of transmission have even less evidence in their support. It is unlikely that direct contact plays an important role for it is quite common for a few animals to contract the disease on the same premises where others in close contact remain healthy. While Records and Vawter (5) found virus in nasal washings of artificially infected horses, virus has not yet been detected in urine,

nasal washings, or even in nasal mucosa of naturally infected animals (13).

The path followed by the virus, once it gains entrance to the host, has not yet been completely worked out. It is easily demonstrated, however, that the virus invades the blood stream soon after its establishment in peripheral tissues. King (14) studied the pathogenesis of the disease and concluded that direct invasion of the brain from the blood stream seems to be the principal method of pathogenesis. Once in the nervous tissue, the virus may spread by different methods. In some cases affected regions bear a striking anatomical relationship; in other cases foci are joined by entirely unaffected anatomical connections. Thus the virus may travel along nerve connections or settle in several distinct areas simultaneously by deposition from the blood stream.

In addition to the problem of transmission during epizootics, we are faced with the question of how these epizootics start. It is one of the characteristics of equine encephalomyelitis that cases never occur after the first killing frost of the fall or before the warm weather of late spring. It is quite unlikely that horses themselves harbor the virus over this winter period. No success has met attempts to detect equine carriers of the virus. Furthermore, many horses are transferred from the eastern to the western zone, and vice versa, without disturbing the geographical distribution of the two types of virus. However, great progress is being made in the detection of other hosts to the virus. The disease has infected humans, monkeys, guinea pigs, mice and other laboratory animals, pheasants and pigeons, to name only a few possible hosts. Should it be proved that the occurrence of encephalomyelitis virus in migratory fowl is not a rare phenomenon, we shall be justified in closely associating birds with the epidemiology of encephalomyelitis. In this connection, susceptibility of human beings must not be overlooked. Outside of laboratory accidents, the source of infection in human cases is as mysterious as that of equine cases. A number of cases reported in Massachusetts in children were of unknown origin since there had never been any contact with horses or any other common source of the virus.

With so little known about the virus and its habits, it is quite natural that the treatment of encephalomyelitis is almost entirely symptomatic. The most important step in treatment, which acts directly against the virus, is the administration of antiserum of which more will be said later. Prerequisite to successful treatment is the provision of a quiet, restful atmosphere. Bright lights, noises, and all factors conducive to excitement are to be avoided. To combat dehydration, water must be administered. If no other way is possible, intravenous injections of water sometimes give good results. Harsh purgatives, because of the loss of water they cause, are contra-indicated, but mild laxatives are sometimes necessary and beneficial. Of course, the animal must be assisted in taking nourishment where paralysis has made chewing and swallowing difficult or impossible. These are the general lines of treatment. Many veterinarians have reported good results with various methods, but all such methods are based on the fundamentals of treating symptoms for immediate relief and injecting

serums to combat the virus. Some veterinarians are skeptical as to the therapeutic value of antiserum. It is true that, unless serum production is very carefully controlled, a product of low protective titre may result, but it is quite possible to produce a serum of high antibody content in animals in which, following vaccination, a state of hyper-immunity has been induced by the repeated injection of living virus. Previous to last year, the hyper-immunizing virus consisted of an emulsion of infected brain tissue. Antiserum produced by this method was of great value in treatment and prophylaxis but was still below the efficiency of antisera of other diseases. With the development of the chick embryo propagated virus, an immunizing agent of far greater potency than was formerly possible has been made available. Chick embryo antigen contains as much as 100,000 times the virus concentration of brain tissue antigens. As a consequence of the greater stimulation of antibody mechanisms in serum-producing horses, anti-encephalomyelitis serum is now, at least in experimental trials, a far better prophylactic and therapeutic agent than it was a short time ago. The sudden dropping off of the incidence of encephalomyelitis this year has made impossible any field comparisons of the old and new serums, and it is consequently impossible to state how the new type serum will act in an actual epizootic. But it can be stated that in place of delicate neutralization tests protection tests are practical for titrating the new serum.

It is in the field of prevention that the greatest advances in the battle against encephalomyelitis have been made. To many the story of the development of a reliable vaccine in an almost incredibly short time is the most interesting phase of any consideration of the disease. Aside from the purely practical angle of saving horses' lives, the history of the vaccine is important because of the methods involved and because of new light shed on virus immunological problems during the investigations.

In 1934 Records and Vawter (15) published work on equine encephalomyelitis immunization through the use of active virus. They found subcutaneous injections of the virus would stimulate immunity but were extremely dangerous in that a great many animals succumbed to the disease during the process of immunization. Their attempts to inactivate the virus without disturbing the antigenic complex were unsuccessful. In the same year Howitt (16) also reported experiments designed to develop a safe yet efficient vaccine. She found that one small dose of live virus would immunize guinea pigs, but the same high incidence of infection followed vaccination that resulted in Records' experiments also occurred in the work of Howitt. Howitt also tried serum virus mixtures for vaccination. Practically no protection resulted from the injection of mixtures of serum and virus unless an excess of virus was present. It was also necessary to follow the initial serum-virus treatment with several injections of active virus. Although this method was safer than the former attempts of the author, the possibility of infection was still far too great to justify field use. In this connection it might be mentioned that Olitsky (17) in 1938 and Gochenour (18) in 1939 have published conclusive evidence that antiserum has a block-

ing effect on the action of vaccine. Gochenour advises against vaccination for a period of at least two weeks after administration of anti-serum and presents experiments with guinea pigs to show that within this period prophylactic doses of serum prevent the action of vaccine.

In 1936 Olitsky and Cox (19) published a paper dealing with the quantitative aspects of vaccination with active virus. They found that active untreated virus, virus absorbed on aluminum gel, and virus precipitated by tannin were all equally efficient as immunizing agents and, furthermore, that 3000 to 30,000 m.i.d.'s of any of these agents were necessary to protect against intracerebral infection. Later in the same year, Olitsky (20), acting on the encouraging results reported by Shahon and Giltner (21), made a further investigation of the possibilities of formalized virus as a vaccine. He found that the method of preparing such vaccines was of great significance, and this may explain the failure of earlier attempts. Fresh tissues of high virus content, not over-formalized, gave consistently good results. Vaccine thus prepared has good protective and keeping qualities as long as the formalin is not neutralized.

Since the formalized virus was completely inactivated and the possibility of accidental infection nullified, field experiments were undertaken with success. As a consequence, a formalized brain tissue vaccine was produced by commercial houses and was used with gratifying but not complete success during the epizootics of 1937 and 1938.

In 1935 Higbee and Howitt (22) had succeeded in growing the virus of encephalomyelitis in the developing chick embryo, and Beard, et al, showed that both types of virus grew on this medium in greater concentrations than had hitherto been possible. It was then found that the chick embryo virus could be completely inactivated in the presence of 0.4 per cent formalin while retaining its antigenic integrity. Therefore, it became possible to make a vaccine which was not only entirely safe but, because of its high virus concentration, was extremely efficient. Guinea pigs inoculated with two doses of the chick embryo vaccine are solidly immune seventeen days or less from the time of the first inoculation. Guinea pigs so protected resist many lethal doses of the virus injected intracerebrally; unvaccinated controls invariably died.

Very briefly, the method of preparation of the chick vaccine is as follows. Fertile eggs incubated ten to twelve days make the best medium for propagation of the virus. A 20 per cent suspension of brain tissue virus may be inoculated by any of the accepted methods for virus propagation in eggs. The embryo is so susceptible to the virus, however, that there seems to be no need for natural or artificial air sac methods. If the virus is placed just beneath the shell of the egg, it will be in close enough contact to the chorioallantoic membrane so that infection and death will result in from 24 to 48 hours. Dead embryos harvested aseptically after the first virus passage and used in a 1 to 5 per cent suspension to inoculate other eggs will consistently cause death within 20 hours for an indefinite number of passages. After three or four egg passages, the virus has become adapted to the egg and fixed and is ready for use in the preparation of vaccine. Both the chick and its membranes are ground as finely as possible and suspended in physiological saline

solution. As is true of all tissue vaccines, the more thoroughly the chick tissue is ground, the more virus may be expected to be released into the diluent. Formalin sufficient to make a final concentration of 0.4 per cent may be added conveniently with the diluent. Forty-eight hours at room temperature is a safe time period to allow for inactivation of the virus, and at the end of this time the vaccine should immediately be refrigerated. Guinea pigs inoculated intracerebrally with the vaccine will serve to show the absence of any active virus. A potency test of satisfactory vaccine against a heterologous strain of the same type of virus should result in 100 per cent protection of principles and 100 per cent death of controls. A 1 to 500 dilution of guinea pig brain virus is the usual exposure dose in potency tests. Such a dose will bring complete prostration or death to controls in five days for the western virus and four days for the eastern virus.

With the new chick vaccine available, an important research tool has been added to the laboratory workers' collection. Studies on epidemiology, etiology, and immunology of the disease should advance more rapidly, now that a safe, sure method of immunization is known. It should be emphasized that the efficiency of the vaccine may prove important, not only in solving problems of equine encephalomyelitis but also in solving the fundamental problems of general virus immunology.

I should like, in conclusion, to mention that the study of the mechanism of immunity to encephalomyelitis is a fascinating research problem which is still to a great degree unsolved. It is quite natural that, in the face of costly and widespread epizootics, most research has been undertaken with a view to halting the spread of the disease. That objective is now well advanced toward accomplishment. The means of control of encephalomyelitis may be found in chick embryo vaccine if extensive vaccination can be practiced over a long period of time. But, in our haste to accomplish the practical end of saving horses' lives, we have to a great extent failed to inquire into the mechanisms of the results we have been able to bring about. For instance, we know that intracerebral exposure is more effective in causing fatal encephalomyelitis than any other method, and King (9) has shown that there is a difference in brain pathology in various types of inoculation. The reason for this difference needs more investigation. Olitsky has pointed out that antiserum is much less effective against intracerebral inoculation than intraperitoneal inoculation and mentions that, when virus enters nerve tissue, antiserum seems of little value. Yet, vaccinated animals resist incredibly strong doses of virus directly into the brain. Does this mean that actively immune animals are protected chiefly by tissue immunity? Does it also mean that serum from hyperimmunized animals protects other animals by destroying virus in the blood stream or at the point of inoculation? Such problems as these need more than philosophical consideration, and it is possible that chick embryo virus, with its ability to stimulate the production of more potent antiserum in hyperimmunized animals and a better active immunity in vaccinated animals, will lead to an experimental solution of many virus immunological problems.

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