survives relatively well in sewage kept at 70° F., in spite of very considerable increases in bacteria during storage.

It is concluded that in our experiments the naturally occurring microorganisms in water from a pond, a lake, and a river failed to yield significant increase in several strains of poliomyelitis virus, and that six strains of Bodo, two of Monas, and one each of Pleuromonas, Oikomonas, Tetrahymena, and Uronema, derived from sewage, failed to support the growth of poliomyelitis virus to an extent that would be significant with reference to the finding of the virus in sewage.

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Influence of Anesthesia on Experimental Western Equine Encephalomyelitis[1]

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Past attempts at therapy in neurotropic virus diseases have been largely unsuccessful. The results have been essentially negative because once the symptoms of a virus disease have become evident, the virus is already well established within the host cell. Therapeutic agents are therefore unable to gain access to the virus, with the result that treatment is of little value. Nevertheless, there have been suggestive positive results with both serotherapy and chemotherapy in this group of diseases. Zichis and Shaughnessy (*14*) reported successful treatment of experimental western equine encephalomyelitis infections using hyperimmune rabbit serum, but their observations have not been fully confirmed (*8*). Wherever positive results have been obtained, early administration of potent antiserum has been found to be essential. Successful chemotherapy has been limited to the so-called lymphogranuloma venereum-psittacosis group (*10*), which, however, some workers place intermediate between true filtrable viruses and rickettsiae. Generally, in true virus diseases, chemotherapy has been ineffective (*5*).

The ideal therapeutic agent for use in the treatment of neurotropic virus diseases would bring about a reversible change in the metabolism of the host cell sufficient in degree and duration to destroy the virus without causing permanent injury to the host cell, and must have predilection for cells of the central nervous system. General anesthetics seem to fall within this category. Our interest in the use of anesthetics in neurotropic virus diseases was further prompted by a number of reports on the influence of anesthesia on the course of several other diseases affecting the central nervous system. Bronfenbrenner and Weiss (*1*) noted that anesthetics, alone and in combination with specific antitoxin, decreased mortality in experimental botulism. Similarly, avertin (or tribromethanol) has been used to alleviate muscular spasms in tetanus (*4, 7*).

The *in vitro* effect of ether on viruses has been observed by several workers. When used in relatively high concentrations, this anesthetic is an effective bactericidal agent and can be used *in vitro* to destroy bacterial contaminants in tissues infected with poliomyelitis (*6*), rabies (*12*), and measles (*9*) without affecting the virus. The method, however, is not applicable in removing contaminants from tissues infected with St. Louis encephalitis or equine encephalomyelitis viruses (*2, 3*).

In the present studies a strain of western equine encephalomyelitis virus, obtained from W. McD. Hammon, was used. The virus was maintained by intracerebral injection of Swiss mice. A 20-per cent suspension of infected mouse brain tissue was prepared using 10 per cent sheep serum-broth as diluent. The supernate, removed after centrifugation, was dispensed in 0.2-ml. amounts in sterile glass ampules which were then sealed, frozen quickly, and stored in the deep-freeze cabinet. Under these conditions the titer of virus remains constant for several months. The quantity of virus present in the stock suspension was determined by intracerebral inoculation of three- to four-week-old mice, and the 50-per cent end point (LD_{50}) was calculated according to the method of Reed and Muench (*11*).

In the experiment to be described each three- to four-week-old Swiss mouse was inoculated intracerebrally under light ether anesthesia with 0.03 ml. of a $10^{-8.5}$ dilution of stock virus suspension (approximately $3 \times LD_{50}$). In order to avoid errors due to sequence of inoculation, all inoculated mice were placed in one large cage and then separated at random into three groups. Group I served as controls and received no subsequent treatment. Group II received three four-hour periods of diethyl ether anesthesia during the first 36 hours after intracerebral inoculation, while Group III received two four-hour periods of ether anesthesia beginning at the time the animals first showed symptoms of encephalitis. The open method was used for the administration of the anesthetic. All animals were observed for central nervous system symptoms and death from encephalitis during

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a period of 10 days. The results are summarized in Table 1. The effect of ether anesthesia on the course of the experimental infection became evident early in the experiment. By the third day, 51.3 per cent of the controls had died, and only 29.1 per cent and 26.7 per cent, respectively, of the anesthetized animals had died. Thus, in addition to the increase in total survivors with ether anesthesia, there is also a delaying effect on the progress of the disease. To determine whether or not the data were statistically valid, the *p* values were calculated. The differences in the mortality rates were highly significant when the control group of animals was compared with each of the treated groups.

These studies are being extended to include experiments with other general anesthetics, which possibly may be more effective than diethyl ether in the treatment of experimental neurotropic virus infections. Several neurotropic viruses are under investigation. A preliminary study concerning the effect of combined ether anesthesia and antiserum on western equine encephalomyelitis in mice has been reported elsewhere (*13*).

Summary. Anesthesia, by ether, is effective in the treatment of western equine encephalomyelitis in mice. Of mice treated with deep ether anesthesia soon after the intracerebral injection of western equine virus, only 58 per cent developed the disease as compared with 92.4 per cent of control animals. When anesthesia was delayed the approximate length of the incubation period, 60 per cent of the animals developed the disease as compared with 92.4 per cent of the controls. In addition, ether anesthesia delays the development of central nervous system symptoms not only when administered soon after the injection of the virus but also when administered after the disease has progressed far enough to cause objective signs of encephalitis.

TABLE 1

INFLUENCE OF ETHER ANESTHESIA ON THE COURSE OF EQUINE ENCEPHALOMYELITIS (WESTERN) IN SWISS MICE

Group	Virus inoculum	Number of mice*	Per cent of animals dying from encephalitis during successive days after virus injection										Mice surviving†	
			1	2	3	4	5	6	7	8	9	10	Per cent	p‡
I (Controls)	$.03 \times 10^{-8.5}$ ($3\ LD_{50}$)	39	0	5.1	46.2 (51.3)§	36.0 (87.3)	0 (87.3)	5.1 (92.4)	0	0	0	0	7.6	
II Anesthetized‖ (immediately)	same	31	0	6.5	22.6 (29.1)	25.8 (54.9)	3.2 (58.1)	0	0	0	0	0	42.0	.001
III Anesthetized¶ (Delayed)	same	30	0	3.3	23.4 (26.7)	30.0 (56.7)	0 (56.7)	3.3 (60.0)	0	0	0	0	40.0	.002

* Animals which died from trauma or ether are not included.
† Survived observation period of 10 days.
‡ Values obtained by reference to appropriate tables in Pearl's *Medical biometry and statistics.* Philadelphia: W. B. Saunders, 1930.
§ Figures in parentheses indicate cumulative per cent deaths.
‖ Three four-hour periods of anesthesia administered beginning immediately after virus injection.
¶ Three four-hour periods of anesthesia administered beginning approximately 40 hours after virus injection.

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