That von Economo's disease and the St. Louis outbreak of encephalitis are due to different etiological agents is quite evident from these experiments and those of Webster and Fite.[2] This is further borne out in the difference in the seasonal and age incidence of the two diseases. Moreover, up to the present, no Parkinsonian-like sequellae have been reported from the 1933 outbreak in St. Louis, nor from similar cases reported in Paris, Ill., in the previous year.

Antiviral substances have been reported in the serums of recovered individuals and in those of physicians and nurses who have been in close contact with cases.[8] The fact that the serums of 9 individuals from St. Louis and 68 from New York, all without a history of contact, had no demonstrable neutralizing substance, suggest that the encephalitis antibody is specific and the result of exposure to the virus. It appears, therefore, that contact with the virus can result in either the disease or immunity, the latter being due, perhaps to reaction with the virus or a subclinical attack of the disease. In the present work there was no evidence to show that exposure to the mouse passage virus gave immunity, although the intracutaneous inoculation of virus into mice gave a high degree of immunity.‡

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Experiments with Virus of the St. Louis Epidemic of Encephalitis.*

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Workers have reported the successful transmission of a virus isolated from cases of the St. Louis epidemic of encephalitis to monkeys[1] and to monkeys and mice.[2, 3] Through Dr. Holden of the

8 Barr, David L., Meeting Amer. Coll. Physicians, Chicago, 1934.

‡ I wish to thank Dr. Theodore C. Hempelmann who furnished the serums obtained from St. Louis residents and Dr. Josephine B. Neal, for her helpful interest in the work.

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1 Muckenfuss, R. S., *Am. J. Pub. Health*, 1933, **23**, 1148.

2 Muckenfuss, R. S., Armstrong, C., and McCordick, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

3 Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

College of Physicians and Surgeons was obtained some of the virus used by Dr. Armstrong which had undergone 13 serial passages in white mice. The intracerebral infective dose of the virus varied between 0.03 cc. $\times 10^{-3}$ to 0.03 cc. $\times 10^{-6}$ during the course of this work.

The present work deals with experimental passage of the virus, its preservation, and its resistance to germicides. White mice showed a fairly uniform susceptibility to the virus. After 3 to 5 days' incubation, the animals developed a hyperirritability, ataxia, convulsions and paralysis. Mice receiving attenuated virus or borderline infective doses developed very mild attacks, with tremors lasting only a day or two. Of 4 such animals tested, one showed immunity against subsequent infection.

Attempts to pass the virus to guinea pigs, rabbits, rats, kittens and ferrets were negative. Intracerebral and intraperitoneal inoculations in 5- to 6-weeks-old rats and 24-hour to 6-months-old guinea pigs were done and in many instances were redone at intervals.

One monkey, given 1 cc. intracerebrally (motor cortex) and 2 cc. intraperitoneally of a 10% suspension of the 26th passage in mice, developed a rise in temperature after 4 days, followed by a general slowness and awkwardness, tremors and ataxia. When sacrificed 3 days later, these symptoms were still manifest. Since others had found the maintenance of the virus strain through monkeys difficult, it was decided to use for serial passage those parts of the monkey's central nervous system that contained the most virus.

To determine this, the following areas were tested for virus by either intracerebral or combined intracerebral and intraperitoneal inoculations in mice:—different areas of the cerebral cortex, *e. g.,* Rolandic, frontal, parietal and temporal regions; cerebellum including both cortex and ganglia; basal ganglia, hippocampus, mid-brain, brain stem, cervical, lumbar and dorsal cord. Only the frontal region, mid-brain and lower cord had demonstrable virus, in small amounts. A concentrated suspension of frontal lobes produced merely tremors and then recovery, or the typical attack only after a prolonged incubation period; while suspensions of the mid-brain and lower cord, both of which produced an attack in mice in the usual incubation period, failed to infect at higher dilution than 1 in 5.

A monkey which received a thick suspension of mid-brain intracerebrally and intraperitoneally, developed no symptoms after this injection nor after a reinjection 9 days later of a similar amount of a suspension of mid-brain and lower cord. Subsequent test showed no antiviral substances in this animal's serum, although it had re-

ceived 2 intraperitoneal injections of virus suspension, thereby corroborating the low virus content of the inoculums. The serums of relatively insusceptible animals, such as the monkey, and of animals refractory to the virus, were tested for neutralizing substances. The tests were carried out as given in the previous paper. The serums of 3 monkeys, 2 rats, 2 kittens, 6 rabbits, and 5 guinea pigs showed no antiviral action for they failed to neutralize from 1-10 infective doses of virus.

To compare various methods of preserving the virus, and to maintain an even distribution for comparative work, the following methods were tried, *viz.*, emulsified brain, kept both at ice-box temperature (3-6°C.) and frozen; brain tissue ground well in an equal amount of glycerine (Schering-Kahlbaum) and kept at ice box temperature or frozen; and whole brain in glycerine at ice box temperature. By the latter method, suspensions were made using several pieces of a few brains.

Mouse brain suspension frozen with or without glycerine retained its infectivity for at least 3 months, although there was a loss of approximately 90% of the potency the first 2 weeks. None of the 3 methods used at ice box temperature were satisfactory, for the virus was non-infective in a month or less, and at the end of 2 weeks approximately 99% of the infectivity was lost.

The resistance of the virus contained in a suspension of infective brain tissue, to both formalin and phenol, was tested; the former at 20-23°C. and at 5-7°C., the latter at 5-7°. Equal parts of a 10% brain suspension and double the required amount of germicide were mixed. The mixture was shaken frequently. Mice were injected intracerebrally, and intracerebrally and intraperitoneally with the germicidally treated virus suspension.

The virus was quite resistant to phenol for it was still infective after being in contact with 1% phenol for 25 days and 0.5% phenol for 50 days, at ice box temperature.

The virus was much more susceptible to the action of formalin. This germicide in a concentration of 0.1% rendered the virus non-infective between 12-18 hours at room temperature and from 7-9 days at 5-7°. Likewise, 0.2% formalin inactivated the virus between 9 and 12 hours at 20° and 35 days at 5°.

These experiments confirm and extend the findings of Webster[3] that the mouse is the only susceptible animal among the common small laboratory animals. Moreover, the mouse is decidedly more susceptible to the virus than is the monkey. The monkey showed a diffuse distribution of virus in the frontal region, mid-brain and

lower cord. No attempt was made to correlate these findings with the histological picture in the monkey central nervous system because an attempt to transmit the virus in serial passage through the monkey failed. However, this finding is in keeping with the diffuse distribution of lesions found in the human.[4] The insusceptibility of other laboratory animals to the virus may be explained by a tissue insusceptibility rather than humoral antiviral action.

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Production of a Hyperimmune Antipoliomyelitic Horse Serum.*

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It is generally accepted that immune serum is of little or no therapeutic benefit in poliomyelitis, as well as in other filterable virus diseases. However, it may be of value prophylactically, but this has not as yet been fully demonstrated. Successful passive immunization against some virus diseases leads one to suppose that such protection may possibly be afforded against poliomyelitis when an antiserum of sufficient potency is produced. Numerous attempts[1-4] with varied degrees of success, have been made to prepare an effective antiviral serum in large animals. The production of a potent antipoliomyelitic horse serum was first accomplished in 1929 in these laboratories[5] and at about the same time in England.[6] More

[4] McCordock, H. A., *Am. J. Pub. Health*, 1933, **23**, 1152.

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[1] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **55**, 662.

[2] Dixon, S. G., and Rucker, J. B., *J. Infect. Dis.*, 1918, **23**, 543.

[3] Pettit, A., *Compt. rend. Soc. biol.*, 1918, **81**, 1087; *Bull. gen. de Therap.*, 1925, **176**, 389.

[4] Neustaedter, M., and Banzhaf, E. J., *J. Am. Med. Assn.*, 1917, **68**, 1531.

[5] Weyer, E. R., Park, W. H., and Banzhaf, E. J., *Am. J. Path.*, 1929, **5**, 517; *J. Exp. Med.*, 1931, **53**, 553.

[6] Fairbrother, R. W., *Brit. J. Exp. Path.*, 1930, **11**, 43.