INFLUENCE OF ANESTHESIA ON EXPERIMENTAL NEUROTROPIC VIRUS INFECTIONS

II. In Vitro Studies with the Viruses of Western and Eastern Equine Encephalomyelitis, St. Louis Encephalitis, Poliomyelitis (Lansing), and Rabies*‡

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The results of previous studies (1, 2) have shown that anesthesia with ether significantly alters the course and outcome of experimental infections with the Western or Eastern equine viruses, or with the St. Louis encephalitis virus. No comparable effect was 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, according to previous reports, appear to be destroyed *in vitro* by this anesthetic (Western and Eastern equine encephalomyelitis (3, 4) and St. Louis encephalitis (4, 5). Similarly, those experimental infections not affected by ether anesthesia are caused by viruses which apparently are not destroyed by ether *in vitro* (poliomyelitis (6), rabies (7)).

The present experiments were undertaken for the purpose of confirming previous observations concerning the *in vitro* activity of diethyl ether upon certain neurotropic viruses. It is also the purpose of this paper to present data which may contribute to an understanding of the mechanism underlying the observed effect of ether anesthesia in reducing the mortality rate in certain experimental neurotropic virus infections. Important considerations include a direct effect of the anesthetic upon the virus and a less direct effect through its alteration of the metabolism of the host cell. Several observations regarding the concentration of ether in blood and tissues of anesthetized animals would tend to minimize the probability of a direct effect of ether upon the susceptible viruses. Haggard (8) and Robbins (9), for example, have reported that the concentration of ether in venous blood never exceeds 187 mg. per cent and that the amount of ether in central nervous system tissues is consistently less than

that in venous blood. Such small concentrations of ether could hardly destroy virus directly. It is conceivable, however, that virus and ether have 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. For this reason, quantitative studies to determine the virucidal effect of ether *in vitro* on certain neurotropic viruses were undertaken and will be presented in this report.

Materials and Methods

Viruses.—The viruses of Western and Eastern equine encephalomyelitis, St. Louis encephalitis, poliomyelitis (Lansing), and rabies were used in these experiments. These are the same strains used in previously published studies (2). The stock suspensions of the equine encephalomyelitis (Western and Eastern), and St. Louis encephalitis viruses were prepared as directed previously (2). Fresh infected tissue was used in experiments to test the *in vitro* effect of ether upon the viruses of rabies and poliomyelitis (Lansing). In the case of the latter virus, both brains and spinal cords were removed from infected mice becoming paralyzed less than 5 days following injection, and spinal cords alone from animals becoming paralyzed more than 5 days following injection. The titer of each virus 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 (10).

Animals.—Three to 4 week old Swiss mice of the Webster strain, obtained from a single breeder, were used throughout these studies.

Procedure.—Appropriate serial broth dilutions of each virus were mixed with varying amounts of diethyl ether (Mallinckrodt anesthetic) to give total volumes of 2.0 ml. (or in some experiments, 3.0 ml.) per tube. The dilution of the virus and the per cent ether indicated in each experiment represent the final concentration of each in the total volume. A control series of virus dilutions without ether was included for determining the LD$_{50}$ under the conditions of the experiment. The tubes containing the virus dilutions were tightly stoppered with short sterile rubber stoppers immediately after adding the ether. These mixtures together with the controls were then incubated in a mechanical shaking machine placed in a small incubator box adjusted to 37°C. After incubation for 1 to 2 hours, the rubber stoppers were replaced by cotton plugs, a volume of broth equal to that of the ether added to each tube, and the ether removed by vacuum distillation which usually required 10 to 15 minutes. The final virus dilution in each tube was therefore the same as before shaking, and no ether was present at the time of animal inoculation. The intracerebral inoculum for each animal was 0.03 ml. Ether-treated suspensions were injected first and controls last in order to minimize rather than emphasize any virucidal activity of ether. In each experiment, ether was actually in contact with the virus for approximately 2 hours.

The virucidal capacity of ether, as indicated in the tabulations, was determined by subtracting the log of the LD$_{50}$ of virus in the control from the log of the LD$_{50}$ of virus in the presence of the different concentrations of ether. This difference is actually the log of the amount of virus inactivated. This method of determining the virucidal capacity of ether is similar to that employed by Morgan (11) in estimating the neutralizing capacity of antiserum.

EXPERIMENTAL

Experiment with the Western Equine Virus.—It has been reported that the virus of Western equine encephalomyelitis is readily destroyed by bactericidal

quantities of ether and that this substance is therefore not an appropriate one for removing bacterial contaminants from infected tissues (3, 4). This experiment was undertaken not only for the purpose of attempting confirmation of these earlier reports but also to study the effect of smaller quantities of ether, some of which are within the range of the total per cent ether found in central nervous system tissues of anesthetized animals.

TABLE I

Effect of Ether upon the Virus of Equine Encephalomyelitis (Western Type) after Mixing for 2 Hours at 37°C.

Dilution of virus	Control	Final per cent ether by volume*					
		0.5 per cent	1.0 per cent	3.0 per cent	5.0 per cent	10.0 per cent	20.0 per cent
$10^{-2.0}$						0/3‡	0/4
$10^{-3.0}$						0/4	0/4
$10^{-4.0}$						1/4	0/4
$10^{-5.0}$						0/4	0/4
$10^{-6.0}$	4/4	4/4	4/4	4/4	4/4	2/3	0/3
$10^{-7.0}$	4/4	3/4	4/4	4/4	1/3	0/3	0/3
$10^{-8.0}$	4/4	4/4	3/3	4/4	3/4	0/4	0/4
$10^{-9.0}$	2/5		2/5	2/5	0/5	0/5	0/4
LD$_{50}$ titer of virus	$10^{-8.8}$	$<10^{-8.0}$	$10^{-8.7}$	$10^{-8.8}$	$10^{-8.0}$	$10^{-2.0}$	$>10^{-2.0}$
Virucidal capacity of ether§		<0.8	0.1	0	0.8	6.8	>6.8

In this and subsequent tables:—

* Virus-ether mixtures shaken slowly at 37°C. for 2 hours before intracerebral injection into mice (inoculum—0.03 ml.).

‡ Number of mice which died from encephalitis/number inoculated (not including those which died from non-specific causes).

§ Value obtained by subtracting the log of the LD$_{50}$ of virus in the control from the log of the LD$_{50}$ of virus in the presence of the concentration of ether indicated.

Diethyl ether was mixed with virus to give final concentrations of 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0 per cent ether, and final tenfold dilutions of virus ranging from $10^{-2.0}$ to $10^{-9.0}$. A final total volume of 2.0 ml. was contained in each tube. The contents of these tubes and of the controls were shaken slowly in a 37°C. incubator for a period of slightly less than 2 hours, as described above. After removal of ether from each virus-ether mixture, 0.03 ml. of the remaining virus suspension was inoculated into each of four or five mice. The control virus suspensions were then inoculated into mice in a similar manner. All animals were observed frequently during the following 10 days for symptoms of encephalitis and death.

As previously observed by Howitt (12) the Western equine virus remains relatively stable at 37°C. The titer of the virus during shaking at 37°C.

for 2 hours dropped less than 0.2 of a log unit from the original LD_{50} of 0.03 $\times 10^{-9.0}$. It is evident from the results summarized in Table I that ether in concentrations of less than 5.0 per cent had little, if any, virucidal activity; on the other hand, 10.0 and 20.0 per cent ether were markedly virucidal. The virucidal capacity of 10.0 per cent ether was 6.8 long units and that of 20.0 per cent ether was even greater than 6.8. Five per cent ether, however, caused a reduction of only 0.8 log unit in the LD_{50} of this virus. It is significant that the dilution containing the highest concentration of virus (10^{-2}) was no longer infective for mice after contact with 10.0 or 20.0 per cent ether.

TABLE II

Effect of Ether upon the Virus of Equine Encephalomyelitis (Eastern Type) after Mixing for 2 Hours at 37°C.

Dilution of virus	Control	Final per cent ether by volume*						
		0.1 per cent	0.5 per cent	1.0 per cent	3.0 per cent	5.0 per cent	10.0 per cent	20.0 per cent
$10^{-3.0}$							0/4‡	0/4
$10^{-4.0}$							0/4	0/4
$10^{-5.0}$	4/4	4/4	4/4	4/4	3/4	1/4	0/4	0/4
$10^{-6.0}$	4/4	4/4	4/4	4/4	2/4	1/4	0/4	0/4
$10^{-7.0}$	4/4	3/4	4/4	3/4	2/4	1/4	0/4	0/4
$10^{-8.0}$	2/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4
$10^{-9.0}$	0/3	1/4	0/4	0/4	0/4	0/4	0/4	0/4
LD_{50} titer of virus.	$10^{-8.0}$	$10^{-8.0}$	$10^{-7.5}$	$10^{-7.3}$	$10^{-6.3}$	$10^{-5.0}$	$>10^{-2.0}$	$>10^{-2.0}$
Virucidal capacity of ether§		0	0.5	0.7	1.7	3.0	>5.0	>5.0

Experiment with the Eastern Equine Virus.—Although no information was available concerning the resistance of this virus to ether or its stability at 37°C., the similarity of this agent to the Western equine virus suggested that it would behave similarly under the conditions of the experiment. It was noted, however, that the virus titer fell approximately 1 to 2 log units during the period of shaking at 37°C. for 2 hours.

Dilutions of ether to give final concentrations of 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0 per cent ether were mixed with virus suspensions to give final tenfold dilutions of virus ranging from $10^{-3.0}$ to $10^{-9.0}$. The final total volume in each tube was 2.0 ml. The remaining procedure (involving shaking of the mixture, removal of ether, and inoculation of animals) was the same as described previously. All animals were observed for a period of 10 days for central nervous system symptoms and death from encephalitis. The results of this experiment are summarized in Table II.

Ether in a concentration of 3.0 per cent had some virucidal activity, causing a reduction of less than 2 log units in the LD_{50} of this virus. On the other hand,

ether in a concentration of 5.0 per cent or greater was markedly virucidal, causing a reduction of 3 log units or more in the LD_{50}. The virucidal capacity of the 10.0 and of the 20.0 per cent ether was more than 5 log units. The dilution containing the highest concentration of virus (10^{-3}) was no longer infective for mice after contact with these concentrations of ether. Consequently, the limit of the virucidal capacities of these concentrations was not determined in this experiment. However, in a previous experiment it was found that a 10.0 per cent suspension of virus in 20.0 per cent ether remained fully infective for mice (all four mice inoculated with such a mixture succumbed to the infection) following 2 hours' mixing at 37°C. On this occasion, however, the LD_{50} of the virus was $0.03 \times 10^{-8.5}$. This would suggest that there is a limit to the virucidal activity of this anesthetic and that is is considerably less effective in the presence of larger amounts of nervous tissue.

Experiment with the St. Louis Encephalitis Virus.—Cook and Hudson (13) investigated the stability of this virus under a variety of conditions and found that in a diluent well buffered near pH neutrality, a 10^{-6} dilution of infected tissue remained active for 2 hours at 37°C. Under the conditions of the experiment to be described, the virus of St. Louis encephalitis was extremely stable as indicated by the identical LD_{50} $(0.03 \times 10^{-6.7})$ determinations before and after 2 hours' shaking at 37°C. As stated previously, the destruction of the virus by bactericidal concentrations of diethyl ether has been observed by numerous investigators in the course of attempts to isolate the virus from bacterially contaminated tissues (4, 5).

In the experiment with the St. Louis encephalitis virus, diethyl ether was mixed with virus to give final concentrations of 0.5, 1.0, 3.0, 5.0, 10.0, and 20.0 per cent ether, and final tenfold dilutions of virus ranging from $10^{-1.0}$ to $10^{-7.0}$. A final total volume of 2.0 ml. was contained in each tube. The remaining procedure was the same as described for the previous experiments. Four mice were injected with each virus dilution. The animals were observed for a period of 15 days for symptoms of encephalitis and death. The results are presented in Table III.

From this experiment it appears that there is a limit to the amount of St. Louis virus which can be destroyed even by 20.0 per cent of ether. This may be due to the presence of large amounts of tissue in the 1.0 and 10.0 per cent virus suspensions. Ether in a concentration of 3.0 per cent had some virucidal activity, causing a reduction of 3.1 log units in the LD_{50} of this virus. A similar effect was observed with the 5.0 per cent concentration of ether. The 10.0 and 20.0 per cent ether concentrations were markedly virucidal, each reducing the LD_{50} titer of the virus by 5.2 log units.

Experiment with the Poliomyelitis (Lansing) Virus.—The different strains of the virus of poliomyelitis appear to be markedly stable under a wide variety of conditions. Schultz and Robinson (14), testing the poliocidal activity of

certain chemicals incubated under conditions similar to those used in the present experiment, noted that controls always retain their infectivity. Also, Clark and his associates (15) reported infectivity of poliomyelitis virus after 4 months in saturated solution of sodium chloride at ice box temperature. In addition, the virus appears to retain its activity over a wide range of pH, being especially resistant to acid environment.

The resistance of poliomyelitis viruses to ether is well established, the bactericidal activity of this anesthetic having been used in the isolation of this virus from nasal washings (6) and from stools and sewage (16). In the course of isolations of virus from stools (17), it was suggested that the virus of poliomyelitis must be somewhat unique in its resistance to ether, since no other

TABLE III

Effect of Ether upon the Virus of St. Louis Encephalitis after Mixing for 2 Hours at 37°C.

Dilution of virus	Control	Final per cent ether by volume*					
		0.5 per cent	1.0 per cent	3.0 per cent	5.0 per cent	10.0 per cent	20.0 per cent
$10^{-1.0}$				4/4‡	3/3	2/4	2/4
$10^{-2.0}$				4/4	4/4	2/4	2/4
$10^{-3.0}$	4/4			4/4	2/4	0/4	0/4
$10^{-4.0}$	4/4	3/4	2/4	0/4	0/4	0/4	0/4
$10^{-5.0}$	4/4	4/4	3/4	1/4	2/4	0/4	
$10^{-6.0}$	4/4	4/4	4/4	0/4	1/4		
$10^{-7.0}$	1/4	2/4	0/4				
LD$_{50}$ titer of virus......	$10^{-6.7}$	$10^{-6.8}$	$10^{-6.1}$	$10^{-3.6}$	$10^{-3.6}$	$10^{-1.5}$	$10^{-1.5}$
Virucidal capacity of ether§..............		0	0.6	3.1	3.1	5.2	5.2

viruses were ever recovered by intracerebral inoculations of monkeys with ether-treated material.

The Lansing strain of poliomyelitis virus was subjected to *in vitro* activity of ether in the same manner as other viruses in this series.

Because of the wide range of dilutions of this virus which result in death of approximately 50 per cent of the animals inoculated, the LD$_{50}$ is not a significant determination. In previous experiments in which animals were inoculated intracerebrally with 0.03 ml. of a $10^{-2.0}$ dilution of virus, the mortality rate varied from 60 to 75 per cent. This dilution was chosen for study of the *in vitro* resistance to ether. Ether was mixed with virus to give a final concentration of 95 per cent ether and a $10^{-2.0}$ dilution of virus. In the control tube, plain broth was used in place of ether. Each tube contained a total volume of 3.0 ml. The procedure used in shaking the mixtures, removing the ether, and inoculating the animals was the same as described previously. The animals were observed frequently during the observation period of 30 days for extent of paralysis and death from poliomyelitis.

The virus proved to be resistant not only to the effect of 2 hours' shaking at 37°C. but also to the effect of ether. While twenty-six of thirty animals succumbed to intracerebral inoculation of virus which had been mixed with ether, twenty-four of the thirty animals injected with the control suspension of virus alone succumbed to the infection.

Experiment with the Rabies Virus.—The virus of rabies, like that of poliomyelitis, is fairly resistant to adverse conditions but to a lesser extent. No data could be found concerning the persistence of virus activity at room temperature or higher. In addition, most of the work concerning the *in vitro* effect of ether upon this virus has been done with intact infected tissue or with very heavy suspensions of such tissue. Prolonged contact with ether will destroy the infectivity of any rabies virus suspension, and such treatment is

TABLE IV

Effect of Ether upon the Virus of Rabies after Mixing for 2 Hours at 37°C.

Dilution of virus	Control	Final per cent ether by volume*			
		5.0 per cent	10.0 per cent	20.0 per cent	30.0 per cent
$10^{-1.0}$			5/5‡	2/2	3/3
$10^{-2.0}$	5/5	4/4	5/5	5/5	3/4
$10^{-3.0}$	5/5	5/5	5/5	5/5	4/4
$10^{-4.0}$	5/5	4/5	1/5	2/5	1/5
$10^{-5.0}$	1/5	1/5			
LD_{50} titer of virus...	$10^{-4.5}$	$10^{-4.5}$	$10^{-3.5}$	$10^{-3.5}$	$10^{-3.5}$
Virucidal capacity of ether§..........		0.1	1.0	0.8	1.1

the basis for the Alivisatos (18) and Hempt (19) vaccines. Remlinger and Bailly (20) found variations in the resistance of different strains of virus to ether. *Virus fixé* is usually more susceptible than street virus to *in vitro* destruction by this agent.

The bactericidal activity of anesthetic ether has found practical application in recovery of rabies virus from brains of infected animals since longer contact with ether is required for virucidal than for bactericidal activity (7). Following contact of 20 per cent infected tissue suspension with 30 per cent ether for a period of 18 hours at 4°C., almost no decrease in virus activity was observed.

In the present experiment, ether was mixed with virus to give final concentrations of 5.0, 10.0, 20.0, and 30.0 per cent ether and final tenfold dilutions of virus ranging from $10^{-1.0}$ to $10^{-5.0}$. Each tube contained a total volume of 3.0 ml. The remaining procedure was the same as described in previous experiments. For each dilution of virus, five mice were used.

All animals were observed for symptoms and death over a period of 18 days, and the results of the experiment are presented in Table IV.

It is evident from a summary of this experiment that ether is considerably less virucidal for the rabies virus than for the virus of Western or Eastern equine encephalomyelitis or St. Louis encephalitis. The virucidal capacity of 30.0 per cent ether for the rabies virus was only 1.1 as compared with 6.8, the virucidal capacity of 10.0 per cent ether for the Western equine virus. The reduction in the titer of the rabies virus by 0.8 log unit in the presence of 20.0 per cent ether could hardly be considered significant.

DISCUSSION

It has already been reported that anesthesia with ether may significantly alter 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 was observed in experimental infections produced with rabies or poliomyelitis (Lansing) viruses. The infections altered by ether anesthesia are those caused by viruses which, according to previous reports, appear to be destroyed *in vitro* by this anesthetic. Similarly, those infections not affected by ether anesthesia are caused by viruses which apparently are not destroyed by ether *in vitro*. These observations alone suggest that direct virucidal activity of ether might be the basis for the *in vivo* effect. However, several other observations regarding the concentration of ether in blood and tissues of anesthetized animals would tend to minimize the probability of a direct effect of ether upon the susceptible viruses. *In vitro* studies to determine quantitatively the direct virucidal effect of ether on these viruses were undertaken, and the results are presented in this report.

It was found that concentrations of ether within the range found in central nervous system tissues of anesthetized animals possess no *in vitro* virucidal activity. While no more than 0.2 per cent ether in brain tissue is compatible with life (8, 9), no less than 3.0 per cent ether was significantly virucidal in this series of experiments. Of the viruses studied, only those of Eastern equine encephalomyelitis and St. Louis encephalitis were markedly affected by 3.0 per cent ether. The LD_{50} of the Eastern equine virus was reduced 1.7 log units in the presence of this concentration of ether after contact for 2 hours at 37°C.; that of St. Louis encephalitis was reduced 3.1 log units. Five per cent ether caused a reduction of only 0.8 log unit in the LD_{50} of the virus of Western equine encephalomyelitis, while the titer of the virus of Eastern equine encephalomyelitis was reduced 3.0 log units and that of St. Louis encephalitis, 3.1 log units. The slight reduction (0.1 log unit) in the LD_{50} of the rabies virus in the presence of 5.0 per cent ether could hardly be considered significant. The virucidal effect of 10.0 per cent and 20.0 per cent ether on these viruses

is considerably more marked than that of lower concentrations. Titrations of virus in the presence of 10.0 per cent ether showed a reduction of 6.8 log units in the LD_{50} of the Western equine virus and 5.2 log units in the case of the virus of St. Louis encephalitis. The limit of the virucidal activity of 10.0 per cent ether was not reached in the case of Eastern equine virus but the reduction in the LD_{50} titer was more than 5.0 log units. The decrease in the titer of the rabies virus (only 1 log unit) after 2 hours' incubation at 37°C. in the presence of 10.0 per cent ether is not comparable to the reductions noted in the more susceptible viruses.

The virucidal activity of 20.0 per cent ether was, in general, the same as that of 10.0 per cent ether. The titer of the Western equine virus could not be determined in the presence of 20.0 per cent ether since no active virus remained in even the lowest dilution. The reduction in the LD_{50} of the rabies virus in the presence of 20.0 per cent ether was less than that observed when 10.0 per cent ether was used; the slight difference is probably within the range of experimental error.

Since the rabies virus had been reported relatively resistant to the virucidal activity of diethyl ether, a determination of its titer was made in the presence of 30.0 per cent ether. The reduction in the titer of the virus was the same as that observed with 10.0 per cent or 20.0 per cent ether. This observation places the rabies virus, along with the mouse-adapted Lansing strain of poliomyelitis virus, in a group of neurotropic viruses not readily destroyed by diethyl ether *in vitro*. The Lansing poliomyelitis virus exhibited no loss of activity following 2 hours' incubation at 37°C. in as much as 95 per cent ether. It is probable that other strains of rabies virus would react differently in the presence of anesthetic ether. A variability in the time of exposure to ether required for inactivation of this virus was noted during preparation of virus for vaccines (21, 22). Furthermore, the resistance of a given strain to the virucidal activity of ether may vary on animal passage (21). In view of these variations, correlated *in vivo* and *in vitro* studies of several strains of rabies virus varying in susceptibility to ether are contemplated.

While the parallelism between the *in vivo* and *in vitro* effect of anesthetic ether upon these neurotropic viruses suggests that direct virucidal activity of ether might be the mechanism of the *in vivo* inhibition, these experiments show rather conclusively that the over-all concentration of ether present in tissues of anesthetized animals is totally ineffective *in vitro* against even the most susceptible viruses. There remains the possibility that the 0.2 per cent ether in brain tissue is not evenly distributed but concentrated at intracellular sites in the immediate vicinity of the virus. While such a possibility would necessitate the concentration of virus at the same site, the presence of large quantities of virus does not appear to limit the virucidal activity of effective concentrations of ether. The possibility of a direct virucidal effect on the

part of ether is rendered unlikely by the additional observation that similar *in vivo* results have been observed with a variety of general anesthetics including some inorganic compounds (23). It seems improbable that such a wide variety of chemical compounds would have direct virucidal activity *in vitro*.

The evidence in this paper does not warrant a definite statement to the effect that direct virucidal activity of ether is not the basis for the *in vivo* inhibition of certain experimental neurotropic virus infections by ether anesthesia. Quantitative aspects of the virucidal activity do, however, render such a mechanism extremely unlikely.

SUMMARY

1. Experimental neurotropic virus infections previously shown to be altered by ether anesthesia are caused by viruses destroyed *in vitro* by anesthetic ether; this group includes the viruses of Eastern equine encephalomyelitis, Western equine encephalomyelitis, and St. Louis encephalitis.

2. Experimental neurotropic virus infections which were not altered by ether anesthesia are caused by viruses which are refractory to the *in vitro* virucidal activity of even large amounts of anesthetic ether; this group includes the viruses of poliomyelitis (Lansing) and rabies.

3. Quantitative studies of the *in vitro* virucidal activity of ether indicate that concentrations of this anesthetic within the range found in central nervous system tissues of anesthetized animals possess no virucidal activity.

4. The lowest concentration of ether possessing significant virucidal capacity is more than fifteen times the maximum concentration of the anesthetic tolerated by the experimental animal.

5. Concentrations of ether 50 to 100 times the maximum amount tolerated by the anesthetized animal are capable of destroying large amounts of susceptible viruses, the average lethal dose (LD_{50}) being reduced more than 5 log units.

6. On the basis of the studies presented in this report, it cannot be concluded that direct virucidal activity of ether is not the underlying mechanism of the inhibition by anesthesia of certain experimental neurotropic virus infections. Indirect inhibition of the virus by the anesthetic through an alteration in the metabolism of either the host cell or the host animal as a whole appears at this point to be a more likely possibility.

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