# VIRUS PROLIFERATION IN HYPOXIC MICE AND CHICK EMBRYOS*

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Various approaches have been explored in an attempt to elucidate the host-virus relationship. Stimulation of protein anabolism by the administration of pituitary growth hormone or testosterone results in an increase in demonstrable influenza virus in mouse lung (1). Virus growth in the same host can be diminished by the administration of small amounts of ACTH or cortisone or by castration, procedures known to enhance catabolic processes (2). Zinsser and Schoenback (3), in studies with Western equine encephalitis virus in tissue culture, demonstrated that oxygen lack may affect virus proliferation. Magill and Francis had previously demonstrated the inability of influenza virus to proliferate in tissue cultures maintained under anaerobic conditions (4). Recently Berry and his coworkers (5) have confirmed the finding that hypoxic mice become more resistant to influenza virus infection. Other physiological factors which are undoubtedly involved in this complex relationship have been discussed in various recent reviews (6, 7). If the growing virus uses the host cell oxidative mechanism as its source of energy, any derangement of this mechanism should impair virus proliferation. A preliminary report indicated this possibility (8). Continuing these studies, the present paper reports the effect of hypoxia on (*a*) influenza virus proliferation in the mouse and developing chick embryo, (*b*) mouse encephalomyelitis, Theiler's GD VII virus in the mouse, and (*c*) the combined infection of both viruses in the mouse.

## *Materials and Methods*

*Mice.*—3- to 4-week-old mice were employed. Although no attempt was made to differentiate the sexes, male mice were used in the majority of cases.

*Chick Embryos.*—11- to 12-day-old embryos were employed for the virus studies and titrations. All eggs were obtained from the same dealer and incubated in the laboratory until the desired age.

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*Viruses.*—The PR8 strain of influenza and the GD VII strain (Theiler's) of mouse encephalomyelitis were employed for these studies. The PR8 strain has been maintained in this laboratory for a number of years. Two lines were employed: a mouse-adapted strain that had been passaged intranasally in this laboratory 68 times ($LD_{50}$ approximately $10^{-6.5}$) and a chick embryo strain that has been maintained by allantoic inoculation of the chick embryo with occasional passages through mice, ($EID_{50}$ approximately $10^{-7.5}$).

The mouse encephalomyelitis virus (Theiler's GD VII strain) was supplied by Dr. M. Theiler in its 188th mouse passage. This virus was passaged intracerebrally through mice 4 times in this laboratory before preparing stock virus. The titer of this material when used was approximately $10^{-6.5}$.

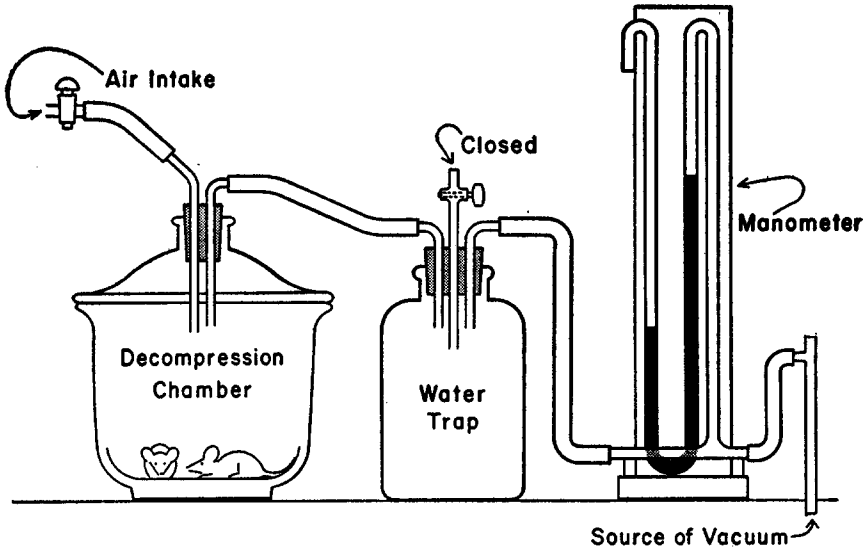


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FIG. 1. Decompression chamber.

For stock material, sufficient mice or embryos were appropriately inoculated and after sufficient time had elapsed to allow for maximal proliferation, the virus-containing tissue was harvested. The lungs or brains were homogenized as a 10 per cent suspension, and aliquots placed in 1.0 ml. glass ampoules which were sealed, quickly frozen, and stored at −60°C. Undiluted allantoic fluids were stored in a similar manner after light centrifugation. Titrations were made in the usual manner and the $LD_{50}$ or $EID_{50}$ determined by the method of Reed and Muench (9). Little, if any, changes in titer occurred during the course of the experiment.

*Decompression Chamber.*—This was a well ventilated vacuum type desiccator (Fig. 1), maintained at room temperature except when chick embryos were placed within. At these times, the desiccator was put in a 37°C. water bath. Infected control embryos were placed in an open jar in this same water bath.

Evacuation of the chamber to a simulated altitude of approximately 30,000 feet (equivalent to an oxygen partial pressure of 6.5 per cent) was usually completed within a few minutes (10). Water and food pellets were given to both groups of animals. At the end of each experiment, the pressure in the chamber was allowed to rise and the animals removed.

For most experiments the mice or embryos were inoculated with virus before introduction

into the chamber and then brought to the desired oxygen pressure within 15 minutes. In a few control experiments, the mice or eggs were decompressed first and inoculated within 15 minutes after removal from the chamber.

TABLE I

*Amount of Influenza Virus (PR8) Present in Normal and Hypoxic Mice at Various Time Intervals*

Each mouse received approximately 100 to 1000 $LD_{50}$.

| Time in mice | Group | Log $ID_{50}$ | Mean |
|---|---|---|---|
| *hrs* | | | |
| 3 | Hypoxic | <2.0*, <2.0, 2.7, 3.3, <2.0, 3.0, 2.5, 2.0, <2.0, <2.0, <2.0, 2.0, <2.0 | *1.2* |
| | Control | <2.0, <2.0, <2.0, <2.0, <2.0, 2.7, 5.5, 2.0, 2.5, <2.0, <2.0, <2.0 | *1.1* |
| 6 | Hypoxic | 3.4, 2.0, 2.0, 3.5, 2.5, 2.0 | *2.6* |
| | Control | 2.7, 4.3, 2.5, 5.5, 5.5, 2.0 | *3.8* |
| 7 | Hypoxic | 4.3, 4.5, 2.0, 2.0, (2.0, 2.7 | *2.8* |
| | Control | 2.7, 5.0, 2.0, 4.3, 5.3 | *3.7* |
| 9 | Hypoxic | 5.0, 4.8, 3.0, 2.5, 4.5, 5.8 | *4.3* |
| | Control | 5.5, 6.0, 2.5, 6.0, 4.8, 6.3 | *5.2* |
| 10 | Hypoxic | 4.5, 3.0, 2.7, 3.7, 3.0 | *3.4* |
| | Control | 5.0, 4.5, 5.7, 3.0, 3.0 | *4.2* |
| 12 | Hypoxic | 4.6, 4.6, 4.8, 4.8, 3.5, 4.5 | *4.5* |
| | Control | 7.3, 7.4, 4.2, 3.0, 3.5 | *5.1* |
| 13 | Hypoxic | 5.0, 2.5, 3.0, 3.5, 3.2 | *3.4* |
| | Control | 5.6, 5.5, 5.3, 3.5 | *5.0* |
| 14 | Hypoxic | 5.0, 6.3, 4.4, 4.2, 3.6, 5.3 | *4.8* |
| | Control | 5.5, 5.0, 5.0, 6.5, 6.0 | *5.6* |
| 15 | Hypoxic | 5.0, 3.8, 4.7, 4.5, 6.5, 5.3, 4.5, 5.3, 3.6, 5.5 | *4.9* |
| | Control | 7.3, 7.6, 7.5, 6.5, 7.0, 6.5, 6.5, 7.0, 6.3, 7.3, 7.2 | *7.0* |
| 17 | Hypoxic | 7.5, 6.8, 6.0, 6.8, 7.0 | *6.8* |
| | Control | 7.4, 7.5, 7.8, 8.3, 7.8 | *7.8* |
| 18 | Hypoxic | 4.7, 4.3, 5.5, 5.5, 4.5, 5.6, 7.0, 6.8, 7.5, 3.3, 4.0 | *5.3* |
| | Control | 6.4, 7.8, 7.2, 7.0, 6.8, 7.0, 7.0, 7.8, 7.3 | *7.2* |
| 21 | Hypoxic | 4.5, 5.8, 3.0., 2.5, 7.5, 6.0 | *4.9* |
| | Control | 8.3, 5.6, 4.4, 6.5, 8.1, 7.5 | *6.9* |
| 24 | Hypoxic | 7.2, 7.3, 6.8, 3.5, 7.5, 7.0, 6.0, 6.0 | *6.4* |
| | Control | 8.3, 8.8, 8.8, 7.3, 8.5, 6.0, 9.0, 8.0, 8.0 | *8.1* |
| 48 | Hypoxic | 8.5, 8.5, 7.5, 6.3, 8.0, 8.0 | *7.8* |
| | Control | 8.3, 8.0, 8.5, 8.5, 6.5, 8.5 | *8.1* |

* All tests for the presence of virus were started at a dilution 1:100 of mouse lung. Any test in which no virus was detected at this dilution was considered negative.

Only 5 to 6 mice or fertile eggs were placed in the decompression chamber at any one time. Each animal was studied as an individual experiment.

*Virus Titrations.*—The amount of virus present in test or control host tissue was determined by titration in developing embryos in the case of influenza virus and in mice for Theiler's GD VII virus.

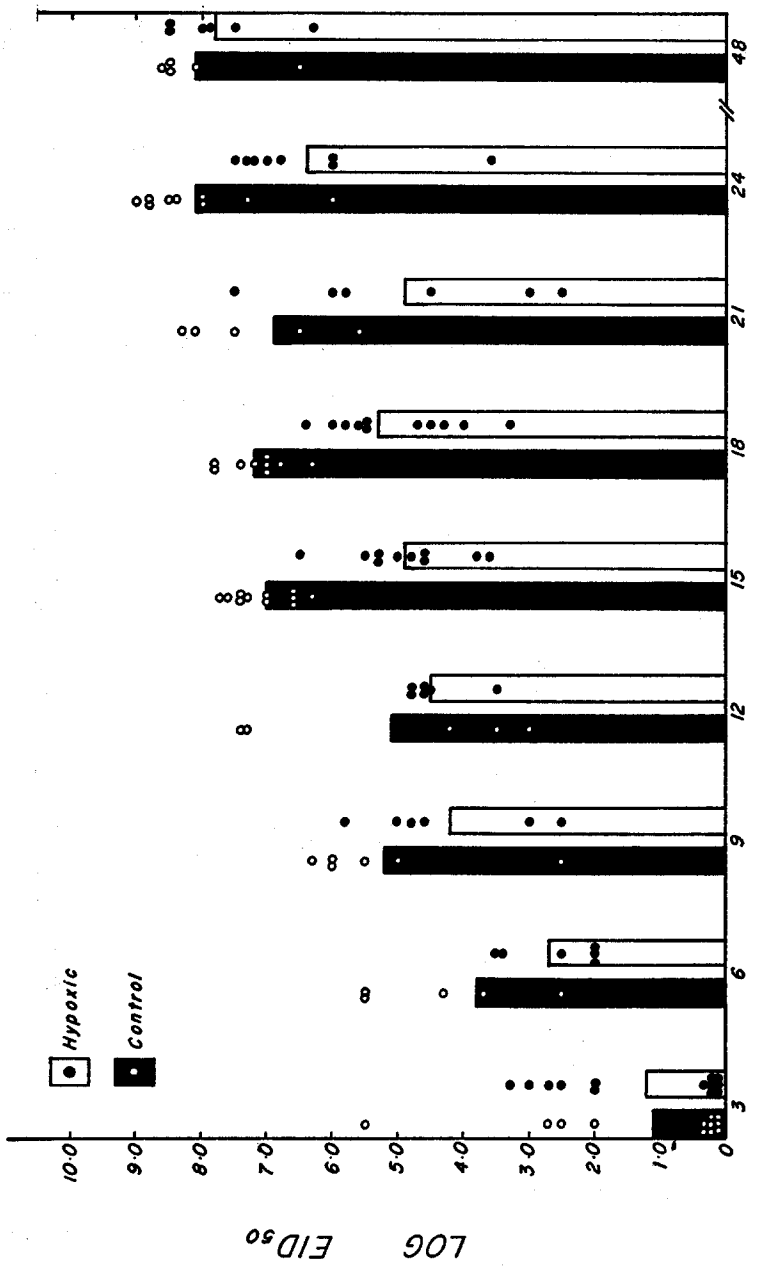


FIG. 2. Amount of influenza virus present in mouse lung at varying time intervals. Each animal received approximately 1000 $LD_{50}$.

Influenza virus in mouse lung was determined by preparing tenfold serial dilutions in buffered saline starting with a 10 per cent suspension of mouse lung under consideration. These dilutions were inoculated into 4 ten-day-old embryos per dilution and the presence or absence of hemagglutinins determined after approximately 40 hours' incubation. The allantoic fluid was tested for the presence of virus after chilling the eggs at 4°C. Influenza virus in embryos was determined in a similar manner. The undiluted allantoic fluid was lightly centrifuged and the supernatant fluid diluted 1:10 prior to the subsequent serial dilutions. Penicillin and streptomycin were added to all influenza tissues to insure sterility.

Theiler's GD VII virus was assayed by preparing 10 per cent mouse brain suspensions in a manner similar to that employed in preparation of the mouse lung. Titrations were made in young (3- to 4-week-old) mice using 4 to 5 mice per dilution. Antibiotics were not employed in these titrations as sterile precautions were maintained throughout the experiment.

TABLE II

*Effect of Hypoxia (18 Hours) Produced in Mice Prior to Inoculation of Virus*

| Time in Mice | Group | Log $ID_{50}$ | Mean |
|---|---|---|---|
| *hrs.* | | | |
| 3 | Hypoxic | 1.7, 2.5, 0, 2.0, 2.5 | *1.7* |
| | Control | 0, 2.3, 2.0, 1.7, 0, 0 | *1.0* |
| 6 | Hypoxic | 0, 2.0, 2.3, 0, 3.0 | *1.5* |
| | Control | 2.0, 1.7, 2.0, 2.0, 0 | *1.5* |
| 12 | Hypoxic | 4.5, 4.0, 4.5, 6.0, 4.0, 5.0 | *4.7* |
| | Control | 3.3, 2.5, 5.0, 4.5, 4.3, 5.0 | *4.1* |
| 18 | Hypoxic | 5.0, 5.3, 4.3, 2.7, 3.5 | *4.2* |
| | Control | 5.7, 6.3, 6.0, 6.7, 5.7 | *5.0* |
| 24 | Hypoxic | 6.3, 6.0, 5.7, 5.5, 5.7, 5.0 | *5.7* |
| | Control | 5.5, 5.3, 5.3, 6.3, 5.5, 5.3 | *5.5* |

## EXPERIMENTAL

*Influenza Virus Proliferation in Hypoxic Mice.*—Mice inoculated with approximately 1000 $LD_{50}$ of influenza virus were placed in an atmosphere of 6 per cent oxygen, to determine the effect of this environment on the amount of virus produced. 3 hours after inoculation, there was no detectable difference between control and hypoxic mice. From 6 to 24 hours, however, less virus was found in the lungs of mice subjected to hypoxic conditions than in the normal controls. This depressed virus growth was most apparent between 15 and 21 hours. At approximately 48 hours, the virus titer in the treated group approached that found in the untreated animals (Table I). Examination of these data indicates a wide individual variation in amount of virus present in each animal. That the difference between the two groups of animals, *i.e.* hypoxic and normal, is significant may be determined by a statistical analysis of the obtained values. In animals subjected to decompression one may observe as much as two logarithmic intervals in growth inhibition. At the 15 hour interval, for example, the mean log $ID_{50}$ for the hyp-

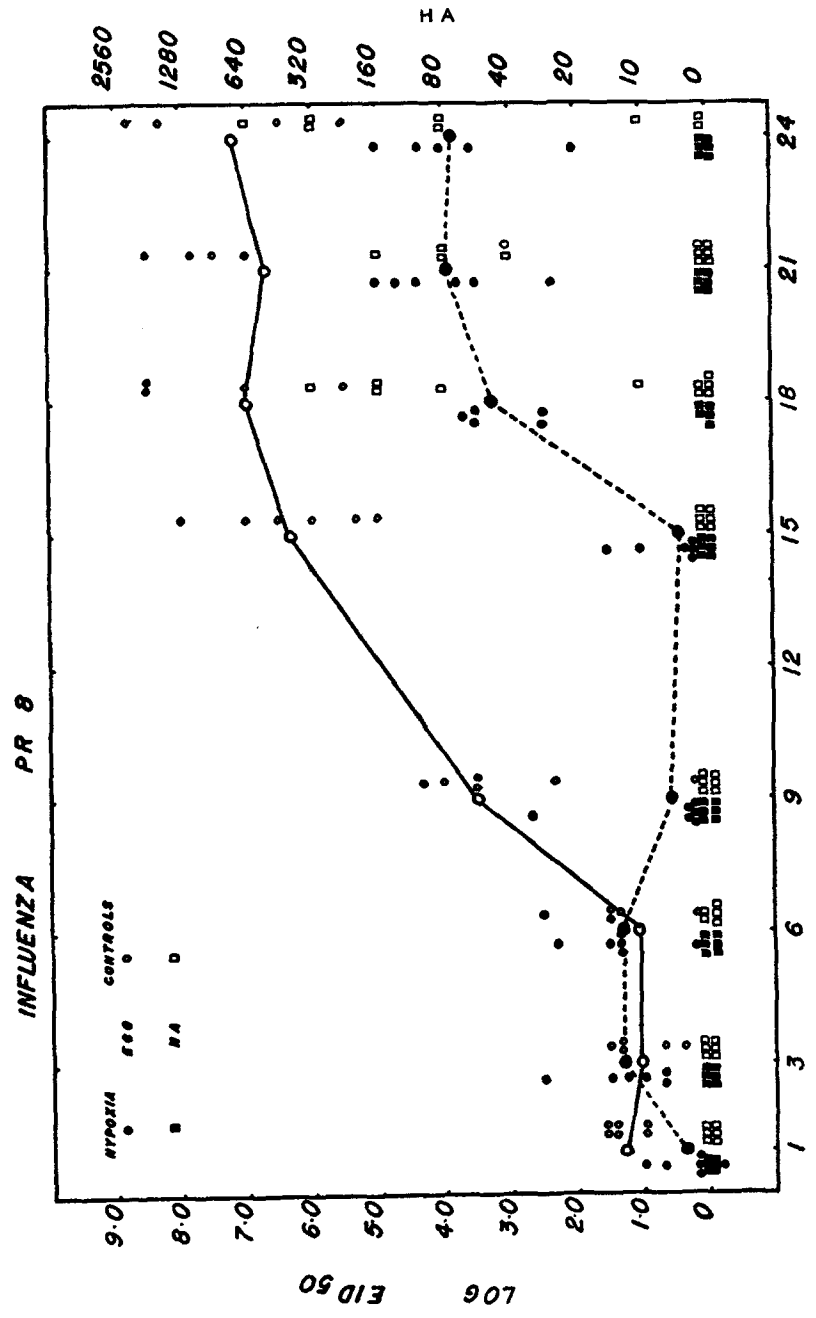


FIG. 3. Amount of influenza virus present in normal and hypoxic chick embryos at varying time intervals. Each embryo received approximately 1000 $LD_{50}$.

oxic animals was 4.87 ± S.E. 0.27 and for the controls 6.97 ± S.E. 0.143. This results in a $t$ value of 5.85 with the probability less than 0.01 that this is due to chance. Fig. 2 compares virus proliferation in normal and hypoxic animals. No attempt was made to construct growth curves for these data as the experiments were done at various times by different individuals. The equivalence in amount of virus in test and control animals at 48 hours is unexplained at the moment. Possibly the hypoxic animals had compensated for

TABLE III

*Amount of Influenza Virus (PR8) Present in Normal and Hypoxic Chick Embryos at Various Time Intervals*

Each embryo received approximately 1000 $ID_{50}$.

| Time in Mice | Group | Log $EID_{50}$ | Mean |
|---|---|---|---|
| *hrs.* | | | |
| 1 | Hypoxic | 0, 1.0, 0, 0, 0, 0.7 | *0.3* |
| | Control | 1.5, 1.5, 1.5, 1.5, 1.0, 1.0 | *1.3* |
| 3 | Hypoxic | 1.3, 0.7, 0.7, 2.5, 1.5, 1.0 | *1.3* |
| | Control | 1.3, 1.3, 1.5, 0.7, 0.3 | *1.0* |
| 6 | Hypoxic | 1.3, 1.5, 1.3, 0, 2.3, 1.3 | *1.3* |
| | Control | 1.5, 2.5, 1.5, 1.0, 0, 0 | *1.0* |
| 9 | Hypoxic | 0, 0, 2.7, 0, 0, 0 | *0.5* |
| | Control | 4.3, 0, 3.5, 4.0, 2.3, 3.5 | *3.5* |
| 15 | Hypoxic | 0, 1.0, 1.5, 0, 0, 0 | *0.4* |
| | Control | 7.0, 6.5, 5.3, 5.0, 6.0 | *6.3* |
| 18 | Hypoxic | 2.5, 2.5, 3.7, 3.5, 3.5 | *4.5* |
| | Control | 5.5, 7.0, 8.5, 8.5 | *7.0* |
| 21 | Hypoxic | 2.3, 4.7, 3.5, 5.0, 3.7, 4.5 | *4.0* |
| | Control | 7.0, 3.0, 7.5, 8.5, 7.7 | *6.7* |
| 24 | Hypoxic | 4.0, 2.0, 5.0, 3.7, 4.3 | *3.8* |
| | Control | 8.3, 6.5, 8.7, 5.5 | *7.3* |

the oxygen deficiency by this time. Because of natural variation in the RBC counts in mice, studies on this red cell level threw no light on this point.

An attempt was made to alter virus proliferation in mice subjected to decreased oxygen tension prior to inoculation of the virus. In this experiment mice were placed in the decompression chamber for periods of between 15 and 18 hours. After removal from the chamber the test and control animals were given a challenge inoculation of approximately 100 $LD_{50}$. At various time intervals, these animals were sacrificed and their lungs tested for virus in the usual manner. A comparison of the amount of virus in these animals at the different time intervals is made in Table II. Essentially little difference was found in the two groups of animals.

*Influenza Virus Proliferation in Hypoxic Chick Embryos.*—A similar series of experiments were made employing the developing chick embryo instead

of the mouse. The results of this experiment are described in Fig. 3 and tabulated in Table III. The growth curve for influenza virus in untreated eggs is as expected. An increase in the amounts of virus became apparent after 6 hours and reached the maximum at about 18 hours. In hypoxic eggs, little, if any, increase was apparent until after 15 hours had elapsed. The experiment was terminated at the 24 hour period as it was difficult to maintain sufficient live embryos at this oxygen tension for this length of time. The depressed virus growth is more apparent in the chick embryos than in mice probably because they have no red cell reserve. At 15 hours approximately 6 logarithmic intervals are apparent and at 24 hours, when maximal virus proliferation is presumed to have occurred, approximately 3 logarithmic intervals are observed.

TABLE IV

*Effect of Hypoxia Produced in Embryonated Eggs Prior to Inoculation of Virus (PR8, 1000 $ID_{50}$)*

All eggs were reincubated for approximately 40 hours prior to testing for amount of virus.

| Time in Chamber | Group | Log $EID_{50}$ | Mean |
|---|---|---|---|
| *hrs.* | | | |
| 3 | Hypoxia | 4.5, 4.5, 4.5, 4.0, 5.5 | *4.6* |
| | Control | 6.5, 6.5, 5.7, 6.5, 6.5, 5.5 | *6.2* |
| 6 | Hypoxia | 2.5, 3.5, 2.5, 3.7, 4.5, 4.0 | *3.5* |
| | Control | 6.5, 7.3, 8.0, 5.5, 5.5, 6.0 | *6.5* |
| 8 | Hypoxia | 4.5, 6.5, 6.0, 4.0, 3.3, 5.0 | *5.0* |
| | Control | 5.5, 5.5, 4.7, 4.0, 5.3 | *5.0* |
| 12 | Hypoxia | 5.0, 4.0, 5.0, 3.7, 3.7, 4.5 | *4.3* |
| | Control | 8.5, 7.3, 6.5, 8.0, 6.3, 2.5 | *6.5* |

The effect on hemagglutination is also evident. Hemagglutinins could be demonstrated only when the control animals had reached an approximate titer of $10^6$ particles. This titer was not attained in hypoxic embryos.

Mice subjected to a low oxygen concentration before inoculation with virus contrasted with fertile eggs treated similarly. The eggs were placed in the decompression chamber for varying periods of time prior to inoculation with approximately 1000 $ID_{50}$. These embryos were then reincubated along with inoculated controls and tested for virus after 36 to 40 hours (Table IV). The data show that virus proliferation was depressed in chick embryos that had previously been exposed to low oxygen tension.

*Theiler's GD VII Virus Proliferation in Hypoxic Mice.*—In order to test these findings with another virus, Theiler's GD VII was studied under conditions similar to those used for influenza virus in mice. Two different inocula were employed and the amounts of virus titrated at 24 and 48 hours with essentially the same results (Table V and Fig. 4). A notable difference in infection due to influenza and Theiler's GD VII was observed in mice after 48 hours in

the decompression chamber. Whereas influenza virus has reached its maximal titer in both groups of animals, a marked inhibition was still apparent with both dilutions of Theiler's GD VII; *i.e.*, 50 or 5000 $LD_{50}$. The general phenomenon of virus depression, however, is similar for both viruses.

*Combined Influenza (PR8) and Theiler's GD VII Infection of Mice.*—An additional experiment was done in order to determine the effect of simultaneous infection with two viruses. In this experiment, after anesthetizing the animals, influenza virus (approximately 100 $LD_{50}$) was inoculated intranasally and Theiler's GD VII (approximately 10 $LD_{50}$) was given intracerebrally within a time interval of less than 5 minutes. Immediately thereafter the animals were placed in the decompression chamber. After 18 hours in the chamber, the animals were sacrificed and the amount of virus present in either the brain or lung tested in the usual manner (Fig. 5 and in Table VI). It will be observed that the inhibition demonstrated in the original experiments also oc-

TABLE V

*Amount of Theiler's GD VII Virus Present in Normal and Hypoxic Mice at Various Time Intervals*

At 24 and 48 hours challenge inocula of either 50 $LD_{50}$ or 5000 $LD_{50}$ were employed.

| Time in Mice | Group | Log $LD_{50}$ | Mean |
|---|---|---|---|
| *hrs.* | | | |
| 3 | Hypoxic | 1.0, 0.7, 2.3 | *1.3* |
| | Control | 1.3, 0.7, 1.5, 1.7 | *1.3* |
| 6 | Hypoxic | 0.5, 0.7, 1.0, 1.0 | *0.8* |
| | Control | 3.0, 1.0, 1.5 | *1.8* |
| 9 | Hypoxic | 0.5, 0.5, 0.7, 1.0 | *0.7* |
| | Control | 4.3, 2.7, 3.3 | *3.4* |
| 12 | Hypoxic | 1.0, 0.5 | *0.8* |
| | Control | 3.0, 3.3, 2.7, 2.0 | *2.8* |
| 15 | Hypoxic | 2.0, 1.7, 3.0 | *2.2* |
| | Control | 2.7, 2.7, 2.5, 2.5 | *2.6* |
| 18 | Hypoxic | 2.0, 3.0, 3.5, 3.5 | *3.0* |
| | Control | 6.0, 6.3, 5.7 | *6.0* |
| 21 | Hypoxic | 3.0, 2.7, 2.7, 3.5 | *3.0* |
| | Control | 4.0, 4.3, 5.3 | *4.4* |
| 24 | Hypoxic | 3.5, 3.3, 2.0, 1.5 | *2.6* |
| | Control | 7.0, 5.0, 4.0, 3.0 | *4.8* |
| | Hypoxic (50 $LD_{50}$) | 3.0, 2.0, 1.3, 1.5 | *2.0* |
| | Control (50 $LD_{50}$) | 4.3, 3.5, 3.5, 2.0, 2.0 | *3.8* |
| 48 | Hypoxic | 3.3, 3.3, 3.5, 3.5, 5.5, 6.5 | *4.3* |
| | Control | 6.7, 6.7, 6.5, 6.5, 6.0, 6.0, 6.0, 5.5, 4.3 | *6.0* |
| | Hypoxic (50 $LD_{50}$) | 5.5, 2.6, 2.3, 1.5, 1.5, 1.0, 1.0, 1.0 | *2.1* |
| | Control (50 $LD_{50}$) | 6.0, 5.5, 5.3, 5.0, 4.7, 4.5, 4.3, 3.5 | *4.9* |

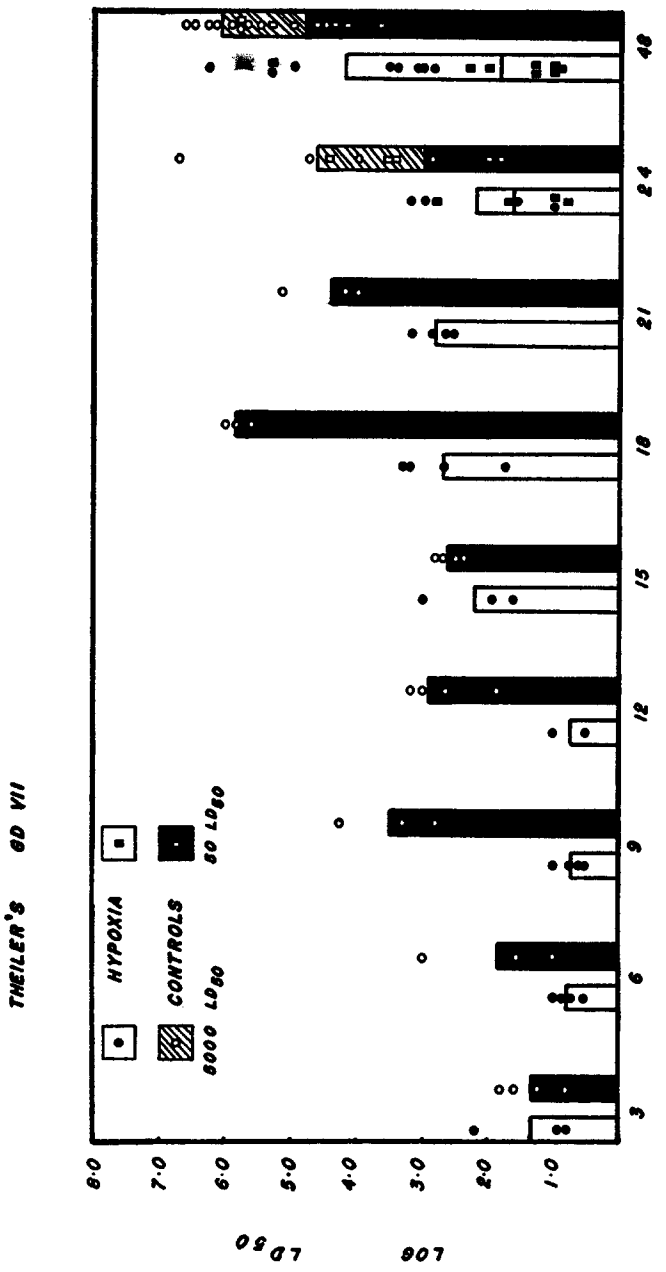


FIG. 4. Amount of Theiler's GD VII virus present in mouse brain at varying time intervals.

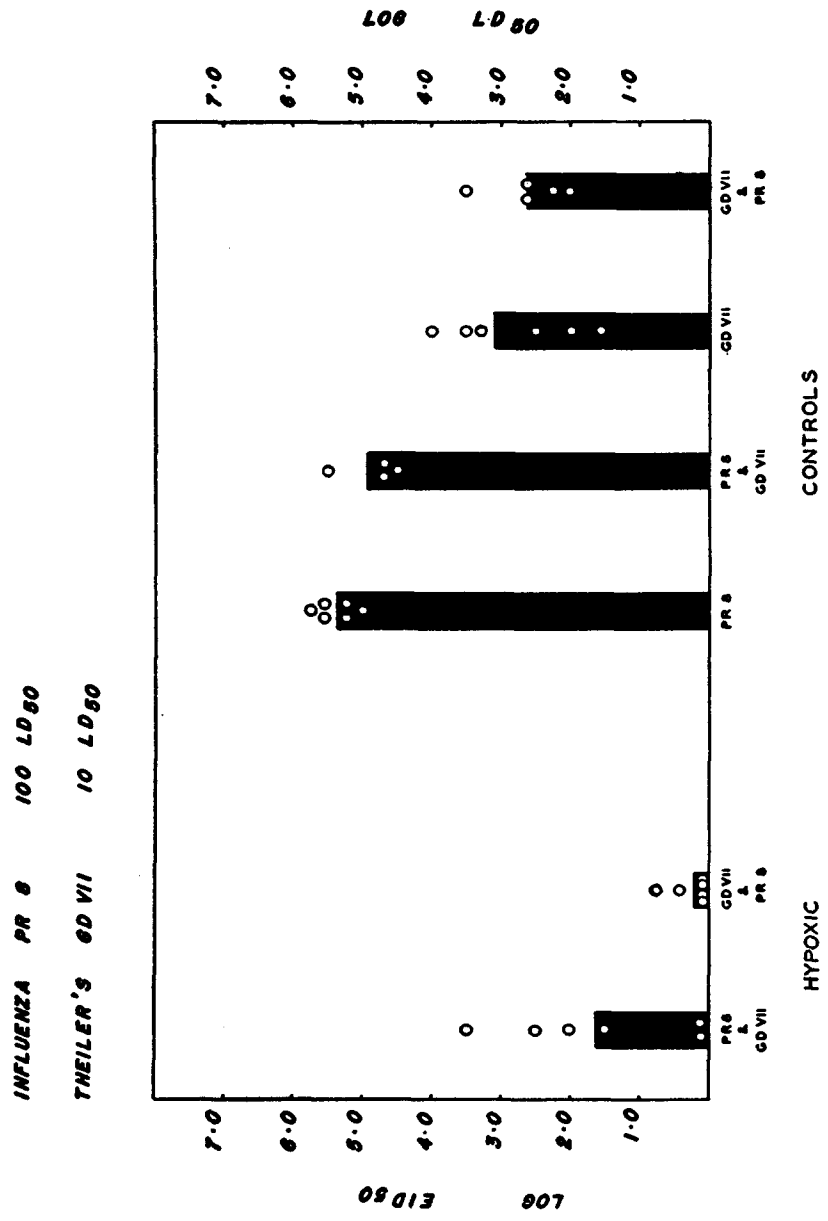


FIG. 5. Combined infection of influenza virus (PR 8) and Theiler's GD VII virus in mice. The animals were kept in the decompression chamber for 18 hours following inoculation with both viruses.

curred here and in a more marked degree. It will also be noted that there tends to be a little less virus present in the animals receiving the two viruses than in their control counterparts.

TABLE VI

*Amount of Virus Present in Hypoxic Mice Inoculated Intranasally with Influenza Virus (PR8) and Intracerebrally with Theiler's GD VII Virus*

All animals were kept in the decompression chamber for 18 hours before determining the amount of virus present in the tissues.

| Animal No. | $LD_{50}$ GD VII in brain | | | | $EID_{50}$ PR8 in lung | | | |
|---|---|---|---|---|---|---|---|---|
| | Experiment No. | | | | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Hypoxic | | | | | | | | |
| 1 | 0 | 0.2 | 0.2 | 0.2 | 0 | 3.0 | 2.0 | 2.3 |
| 2 | 1.0 | 0.5 | 0.3 | 0.5 | 0 | 3.0 | 2.3 | 2.0 |
| 3 | 0 | 1.3 | 1.0 | 0.5 | 2.5 | 3.0 | 3.0 | 0 |
| 4 | 0.5 | 0 | 1.0 | 0.2 | 1.5 | 0 | 3.0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 2.0 | 2.5 | 3.0 | — |
| 6 | 0 | — | 0 | 0 | 3.5 | — | 2.5 | — |
| Controls | | | | | | | | |
| 1 | 2.0 | 3.5 | 1.0 | 1.5 | 4.7 | 3.5 | 3.7 | 2.5 |
| 2 | 2.3 | 2.5 | 1.0 | 2.0 | 4.7 | 5.0 | 6.5 | 3.0 |
| 3 | 3.5 | 4.0 | 2.0 | 2.5 | 4.5 | 4.7 | 5.5 | 2.5 |
| 4 | 2.5 | 1.5 | 2.5 | 2.8 | 5.5 | 6.5 | 5.3 | 4.5 |
| 5 | 2.5 | — | 2.5 | 2.5 | — | — | 3.0 | 2.0 |
| 6 | — | — | 2.5 | 1.0 | — | — | 5.3 | — |
| | Controls | | | | | | | |
| | No. PR8 virus | | | | No GD VII virus | | | |
| 1 | 3.5 | 0 | 1.0 | 3.5 | 5.3 | 4.7 | 3.3 | 6.3 |
| 2 | 1.5 | 1.0 | 2.0 | 2.5 | 5.5 | 6.0 | 2.5 | 5.5 |
| 3 | 3.3 | 3.0 | 1.5 | 3.5 | 5.3 | 3.0 | 2.5 | 5.7 |
| 4 | 4.0 | 2.5 | 2.5 | 2.5 | 5.7 | 2.5 | 2.7 | 4.5 |
| 5 | 2.0 | | 2.5 | 3.0 | 5.0 | — | 4.3 | — |
| 6 | 2.5 | | 2.5 | 2.5 | 5.5 | — | 1.7 | — |

## DISCUSSION

From the above data it may be concluded that virus proliferation in the intact animal is partially dependent upon the oxygen supply to the host tissue. This is in agreement with the tissue culture findings of other investigators (3, 4). When the oxygen supply to the lung or the brain is decreased, the rate of virus proliferation in these two tissues is markedly reduced.

Although the effect of hypoxia upon the growth of the two viruses was simi-

lar, there was one important difference. It was observed that the influenza titer in test and control animals was approximately the same at 48 hours, whereas with Theiler's GD VII virus there was still marked inhibition. At the present time we can only speculate on the cause of this difference. Nervous tissue is least capable of all the tissues to withstand oxygen want. The brain tissue is also the first tissue to be affected in anoxia. It thus seems probable that virus proliferation within such tissue is more readily affected than virus growing in a tissue as well oxygenated as the lung.

At present little information is available concerning the relationship between tissue anoxia and virus proliferation. In our previous report (8) it was suggested that this inhibition may be due to an impairment of the host's cellular oxidative mechanism and a corresponding reduction in tissue protein synthesis. This reduction in protein synthesis would be reflected in decreased virus proliferation. This would be substantiated by the findings of Eaton *et al.* (11) who found that 3,5-diiodo-4-hydroxybenzoic acid inhibited the proliferation of influenza virus in tissue culture. This compound resembles 2,4-dinitrophenol (DNP) in activity, thus indicating that the high energy bonds obtained from oxidative phosphorylation entered into the mechanism of virus proliferation. It was also stated that the butyl esters of 3,5-diiodo-4-hydroxybenzoic acid reduced oxygen tension by 15 to 30 per cent. The investigations of Mann and Gruschow (12) with radiophosphorous would also indicate this mechanism of action. These investigators demonstrated that phosphate incorporation into nucleic acid and phosphoprotein was inhibited in the absence of oxygen. The findings of Berry *et al.* (5) concerning the reduction in amount of citric acid present in the hypoxic mouse lung are also compatible with the suggestion that any impairment of the host metabolic activity would result in a similar derangement of virus synthesis.

From the above, the following hypothesis concerning the virus proliferation may be presented. Upon entering the host cell the virus particle is changed into components as yet unrecognizable. These components probably reduplicate in a similar manner to other components within the host cell. This synthesis of essential virus materials parallels that of the synthesis of normal components of the host cell. When the energy-yielding mechanisms concerned with this synthesis are so altered that protein synthesis is retarded then the virus proliferation is likewise depressed.

## SUMMARY

Virus proliferation in hypoxic animals was markedly reduced. Little difference in this respect between influenza virus (PR8) and mouse encephalomyelitis virus (Theiler's GD VII) was observed except that there was a longer depression of the latter virus. The possible mechanisms involved are discussed.

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