1941

Bovine Encephalitis

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Sporadic bovine encephalitis proven an infectious disease by use of chick embryo

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IN THE study of sporadic bovine encephalitis it has been necessary to propagate the causative agent by animal passage. This has been rather expensive and time consuming where calves were employed and only slightly less so when guinea pigs were used. In an effort to find a more satisfactory method, chick embryos were inoculated with the peritoneal exudates or the ground liver and spleen of guinea pigs killed during the height of the disease. These materials were known to be high in concentration of the infectious agent. The methods of embryo inoculation were those usually employed.

First Trials

In the first trials, the inoculations were made into the allantoic sac of 6 to 12 day old embryos. Insofar as could be determined, this method of inoculation produced no effect whatever on the embryo or its membranes. If one is to use embryos for propagation of infectious materials, it is highly desirable that the embryos show some recognizable change whereby one can determine whether or not there has been multiplication. This is not always possible, but in order to determine if it could be done in this case, a second method of inoculation was tried. This proved to be satisfactory.

The successful method by which the causative agent was cultivated on chick embryos is known as yolk sac inoculation. The developing embryos were candled at the desired age and the position of the embryo and the natural air cell were marked. Some workers using this method locate the position of the yolk or yolk sac before inoculation, but this has not been necessary since that portion of the egg lies on the side opposite the actual embryo and at a slightly lower level. Thus, if the position of the embryo is known, the location of the yolk sac is obvious. Eggs with floating or mislocated air cells were discarded. A small hole was bored through the shell over the air cell and after the application of a disinfectant to this area, a twenty gauge needle was inserted through the hole and shell membranes. The needle was directed toward the side opposite the embryo and an injection of not more than 0.2 cc was made at a depth of nearly an inch, after which the eggs were returned to the incubator and candled daily thereafter. Embryos of from six to nine days were employed. The eggs were incubated under good hatchery conditions as regards to temperature, moisture, rotation of the eggs, etc. The first inoculation of infectious material into embryos made in this manner resulted in death of about 50% of the embryos within nine days. A mixture of yolk and embryonic fluids from such dead embryos was again injected into the yolk of fresh seven day old embryos and in this way the infection has been propagated in series through 48 passages or subcultures. The interval between inoculation and death of the embryo gradually lessened until 70% of the inoculated embryos died between the third and fourth days and all were opened and examined at that time. Occasionally, the concentration of infection in some of the live embryos was so small that death of freshly inoculated embryos was delayed, although often such live infected embryos contained as much of the causative agent as did the dead...
embryos. Inoculated eggs were candled on the first and second day after injection, and all embryos found dead at that time were discarded because such deaths were usually due to accidents or bacterial contamination. Cultures and stained smears were made from all embryos in order to eliminate bacterial contamination. Material from infected embryos was occasionally injected into guinea pigs as an added proof that the infectious agent of bovine encephalitis was the cause of the embryo death. Such material has always produced a more severe disease with earlier symptoms than where infectious animal tissues were employed. Such findings indicate an increased concentration in the embryos, and tests are now underway to determine whether or not this is actually true.

Results

In the study of sporadic bovine encephalomyelitis, developing chick embryos are also being injected with bacteria-free filtrates of infectious material to determine whether or not such filtrates contain the causative agent. Guinea pigs have been found to be more satisfactory for such tests, but this may be due to the fact that the guinea pigs are given a relatively large inoculation, whereas the embryo yolk sac inoculations have been small. Chick embryos have also been employed in attempts to assay the concentration of infection in various materials. Preliminary trials have given irregular but very promising results. Takes have been obtained when as little as 0.2 cc of a 1:100,000 dilution have been injected into the yolk sac of developing embryos. When the technic has been worked out, it is thought that takes will be possible when using a dilution of 1:100,000 or even higher.

After showing that sporadic bovine encephalomyelitis was an infectious disease, efforts have been mainly concerned with attempts to identify the infection. In this, the encephalomyelitis has been compared with known similar diseases; attempts have been made to grow the causative agent in culture media; material has been stained in an endeavor to find inclusion bodies; filtrations have been made to identify a virus; and chick embryos have been employed. The disease has been shown not to be related to equine encephalomyelitis, Listerellosis, rabies, Aujesky's disease, or malignant catarrhal fever. It has been impossible to grow bacteria from tissues of the affected animals. In culturing, a study using aerobic methods has been quite exhaustive. Trials by anaerobic methods have not been so complete, but all those so far employed have been equally negative. Efforts to stain inclusion bodies like those seen in some virus diseases, but especially those found in the Rickettsia diseases and in contagious pleuro-pneumonia, have met with total failure. Thus, attempts to identify the causative agent by the above three methods have failed. Infectious material has been passed through the coarse and medium grades of Berkefeld filters. This has resulted in 19 filtrates that were considered satisfactory. Nine of these gave positive results and 10 gave negative results when injected into susceptible animals. The filters used in the nine positive instances held back bacteria that were known to be present in the material before filtration, and at the same time allowed the infectious agent of the encephalitis to pass. Other methods of identification have so far failed entirely, whereas this method has given a strong promise of success. The work with infected chick embryos has been highly satisfactory and furnishes a cheap method of propagation whereby large amounts of infectious material can be obtained at frequent intervals. A study of the infected embryos and the different egg parts is continuing, but here again efforts to identify the causative agent by this study have so far failed.

Bibliography