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Isolation of St. Louis Encephalitis Virus During Inter and Epidemic Periods.

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In the interval between the 1933 encephalitis epidemic and the present outbreak of the disease, neutralization tests have been made to determine the presence of virucidal antibodies in serum, obtained during convalescence, from 10 patients whose illness had been diagnosed as acute encephalitis. In none of these instances was the test positive. Efforts have also been made to isolate the virus from human brain material obtained at autopsy from 6 fatal cases in which encephalitis was regarded as the cause of death. Intracerebral injections in mice* [1, 2] which proved susceptible in 1933, failed to demonstrate the virus in any of these 6 attempts. Pathological lesions consistent with encephalitis were observed in 3 of these fatal cases, 2 of them being in children.

The clinical diagnosis in the cases without brain lesions might be regarded as incorrect. Concerning those which showed pathological lesions, however, another possibility is that these sporadic cases of the disease were produced by a virus of altered virulence which was not infective for mice in the dilutions employed.

Recalling this interepidemic experience, it was surprising to note the ease with which the virus was isolated from the fatal cases occurring this summer. Using again the same method of intracerebral injection of human brain emulsion into mice, 7 different strains of the virus have already been obtained from 19 different fatal cases. This incidence of the infectivity of brain tissue is almost the same as that observed in 1933 when the virus was first isolated. At that time 7 out of 15 brains were shown to contain active virus by producing the disease when injected into *Macacus rhesus* monkeys.[1] Webster[2] obtained 5 strains out of 11 trials in mice. He was, however,

* Strain C57 Black, first obtained from Dr. Leo Loeb, has been used in this laboratory for the study of encephalitis since 1934. Special strains are apparently not even necessary for the primary isolation of virus from human brain tissue.

1 Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, U. S. P. H., 1933, **48**, 1341.

2 Webster, L. T., and Fite, G. L., *J. Exp. Med.*, 1934, **61**, 103.

using material from selected cases while in our present experiments every case diagnosed encephalitis was used.

The first strain of virus was isolated on September 8 from material injected September 3. Two other strains were obtained 6 days after inoculation and the remaining 4 on the seventh day.

The results of neutralization tests indicate that the 1937 strains are immunologically similar with those isolated in 1933. Sera from several 1937 patients were tested against the 1933 virus. Tests were also performed using the 1937 virus and 1933 sera. Two samples of 1933 sera were used, a hyperimmune rabbit serum, and serum recently obtained from an individual who survived a severe attack of the disease in 1933. A sample of human convalescent serum stored on ice since 1934 was used in some of the tests, and although originally of high titer it now affords no apparent protection. In each set of neutralization tests, one 1933 positive and 2 negative control sera were used. The dilutions employed were 10^{-3}, 10^{-4}, and 10^{-5}.

Titration experiments to determine the potency of the virus have been carried out on 2 of the recently isolated strains after the fourth passage through mice. One strain is active in dilutions as high as 10^{-5} and the other to only 10^{-4}, when injected in .025 cc. amounts. It is probable that the virulence of these strains will increase after further passage in mice as was the case with the 1933 strains. At present this difference in virulence has remained constant after several additional animal passages. A similar slight but constant difference in virulence has also been observed among 4 strains of 1933 virus maintained in this laboratory.

The virulence of the two 1937 strains studied does not, however, differ greatly from that previously observed here and reported by Webster.[3,4] Using an already established virus in unselected stock mice, Webster reports an infectivity at varying levels between 10^{-4} and 10^{-6}, when injected in 0.03 cc. amounts. With selected Swiss mice the titer was higher, namely, 0.03 cc. of a dilution of 10^{-7}. The variation in virulence of different strains of the virus isolated during the two epidemics is now being studied.

So far the virus has not been demonstrated in nasal washings using broth, pH 7.6, nor in the spinal fluid from active cases. Guinea pigs have been injected intracerebrally and intraperitoneally with brain tissue and spinal fluid removed early in the disease to test for the virus of choriomeningitis, without positive results. Sufficient time has not yet elapsed since some of these animals were injected to be sure of negative results.

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3 Webster, L. T., Fite, G. L., and Clow, A. D., *J. Exp. Med.*, 1935, **62**, 827.

4 Webster, L. T., and Fite, G. L., *J. Exp. Med.*, 1934, **61**, 411.

The pathological lesions observed in the brains of fatal human cases are identical with those described in the previous epidemics.[5, 6] The changes produced in mice by the 1937 strains are also the same as those reported by Smadel and Moore[7] in the case of the 1933 virus.

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Photoelectric Plethysmography of the Nasal Septum in Man.

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Advantage has been taken of the fact that the opacity of tissues to light varies with the blood content, to record photoelectrically the changes in the blood content of the nasal septum.

The arrangement used (shown schematically in Fig. 1) is essentially an adaptation of the photoelectric plethysmograph for the finger, previously reported.[1] Illumination of the septum is provided by a small ophthalmoscope bulb inserted in one nares. Local heating is largely prevented by a heavy metal cap which, placed over the bulb, conducts the heat away to the lamp carrier. Local heating may be practically eliminated by using a mirror arrangement which permits the light source to be placed some distance from the nares. The latter method of providing illumination has the additional advantage of offering opportunity to control amplification, independent of septal luminosity, through predetermined decrements in light intensity by means of suitable filters. The light transmitted by the septum is reflected out the other nares by the mirror to the photoelectric cell. The entire assembly is mounted on a dental impression plate which, carried between the teeth, not only provides a rigid mount but also guarantees constancy of alignment with the nasal septum. The mirror and light tubes may be varied in diameter to fit varying nares. Any desired penetration is readily provided. The apparatus is light and comfortable and may be worn for hours without discomfort. Breathing through either the mouth or nose is equally feasible. The photoelectric oscillations are recorded galvanometrically on the photokymograph after amplification.

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[5] McCordock, H. A., *Am. J. Public Health*, 1933, **23**, 1148.

[6] McCordock, H. A., Collier, Wm., and Gray, S. H., *J. Am. Med. Assn.*, 1934, **103**, 822.

[7] Smadel, J. E., and Moore, E., *Am. J. Path.*, 1934, **10**, 829.

[1] Hertzman, A. B., and Spealman, C. R., *Am. J. Physiol.*, 1937, **119**, 334.