INFLUENCE OF ANESTHESIA ON EXPERIMENTAL NEUROTROPIC VIRUS INFECTIONS*

I. In vivo Studies with the Viruses of Western and Eastern Equine Encephalomyelitis, St. Louis Encephalitis, Poliomyelitis (Lansing), and Rabies

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Past attempts to find an adequate method of treatment for the neurotropic virus diseases have been largely unsuccessful. The results have been essentially negative because treatment was not administered until symptoms appeared, at which time the virus is already well established within the host cell. Antibodies are unable to gain access to the virus, with the result that antiserum therapy is of no value. The close association between the virus and the host cell also renders chemotherapy difficult, for an agent in concentration sufficient to destroy the virus would probably injure the host cell (1). Nevertheless, there are records of suggestive positive results with both serotherapy and chemotherapy in certain virus diseases.

The effective administration of antiserum to experimental animals infected with influenza (2) and psittacosis (3) has been reported. Also, there is clinical evidence that measles can be modified by antiserum even after the appearance of preeruptive symptoms (4). Zichis and Shaughnessy (5) reported successful treatment of experimental Western equine encephalomyelitis infection using hyperimmune rabbit serum, but their observations have not been fully confirmed (6). It has been suggested that results of experiments with hyperimmune serum therapy have been inconsistent because of variations in the virulence and incubation periods of the virus strains employed and differences in the potency of antisera (7). However, in all experiments in which positive results were obtained, early administration of potent antiserum was found to be essential. Reports with chemotherapeutic agents have been discouraging (1, 8). Successful results have been limited to the so called lymphogranuloma-psittacosis group (9), which, however, is considered by some workers to be intermediate between true filterable viruses and the rickettsiae. Certain antibiotics have likewise proved ineffective in experimental neurotropic virus infections (10, 11).

The ideal therapeutic agent would bring about destruction of the virus without causing permanent injury to the host cell. Such an agent should have

the same tissue predilection as the virus. General anesthetics seem to fall within this category. There have been a number of reports on the influence of anesthesia on the course of several toxic diseases affecting the central nervous system. Bronfenbrenner and Weiss (12) noted that anesthetics, alone and in combination with specific antitoxin, decreased mortality in experimental botulism. Similarly, avertin (or tribromethanol) has been used by Lawen (13) to alleviate muscular spasms in tetanus. Kaspar (14) later found that small amounts of tetanus antitoxin alone saved only 4 per cent of intoxicated animals, while avertin and antitoxin together saved 37 per cent. In addition, the *in vitro* effect of ether on certain viruses has been observed. This anesthetic when used in relatively high concentrations is an effective bactericidal agent and can be used *in vitro* to destroy bacterial contaminants without affecting certain viruses (poliomyelitis (15), rabies (16), and measles (17)). The method, however, is not applicable in removing contaminants from tissues infected with the St. Louis encephalitis or equine encephalomyelitis viruses (18–21). It therefore was desirable to investigate the effect of anesthesia on the course and outcome of these and certain other experimental neurotropic virus diseases.

Material and Methods

Viruses.—Five neurotropic viruses were included in this series of experiments: 3 which are destroyed by ether *in vitro* (St. Louis encephalitis, Western and Eastern equine encephalomyelitis), and 2 which are unaffected (poliomyelitis and rabies). Other differences between these 2 groups of neurotropic viruses will be discussed later.

The Western equine virus, labeled "Olitsky," was provided by Dr. W. McD. Hammon, and has been maintained by frequent intracerebral passage in Swiss mice. The Ten Broeck strain of Eastern equine virus was obtained from Dr. Carl G. Harford. The St. Louis encephalitis virus (Hubbard strain) was furnished by Dr. Albert Milzer. It has been transferred twice in this laboratory, immediately preceding the experiments to be described. The Lansing strain of poliomyelitis virus, adapted to mice by Armstrong (22, 23), was also provided by Dr. Milzer. The rabies virus (strain 3557) was isolated by one of us in 1940 and has been maintained by serial passage in Swiss mice; the virus used in these experiments was in its twelfth mouse passage.

The stock suspensions of the St. Louis encephalitis, equine encephalomyelitis (Eastern and Western), and rabies viruses were prepared by the following procedures. In each case, a minimum of 3 infected mouse brains was used as the source of virus. A 20 per cent suspension of infected mouse brain tissue was made, using 10 per cent sheep serum broth as the diluent. The supernate, removed after centrifugation at 2000 R.P.M. for 3 minutes, was dispensed in 0.2 ml. amounts into 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 3 to 4 week old mice, and the 50 per cent end point (LD$_{50}$) was calculated according to the method of Reed and Muench (24). In preparing the stock suspension of the Lansing strain of poliomyelitis virus, several mice were simultaneously infected by the intracerebral route, and the cords and medullae from animals which succumbed to the infection within 5 days were harvested. A 20 per cent suspension of the infected tissue was prepared in nutrient broth, titrated for infectious potency, and stored in the deep-freeze unit.

In each experiment, the animals were inoculated intracerebrally under light ether anesthesia. In order to avoid errors due to sequence of inoculation, they were then placed in one large cage and were separated at random into the respective groups.

Animals.—Three to 4 week old Swiss mice of the Webster strain were used throughout these studies. In one experiment with the virus of poliomyelitis 5 week old mice were used since it had been shown that age was not an important factor in susceptibility of mice to this experimental infection (25).

Administration of Anesthetic.—Mallinckrodt anesthetic ether was used in these experiments. A closed system, involving the use of an air-tight glass container to which a McKesson anesthesia machine was attached, resulted in a high mortality rate. Concentrations of ether sufficient to cause death of a large number of mice failed, however, to produce even a light anesthesia in a considerable number of others. Because of these individual variations in ether tolerance, it was necessary to resort to an open method for the administration of the anesthetic. Animals were placed in an ether chamber until they reached a state of deep anesthesia (complete muscular relaxation). They were then laid on their backs in which position they remained until they were sufficiently aroused to right themselves. The anesthetic was administered in this manner several times during each period of anesthesia. The time and duration of anesthesia were decided somewhat arbitrarily, the objective being to continue anesthesia over most of the time during which virus would presumably be rapidly increasing in quantity. Each "period" of anesthesia consisted of 4 hours. It is possible that a shorter period would have proved equally effective, but this point has not been investigated. A minimum of 2 periods of anesthesia was employed; the exact procedure used in the respective experiments is indicated in the protocols.

EXPERIMENTAL

Experiments with the Western Equine Virus.—In a preliminary experiment, it was noted that while all the control animals receiving virus alone died from encephalitis within 3 days after the intracerebral inoculation, a small per cent of animals receiving ether anesthesia immediately after virus inoculation survived. Although an overwhelming amount of virus was used, this observation was sufficiently significant to warrant further investigation. In the subsequent experiments, an effort was made to use that amount of virus which would produce encephalitis in approximately 90 per cent of the control animals; such amounts were estimated on the basis of preliminary titrations.

Experiments 1 and 5.—A single group of control animals was used for these 2 experiments. The LD_{50} of the stock virus suspension was $10^{-9.0}$. Each animal was inoculated intracerebrally with 0.03 ml. of a $10^{-8.5}$ dilution of the stock suspension (approximately 3 LD_{50}). In Experiment 1, animals were given a period (4 hours) of anesthesia immediately following virus inoculation, allowed to rest for 1 hour, and then given another period of anesthesia. About 18 hours later another period of anesthesia was given. In Experiment 5, animals were not anesthetized until the most susceptible mice began to show the first symptoms of encephalitis, which consisted of ruffled fur, ataxia, slowness, unsteadiness, and progressive hyperexcitability. These signs occurred from 36 to 40 hours following intracerebral inoculation of virus. At this time the animals were given a period of anesthesia, allowed to remain unanesthetized for 1 hour, and then given another period. All animals were observed for 10 days for central nervous system symptoms and death.
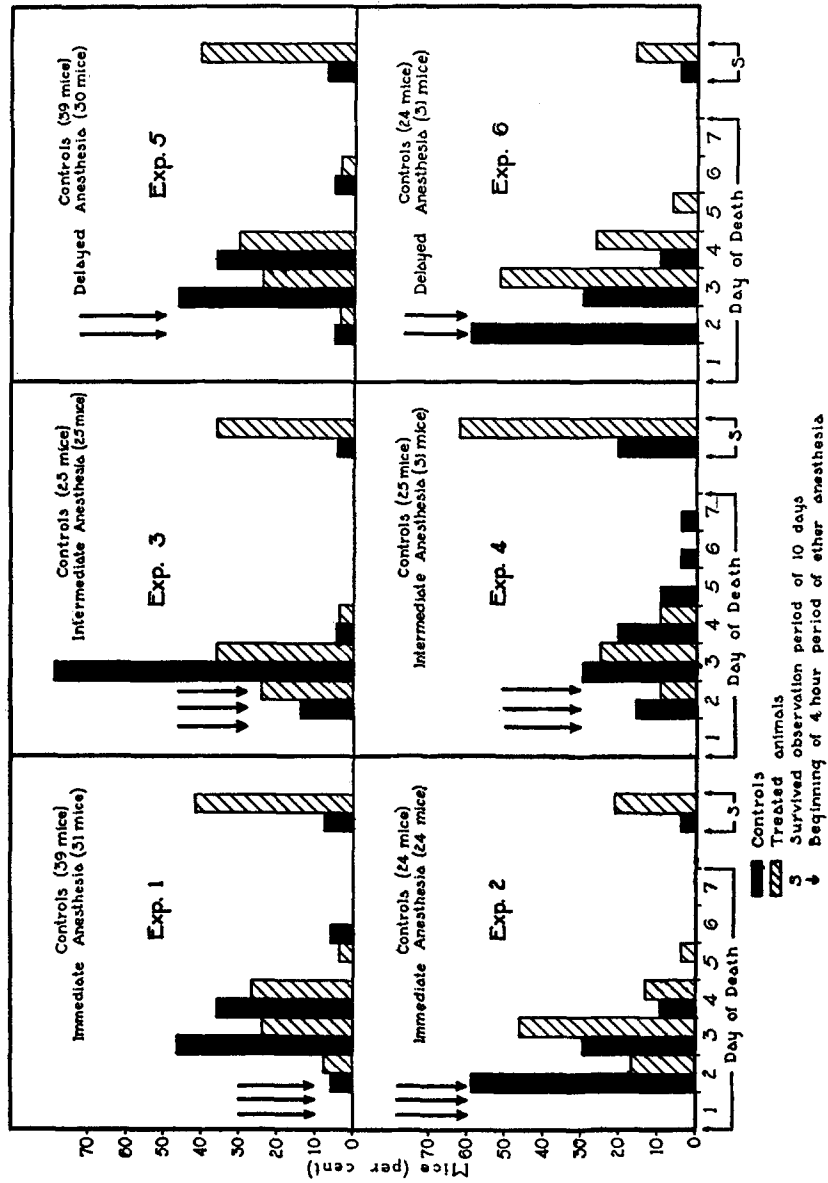
FIG. 1. Influence of ether anesthesia on course of disease produced in Swiss mice infected with equine encephalomyelitis virus (Western type).

The results of these experiments are presented graphically in Fig. 1, and a statistical analysis of the final outcome of each experiment is summarized in Table I. The number of animals used in each experiment is indicated; those which died from trauma or ether are not included in these summaries. It can be seen that only 7.6 per cent of the 39 untreated controls survived the observa-

TABLE I

Effect of Ether Anesthesia on Outcome of Disease Produced in Swiss Mice Infected with Equine Encephalomyelitis Virus (Western Type)

Experiment No.	Virus inoculum (injected intracerebrally)	Method of treatment*	No. of Mice‡	Mice surviving§	
				Per cent	p‖
1	$0.03 \times 10^{-8.5}$ (3 LD$_{50}$)	Immediate anesthesia No treatment	31 39	42.0 7.6	0.001
2	$0.03 \times 10^{-8.5}$ (3 LD$_{50}$)	Immediate anesthesia No treatment	24 24	20.8 4.2	0.089
3	$0.03 \times 10^{-8.2}$ (6 LD$_{50}$)	Intermediate anesthesia No treatment	25 23	36.0 4.3	0.007
4	$0.03 \times 10^{-8.7}$ (>2 LD$_{50}$)	Intermediate anesthesia No treatment	26 25	61.5 20.0	0.003
5	$0.03 \times 10^{-8.5}$ (3 LD$_{50}$)	Delayed anesthesia No treatment	30 39	40.0 7.6	0.002
6	$0.03 \times 10^{-8.5}$ (3 LD$_{50}$)	Delayed anesthesia No treatment	31 24	16.1 4.2	0.161

* Anesthesia administered immediately or beginning 18 hours (intermediate) or 40 hours (delayed) after intracerebral inoculation of virus.

‡ Animals which died from trauma or anesthesia are not included in this and subsequent tables.

§ Survived after observation period of 10 days.

‖ In this and subsequent tables, values are obtained by reference to appropriate tables in Pearl, R., Medical Biometry and Statistics, Philadelphia, W. B. Saunders Co., 1930.

tion period; but of the 31 animals anesthetized during the incubation period (Experiment 1), 42 per cent survived, and of the 30 animals anesthetized following the onset of symptoms, 40 per cent survived. The effect of ether anesthesia on the course of the experimental infection became evident early in the experiment. By the 3rd day, 51.3 per cent of the controls had died while only 29.1 per cent of the animals receiving immediate and 26.7 per cent receiving delayed anesthesia had died. In both experiments, the peak in mortality among anesthetized animals occurred about 24 hours following that among control

animals. Thus, in addition to the increase in total survivors with ether anesthesia, there appeared to be a delaying effect upon the progress of the infection. To determine whether or not the data are statistically valid, 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 etherized groups (Table I). These data have been presented in a preliminary note (26).

Experiments 2 and 6.—Experiments similar to those just described were carried out about 3 months later using the same stock virus suspension. The virus inoculum was 0.03 ml. of a $10^{-8.5}$ dilution (3 LD_{50}). A single group of 24 unanesthetized animals served as controls for both experiments.

The results of these experiments are shown in Fig. 1 and Table I. The survival rate was much lower in both treated and control animals, and peaks in mortality occurred earlier than in Experiments 1 and 5. As might be expected with a larger inoculum of virus, anesthesia proved less effective whether administered immediately after inoculation of virus or when symptoms became evident. The differences observed in mortality rates were not as significant when analyzed statistically. Nevertheless, an obvious effect upon the mortality rate was noted. Following a 10 day observation period, only 4.2 per cent of the 24 control animals survived, while 20.8 per cent of the 24 animals receiving immediate anesthesia (Experiment 2) and 16.1 per cent of the 31 animals receiving delayed anesthesia (Experiment 6) survived. Again, the peak in mortality among the etherized animals occurred a day later than that of controls, and deaths among anesthetized animals occurred later in the observation period than did deaths among control animals.

It was evident from these data that anesthesia, by ether , modified the course and outcome of the experimental infection with the Western equine virus. These effects of ether anesthesia could be observed not only when the anesthetic was administered soon after the injection of the virus, but also when it was administered after the disease had progressed far enough to cause objective signs of encephalitis.

Since there was no appreciable difference in survival rate among animals receiving anesthesia immediately after inoculation of virus as compared with those receiving "delayed" anesthesia, in subsequent experiments animals were anesthetized beginning 18 hours after intracerebral inoculation of virus. This procedure is referred to as "intermediate" anesthesia.

Experiment 3.—In this experiment, each animal was inoculated intracerebrally with 0.03 ml. of a $10^{-8.2}$ dilution of the stock virus suspension (6 LD_{50}). The animals to be treated were then given three 4 hour periods of anesthesia beginning 18 hours after virus inoculation, with an hour interval between the first 2 courses. The third period of anesthesia was given the following day.

Experiment 4.—This experiment was carried out at a later date, using a slightly higher dilution of the same stock suspension. The virus inoculum consisted of 0.03 ml. of a $10^{-8.7}$ dilution (approximately 2 LD_{50}).

The results of Experiments 3 and 4 are also summarized in Fig. 1. It can be seen that in Experiment 3, only 4.3 per cent of the 23 control animals survived the 10 day observation period as compared with 36.0 per cent of the 25 anesthetized animals. Of the 25 control animals in Experiment 4, 20 per cent survived as compared with 61.5 per cent of the 26 animals which received "intermediate" anesthesia. Again, it seemed that with a smaller inoculum of virus, more significant results were obtained (Table I). No delay in the progress of the infection occurred as a result of "intermediate" ether anesthesia, although such anesthesia had a clearly significant effect on the final outcome in each of the 2 experiments.

The effectiveness of ether anesthesia is apparently not greatly dependent upon the time of initiation of treatment. However, from a comparison of Experiments 2 and 6 with Experiment 3, "intermediate" anesthesia would seem to be slightly more effective than either "immediate" or "delayed" against a similar amount of virus. Although the mortality rates in the control groups are identical, a greater percentage of these control animals died earlier in Experiments 2 and 6 than in Experiment 3. It is likely that the absence of apparent delay in the groups receiving "intermediate" anesthesia is due merely to chance and that such an effect would become apparent if more experiments were carried out.

Experiments with the Eastern Equine Virus.—Because of the similarity between this virus and that of Western equine encephalomyelitis, it was expected that similar results would be obtained from *in vivo* studies. Since "intermediate" anesthesia in the previous experiments seemed to be more effective than the other methods used, this was the procedure first employed with the Eastern equine virus.

Experiment 7.—The LD_{50} of the stock virus suspension used in this experiment was $10^{-9.8}$ and the inoculum used was 0.03 ml. of a $10^{-8.5}$ dilution (6 LD_{50}). Because of the somewhat shorter incubation period (approximately 36 hours), only two 4 hour periods of anesthesia were administered, the first beginning 14 hours following the virus inoculation. The animals were allowed to rest for 1 hour and then were given another "period" of anesthesia. The anesthetized animals, as well as the controls, were observed for a period of 10 days.

Experiment 8.—In a second experiment with this virus, using a similar inoculum, three 4 hour periods of anesthesia were administered, the first beginning immediately after virus inoculation and the second following a rest period of 1 hour. The third period of anesthesia was begun 24 hours after inoculation of the virus.

The results of these 2 experiments are summarized in Fig. 2. In Experiment 7, in which 2 periods of anesthesia were used, 25.7 per cent of the 35 anesthetized animals survived the observation period of 10 days, while 10 per cent of the 30

untreated controls survived. Not only was this difference in mortality rate doubtful statistically, but also there was no apparent delay in development of symptoms and death among the etherized animals. For this reason, three 4 hour periods of anesthesia were administered in Experiment 8. In this experiment, only 3.4 per cent of the 30 control animals were still living at the end of the observation period, while 26 per cent of the 27 anesthetized animals survived. As indicated in Table II, the significance of this difference was somewhat more in evidence than was that of the preceding experiment (Experiment 7). These 2 experiments indicate that anesthesia, by ether, was able to alter infection with the Eastern equine virus to a slight degree, but the effect was less marked than in the case of the Western virus.
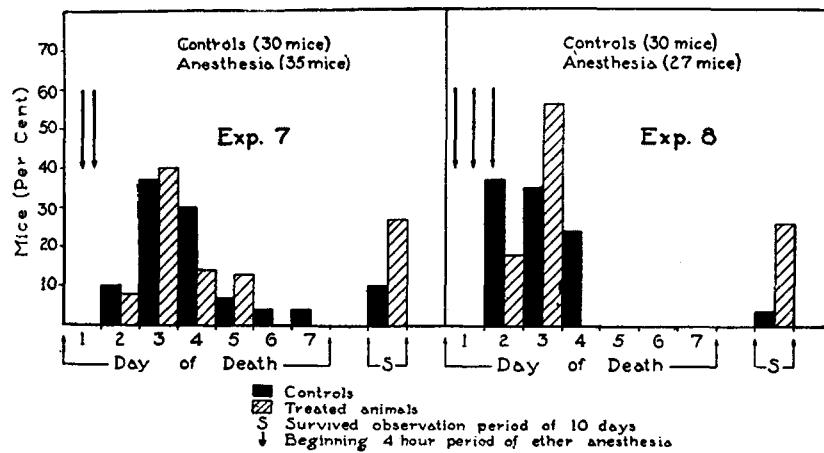

Fig. 2. Influence of ether anesthesia on course of disease produced in Swiss mice infected with equine encephalomyelitis virus (Eastern type).

Experiments with the St. Louis Encephalitis Virus.—It was expected that results similar to those with the equine viruses would be obtained since this agent is also destroyed by anesthetic ether *in vitro* (21). With this virus the incubation period is somewhat longer (approximately 100 hours) than that observed with the equine viruses, and for this reason the administration of ether was accomplished by short periods of anesthesia during the incubation period. No direct information could be found which would indicate the period of most rapid increase of virus.

Experiment 9.—The LD$_{50}$ of the stock suspension of virus used was $10^{-6.7}$. The inoculum used in the experiment was 0.03 ml. of a 10^{-5} dilution. Three 4 hour periods of ether anesthesia were given, the first beginning 18 hours following intracerebral inoculation of virus, with subsequent periods of anesthesia at 24 hour intervals on each of 2 successive days. Observations were made at least twice daily for 15 days, at the end of which time the experiment was

terminated. Unfortunately, an overwhelming amount of virus was used (approximately 50 LD$_{50}$), based on titrations for infectious potency of the stock virus suspension at the time the experiment was done.

As can be seen from the summary of this experiment presented in Table II, all of the 28 control animals died within the observation period, and only 1 of the 33 anesthetized animals survived. On the 5th day of the experiment (the 1st day on which deaths occurred), 63.6 per cent of the anesthetized animals

TABLE II

Effect of Ether Anesthesia on Outcome of Disease Produced in Swiss Mice Infected with the Viruses of Equine Encephalomyelitis (Eastern Type) and St. Louis Encephalitis

Virus	Experiment No.	Virus inoculum (injected intracerebrally)	Method of treatment	No. of mice	Mice surviving*	
					Per cent	p
Eastern equine	7	$0.03 \times 10^{-8.5}$ (6 LD$_{50}$)	Ether anesthesia‡ No treatment	35 30	25.7 10.0	0.109
	8	$0.03 \times 10^{-8.5}$ (6 LD$_{50}$)	Ether anesthesia§ No treatment	27 30	26.0 3.4	0.016
St. Louis encephalitis	9	0.03×10^{-5} (50 LD$_{50}$)	Ether anesthesia‖ No treatment	33 28	3.2 0	0.368
	10	$0.03 \times 10^{-6.3}$ (2.5 LD$_{50}$)	Ether anesthesia‖ No treatment	30 29	63.3 24.2	0.003

* Survived observation period of 10 days for Eastern equine encephalomyelitis and 15 days for St. Louis encephalitis.

‡ Two periods of anesthesia administered beginning 14 hours after injection of virus.

§ Three periods of anesthesia administered beginning immediately after injection of virus.

‖ A period of anesthesia administered on each of 3 successive days beginning 18 hours after inoculation of virus.

died as compared with 86.7 per cent of the controls; and at no time during the 4 days on which death occurred did the mortality rate of the etherized animals reach that of the controls.

Experiment 10.—This experiment was carried out at a later date using a higher dilution of the same stock virus suspension. The virus inoculum (0.03 ml. of $10^{-6.3}$) was injected intracerebrally, and consisted of that amount of virus (2.5 LD$_{50}$) which, on the basis of titrations for infectious potency, was estimated to produce death from encephalitis in approximately 80 per cent of the control animals. The animals received 1 period of anesthesia on each of 3 successive days beginning 18 hours after inoculation of virus.

The results of this experiment, summarized in Table II, show that 24.2 per cent of the 29 control animals survived the 15 day observation period while

63.3 per cent of the 30 anesthetized animals survived. Statistical analysis showed that this difference in survival rate was significant. The results of this experiment are somewhat similar to those with the virus of Western equine encephalomyelitis (Experiment 4). While there was no noticeable evidence of delay in the progress of the infection in either of these experiments, there was a significant difference in the final outcome of the disease in the anesthetized animals as compared with the unanesthetized controls. The results of Experiment 10 with the St. Louis encephalitis virus are presented graphically in Fig. 3.

Experiments with the Lansing Strain of Poliomyelitis Virus—This virus was selected for study because it represents a neurotropic virus not destroyed by
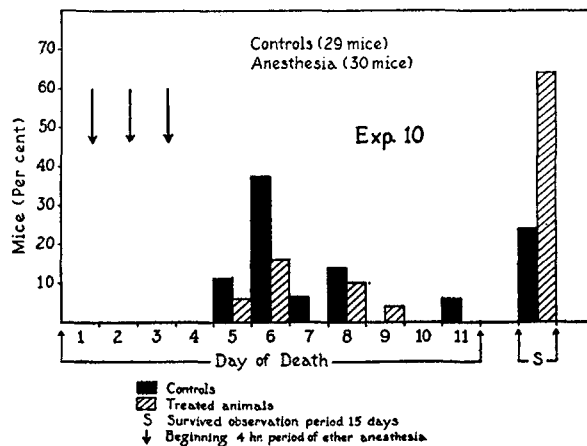

Fig. 3. Influence of ether anesthesia on course of disease produced in Swiss mice infected with St. Louis encephalitis virus.

ether *in vitro* (15). Resistance to ether, however, is not the only fundamental difference which exists between this agent and the viruses of equine encephalomyelitis and St. Louis encephalitis. In the first place, the incubation period in experimental poliomyelitis is much longer. For this reason, it is difficult to know when and in what cell or group of cells the virus is increasing most rapidly. Secondly, in poliomyelitis the cells principally affected are in the spinal cord (especially after 5 days following injection of virus (27)), while those principally affected by the encephalitis viruses are in the cortex and basal ganglia of the brain. Only occasionally do lesions produced by the encephalitis viruses extend as far as the cervical region of the cord. These differences are important because general anesthesia is known to affect first the cortical cells, extending irregularly in the brain and cord, while the anterior horn cells are affected by the anesthetic much later (28).

Experiment 11.—The virus inoculum in this experiment was injected intracerebrally, and consisted of 0.03 ml. of a 10^{-2} dilution of the stock suspension. Two 4 hour periods of ether anesthesia were administered beginning 16 hours after virus inoculation with an hour interval between the 2 periods. A third period was given on the next day.

Titration of this virus is less clear-cut than that of the other neurotropic viruses studied, a wide range of dilutions being capable of producing deaths in approximately 50 per cent of the animals. For this reason, no LD_{50} was determined. Animals were observed frequently during the observation period of 30 days for extent of paralysis and deaths from poliomyelitis.

TABLE III

Effect of Ether Anesthesia on the Course and Incidence of Disease in Swiss Mice Infected with Poliomyelitis Virus (Lansing Strain)*

Experiment no.		11		12	
No. of mice		38	35	31	35
Treatment		Ether anesthesia‡	None	Ether anesthesia§	None
	1–4	3 (7.9)‖	7 (20.0)	4 (12.9)	2 (5.7)
	5–7	10 (34.2)	7 (40.0)	1 (16.1)	5 (20.0)
	8–10	2 (39.4)	0 (40.0)	2 (22.6)	3 (28.6)
No. infected and dying from	11–13	2 (44.7)	2 (45.7)	1 (25.8)	7 (48.6)
poliomyelitis (days after	14–16	3 (52.6)	3 (54.3)	5 (41.9)	2 (54.3)
injection of virus)	17–19	1 (55.2)	2 (59.9)	2 (48.4)	0 (54.3)
	20–22	2 (60.5)	2 (65.7)	0 (48.4)	2 (60.0)
	23–25	5 (73.6)	1 (68.6)	2 (54.8)	1 (62.9)
	26–30	2 (78.9)	1 (71.4)	3 (64.5)	4 (74.3)
Survivors, *per cent*		21.1	28.6	35.5	25.7

* Virus inoculum—0.03 ml. of a 10^{-2} dilution of stock suspension injected intracerebrally (probably 3 LD_{50}).

‡ Three periods of anesthesia administered beginning 16 hours after injection of virus.

§ One period of anesthesia administered on each of 6 successive days beginning 18 hours after inoculation of virus.

‖ Figure in parentheses indicates cumulative per cent deaths.

The results of this experiment, as summarized in Table III, show that during the first 4 days only 7.9 per cent of the 38 anesthetized animals died, while 20.0 per cent of the 35 controls became paralyzed and died from poliomyelitis during this same period. However, when the experiment was terminated 30 days following virus inoculation, 78.9 per cent of the anesthetized animals had died and 71.4 per cent of the control animals had died. This slight difference in mortality rate is probably not significant.

Experiment 12.—Because of the extended incubation period, it seemed logical to administer anesthesia over longer periods of time. One 4 hour period of anesthesia was administered on

each of 6 successive days beginning 18 hours after inoculation of virus. The inoculum was 0.03 ml. of a 10^{-2} dilution as in Experiment 11.

The results of Experiment 12 are summarized in Table III. While it appears that more anesthetized animals succumbed during the first 4 days, 35.5 per cent of the 31 etherized animals survived the entire observation period as compared with 25.7 per cent of the 35 unetherized controls. In view of the results obtained in these 2 experiments (Experiments 11 and 12), the only valid conclusion appears to be that ether anesthesia had no effect upon the course of this experimental infection in mice.[1]

Experiments with the Rabies Virus.—This virus was chosen because of its resistance to the *in vitro* effect of ether (16) Although this virus affects cells of the higher central nervous system, like the virus of poliomyelitis it tends to become disseminated throughout the cord. Furthermore, peripheral nerves are involved. Hence, this virus as well as that of poliomyelitis differs from the encephalitis viruses in regard to its pathogenesis in the animal host and also in its *in vitro* resistance to ether.

Experiments 13 and 14.—The LD_{50} of the stock virus suspension used in these experiments was $10^{-4.7}$, and the inoculum used was 0.03 ml. of a $10^{-3.9}$ dilution (6 LD_{50}). A single group of 28 unanesthetized animals served as controls for both of these experiments. In Experiment 13, animals received three 4 hour periods of ether administered beginning 13 hours after intracerebral inoculation of the virus. Two 4 hour periods were administered on 1 day with an hour intervening, and the third period of anesthesia was administered the following day. In Experiment 14, four 4 hour periods of anesthesia were administered at 24 hour intervals beginning 60 hours after injection of the virus. All animals were observed for symptoms during an observation period of 18 days.

The results of these 2 experiments (Experiments 13 and 14) are given in Table IV. It can be seen that when the anesthetic was administered beginning 13 hours after virus injection (Experiment 13), the mortality rate among the controls during the first 4 days on which death occurred (the 8th through the 11th day following virus inoculation) was consistently higher than that among the anesthetized animals; and by the 11th day 53.6 per cent of the 28 control animals had died as compared with 20 per cent of the 30 etherized animals. Nevertheless, after an observation period of 18 days, 86.6 per cent of the 30 anesthetized animals had died while only 71.5 per cent of the 28 control animals had died. When anesthesia was delayed (Experiment 14), similar results were obtained. The mortality rate during the 18 day observation period was greater among the anesthetized animals than among the untreated controls (81.5 per cent of the 27 etherized as compared with 71.5 per cent of the 28

[1] During the course of these studies, a clinical study by Gamboa (29) came to our attention. This worker noted improvement in 6 of 8 clinical cases of poliomyelitis following treatment with general anesthesia by ether and/or chloroform.

control animals). While the final outcome of these 2 experiments indicates that ether failed to influence the course and incidence of disease in mice infected with the rabies virus, a statistical analysis indicates that the difference in mortality rates between the controls and the etherized animals early in the observation period in Experiment 13 was significant.

TABLE IV

*Effect of Ether Anesthesia on the Course and Incidence of Disease in Swiss Mice Infected with Rabies Virus**

Experiment No.		13	14	Controls
No. of mice		30	27	28
Treatment		Early anesthesia‡	Delayed anesthesia§	None
	8	0 (0)‖	1 (3.7)	1 (3.6)
	9	2 (6.7)	2 (11.1)	2 (10.7)
	10	0 (6.7)	7 (37.0)	5 (28.6)
	11	4 (20.0)	4 (51.9)	7 (53.6)
No. infected and dying from	12	6 (40.0)	1 (55.5)	2 (60.8)
rabies (days after inoculation of	13	5 (56.7)	3 (66.7)	1 (64.3)
virus)	14	2 (63.3)	1 (70.4)	0 (64.3)
	15	4 (76.6)	1 (74.1)	1 (67.9)
	16	1 (80.0)	0 (74.1)	0 (67.9)
	17	2 (86.6)	2 (81.5)	1 (71.5)
	18	0 (86.6)	0 (81.5)	0 (71.5)
Survivors, *per cent*		13.4	18.5	28.5

* Virus inoculum −0.03 × $10^{-3.9}$ given intracerebrally (6 LD_{50}).

‡ Three periods of anesthesia administered beginning 13 hours after virus injection.

§ One period of anesthesia administered on each of 4 successive days beginning 60 hours after virus injection.

‖ Figure in parentheses indicates cumulative per cent deaths.

DISCUSSION

Earlier reports have indicated that certain neurotropic viruses are destroyed by anesthetic ether *in vitro*. Among these viruses are the etiologic agents of Western equine encephalomyelitis and St. Louis encephalitis. Our own observations have shown that the virus of Eastern equine encephalomyelitis is likewise destroyed by ether (30). On the other hand, the viruses of poliomyelitis and rabies are not readily destroyed even by large amounts of ether *in vitro*. The experiments reported in this paper indicate that the *in vivo* effect of ether parallels this *in vitro* effect; that is, the viruses which have been reported to be destroyed *in vitro* are also inhibited *in vivo*, while those which resist the action of ether *in vitro* produce disease in anesthetized animals as well as in untreated

controls. Although it appears that the direct effect of this anesthetic upon the virus may be the underlying mechanism in the *in vivo* as well as in the *in vitro* experiments, other possible explanations for this apparent parallelism should be considered.

The viruses of equine encephalomyelitis and St. Louis encephalitis tend to remain localized in the brain, while those of poliomyelitis and rabies become disseminated throughout the brain and cord early in the infection. Similarly, the effects of anesthesia occur first in the cells of the higher central nervous system and proceed irregularly in the spinal cord, finally reaching the anterior horn cells, so that those cells which become infected with viruses readily destroyed by ether are also those which become anesthetized first. Any significance attached to this coincidence must be considered an effect of anesthesia rather than one of ether *per se*, since all cells of the central nervous system probably are exposed to an equal concentration of ether at a given time; it is rather the manner in which the cells react to the ether that is different at the various levels of the central nervous system.

Recorded data concerning concentrations of ether in blood and tissues of anesthetized animals cast further doubt on the importance of the direct virucidal activity of ether. Van Mechelen (31), for example, found that the concentration of anesthetic ether during induction is higher in arterial blood than in venous blood. Also, it is higher in venous blood than in the tissues. These relative concentrations hold true with respect to nervous tissue, despite its higher lipid content (32). It has further been determined (32, 33) that the concentration of ether in venous blood never exceeds 187 mg. per cent. It seems unlikely, then, that such small concentrations of ether could destroy virus directly, although it is conceivable that both virus and ether have a predilection for similar sites within the host cell, in which case the virus would be subjected to a concentration of ether exceeding the total concentration in nervous tissue. *In vitro* studies to determine quantitatively the virucidal effect of ether on these viruses will be reported subsequently (30).

Other observations which further minimize the probability of a direct effect of ether upon the susceptible viruses are still under investigation. Anesthetics other than ether have been shown to influence the course and outcome of experimental infections with the Western equine virus. Also, some evidence is available to indicate that the concentration of this virus in the brains of anesthetized animals decreases for several hours following a period of anesthesia. Ether, however, is known to be quantitatively eliminated from the body by way of the lung during the first few minutes following cessation of ether administration.

Possible mechanisms which have not been investigated may concern the biochemical effect of ether upon cells which are infected as well as non-infected cells outside the central nervous system. Anesthetized cells are not capable of

oxidizing certain substrates such as glucose, lactate, and pyruvate (34). This decreased activity on the part of the cells is hardly favorable to increase of the virus, which has an obligate association with the cells. While the most noticeable effect of ether anesthesia is the altered activity of the cells of the central nervous system, other tissues are known to be affected.. For example, the development of hyperglycemia and acidosis upon induction with anesthetic ether and the appearance of ketosis upon prolonged anesthesia are presumably due to alteration in liver function.. There is considerable evidence correlating the presence of intermediary products of carbohydrate metabolism and interference with the establishment of a virus in its host cell. Our own unpublished data indicate that certain intermediary metabolic products are capable of direct virucidal activity.

It is not the purpose of this paper to provide experimental data to elucidate the mechanism for the observed inhibitory effect of ether upon the course and outcome of certain experimental neurotropic virus infections. The results of experiments which indicate the extent of this effect have been presented, but their interpretation must await further investigation.

SUMMARY

Anesthesia with diethyl ether significantly alters the course and outcome of experimental infections with the equine encephalomyelitis virus (Eastern or Western type) or with the St. Louis encephalitis virus. No comparable effect is observed in experimental infections produced with rabies or poliomyelitis (Lansing) viruses.

The neurotropic virus infections altered by ether anesthesia are those caused by viruses which are destroyed *in vitro* by this anesthetic, and those infections not affected by ether anesthesia are caused by viruses which apparently are not destroyed by ether *in vitro*. Another striking difference between these two groups of viruses is their pathogenesis in the animal host; those which are inhibited *in vivo* by ether anesthesia tend to infect cells of the cortex, basal ganglia, and only occasionally the cervical region of the cord. On the other hand, those which are not inhibited *in vivo* by ether anesthesia tend to involve cells of the lower central nervous system and in the case of rabies, peripheral nerves. This difference is of considerable importance in view of the fact that anesthetics affect cells of the lower central nervous system only in very high concentrations.

It is obvious from the complexity of the problem that no clear-cut statement can be made at this point as to the mechanism of the observed effect of ether anesthesia in reducing the mortality rate in certain of the experimental neurotropic virus infections. Important possibilities include a direct specific effect of diethyl ether upon the virus and a less direct effect of the anesthetic upon the virus through its alteration of the metabolism of the host cell.

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