THE EFFECT OF DIET ON THE SUSCEPTIBILITY OF THE MOUSE TO PNEUMONIA VIRUS OF MICE (PVM)

I. Influence of Pyridoxine in the Period after the Inoculation of Virus*

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Variability in the susceptibility of different strains of mice to infection with pneumonia virus of mice (PVM) has been recognized for many years (1). Young mice appear to be more easily infected than older animals.

Recently, in this laboratory, lower infectivity titers of PVM suspensions than anticipated were found repeatedly in titrations of this virus in mice. These titers were lower than those found for the same lot of virus in mice of the same strain and age in the laboratory of Dr. Frank L. Horsfall, Jr. at The Rockefeller Institute for Medical Research.

Evidence has accumulated that the nutrition of the host may influence its susceptibility to infection. In general it appears that deficiency states increase host susceptibility to a variety of bacterial infections (2–4). In virus infections the reverse has been observed, and host susceptibility to a number of viruses may be decreased under conditions of dietary deficiency (5–22). Similar results were found in tissue cultures (23) and with bacteriophage (24).

The most apparent difference in the experimental conditions for testing infectivity titers of PVM at the Rockefeller Institute and in our laboratory was dietary. At the Rockefeller Institute the mice were fed a mixture of cracked wheat, bread, powdered milk, and fresh milk; whereas the mice in our laboratory were maintained on Purina dog chow and tap water. It was decided to investigate the effects of variation in diet on the susceptibility of mice to PVM in an attempt to explain the lower infectivity titers observed in our laboratory. The present paper is concerned with short term experiments involving a 12 day period between the inoculation of PVM and the sacrifice of the mice. The results of longer term experiments in which mice were fed different diets for varying periods, before PVM inoculation as well as after, are presented in the accompanying paper (25).

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Materials and Methods

Mice.—Albino Swiss mice between 2½ and 4½ weeks of age (8 to 11 gm. average body weight) were used throughout this study. They were obtained each week from two commercial breeders.[1] They were weaned shortly before shipping by the breeder and ate bread and white potatoes in transit. Most of these mice appeared to be perfectly healthy, and numerous groups held for observation for several weeks failed to develop any signs of illness. During a period in the spring and summer of 1948 some of the mice from one of the breeders were found to be carriers of *Salmonella enteritidis*. A few animals developed obvious infection with this bacterium.

There was no apparent difference in the susceptibility of the two strains of mice to PVM. In the experiments reported below the mice were inoculated within 24 hours after arrival. After inoculation groups of mice which received the same amount of virus were kept together in glass jars with wire screen covers. All mice were kept on pine shavings. No spontaneous deaths with pulmonary consolidation were observed in uninoculated mice. The diet and fluid administration are described in detail below.

Virus.—Pneumonia virus of mice (PVM), Rockefeller Institute strain 15,[2] was used throughout this study. The virus was maintained by occasional passage in young mice. Groups of mice were inoculated intranasally, under light ether anesthesia, with 0.05 cc. each of a dilute virus suspension. Six or 7 days later these mice were sacrificed, the lungs removed with aseptic precautions, weighed, and ground in a mortar with alundum. Sufficient sterile trypticase soy broth[3] was then added to make a 10 per cent suspension by weight. After centrifugation at 1500 R.P.M. for about 10 minutes, the supernatant fluid containing suspended virus was separated and stored in lusteroid tubes with metal screw caps at −70°C. in a cabinet containing solid CO_2. Such a virus suspension will hereinafter be referred to as 10^{-1} dilution of virus. Fresh virus suspensions were made only when additional virus was needed, usually every 3rd or 4th month. When required for use, a tube of virus suspension was thawed in lukewarm water, and after the required amount was removed the remainder was immediately refrozen in a mixture of alcohol and solid CO_2 and returned to the storage chest.

Virus Titrations.—Four appropriate tenfold serial dilutions of the freshly thawed virus suspension were prepared in test tubes containing sterile trypticase soy broth. The tubes were kept in ice water until inoculation was completed. Mice were inoculated from these tubes, each mouse under light ether anesthesia, receiving 0.05 cc. of one of the virus dilutions intranasally. The highest dilution was inoculated first. In each individual titration 5 or usually 6 mice received each dilution. In all experiments simultaneous titrations, using the same tubes of virus dilution, were carried out in two or more comparable groups of 24 mice each. In such multiple titrations all mice receiving any one virus dilution were inoculated before proceeding to the next lower dilution.

All inoculated mice were observed daily; the lungs of those which died were examined for the presence and extent of pneumonia. On the 12th day after inoculation all surviving mice were sacrificed and examined for the degree of pneumonic consolidation.

All end points in virus titrations were calculated by the method of Reed and Muench (26) as the 50 per cent maximum score (M.S. 50) (27), which was found by Horsfall and Curnen (28) to be more reproducible with PVM than either the 50 per cent mortality or the 50 per cent pulmonary lesion end point. The M.S.50 end point reflects not only the frequency with which fatal and non-fatal pneumonia is induced in mice, but also the extent of the pulmonary consolidation in each animal.

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[1] Mr. Victor Schwentker and Mrs. Flora O'Grady.

[2] This strain of virus was kindly supplied by Dr. Frank L. Horsfall, Jr.

[3] Obtained from Baltimore Biological Laboratories, Baltimore.

Hemagglutination Tests.—Suspensions of PVM were heated at 70°C. for 30 minutes and the clear supernates, after centrifugation, were used for studies of agglutination of mouse erythrocytes. The methods of preparing erythrocyte suspensions and of performing hemagglutination and hemagglutination-inhibition tests were identical to those described by Curnen and Horsfall (29).

Neutralization Tests.—Sera for neutralization tests were stored at 4°C. and inactivated by heating at 56°C. for 30 minutes before testing. The method of testing for neutralizing antibodies against PVM in mouse serum was to mix a constant amount of virus suspension (approximately the M.S.50 dose) with varying dilutions of serum in trypticase soy broth. The virus-serum mixtures were kept in ice water for one-half hour prior to inoculation. The mixtures were then inoculated intranasally into each of 5 or 6 mice per dilution.

Diets.—Two natural diets and several partially synthetic diets that were fed the mice are described below:

1. Natural Diets.—(*a*) Milk-wheat diet: cracked whole wheat grain, 1 part; powdered milk (parlac), 1 part; white bakers' bread, 2 parts; and whole fresh milk, 1 part. These ingredients were mixed thoroughly. (*b*) Purina dog chow:[4] a commercial diet in the form of dry pellets.

2. Partially Synthetic Diets.—These were modifications of the mouse diets of Fenton and Cowgill (30) having the following constituents per 100 gm.:

	Diet			
	S-1	S-2	S-3	S-4
Casein[5] (vitamin-free), *gm.*	30	10	30	4
Dextrose, *gm.*	52.5	72.5	52.5	78.5
Corn oil (mazola), *gm.*	10	10	10	10
Salt mixture (31), *gm.*	5.0	5.0	5.0	5.0
Oleum percomorphum, *gm.*	0.2	0.2	0.2	0.2
Choline, *mg.*	150	150	150	150
PABA, *mg.*	100	100	100	100
Inositol, *mg.*	100	100	100	100
Thiamin, *mg.*	0.5	0.5	0.5	0.5
Riboflavin, *mg.*	1.0	1.0	1.0	1.0
Nicotinic acid, *mg.*	1.0	1.0	1.0	1.0
Calcium pantothenate, *mg.*	3.0	3.0	3.0	3.0
α-Tocopherol, *mg.*	6.0	6.0	6.0	6.0
Pyridoxine hydrochloride,[6] *mg.*	0.5	0.5	—	0.5

After thoroughly mixing most of the dry ingredients in a food mill they were added to the oils, choline, and casein which had been mixed by hand and the whole combined with sufficient water to make small cakes. The milk-wheat and Purina diets were placed directly on the shavings; the partially synthetic (S) diets were dispensed in the jars in small cylindrical food cans which excluded the shavings so that it was possible to weigh the diet when desired. An excess of the appropriate diet was available to the mice at all times during the experiments. In all cases distilled water was provided *ad libitum* from glass bottles.

It was frequently more convenient to administer pyridoxine in the drinking water than to administer it mixed in the diet. Pyridoxine 1 mg. per 100 cc. of the drinking water provided a daily pyridoxine intake per mouse equivalent to that in diet S-1. The term "diet S-1" is

[4] Obtained from the Ralston Purina Company, St. Louis.

[5] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

[6] This was kindly supplied by Squibb and Co., Inc.

used in this report to designate either diet S-1 as described above or diet S-3 plus pyridoxine in the above concentration in the water. All pyridoxine solutions were stored in the dark at 4°C. Desoxypyridoxine[7] was given in the drinking water in certain experiments.

EXPERIMENTAL

The Effect of Certain Diets on Growth.—

Fifteen freshly weaned mice about 2½ to 3 weeks of age, with an average weight of 8.2 gm., were divided into three groups of 5 mice each. Each group was fed a different diet, milk-

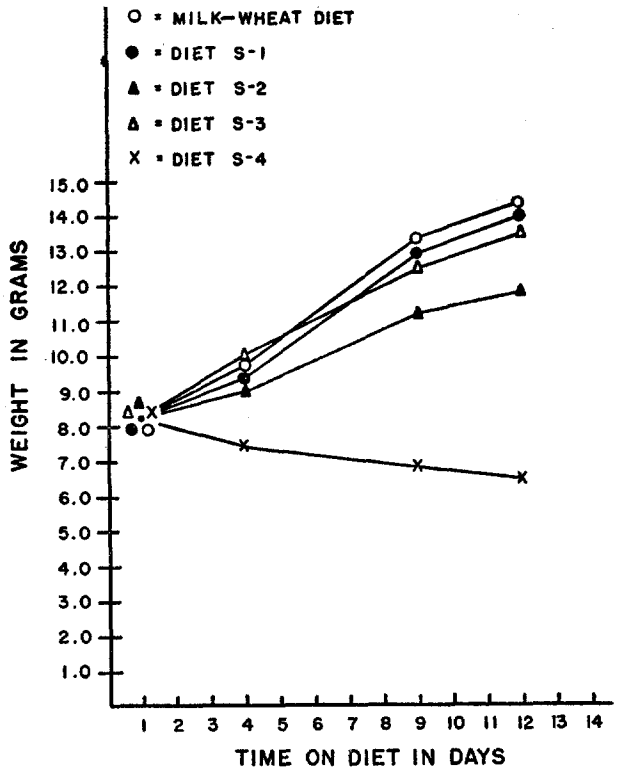

FIG. 1. Weight curves of mice fed various diets. The indicated weights are the averages of 5 mice fed each diet. Mice were approximately 3 week old weanlings at the beginning of the experiment. The diet was in each case supplied in excess at all times.

wheat, S-1, S-2, S-3, or S-4 respectively for 12 days. During this time, each diet group of 5 mice was weighed five times at intervals. Weight curves on these five diets are shown in Fig. 1.

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[7] Obtained from Merck and Co., Inc.

It is apparent that the weight curves of mice receiving milk-wheat, S-1, or S-3 diets, do not significantly differ. The mice receiving diet S-2 (10 per cent casein) will be seen to have gained weight, but at a retarded rate; while those on diet S-4 (4 per cent casein) lost weight steadily.

Reproducibility of Virus Titration End Points.—Curnen and Horsfall (28) reported a geometric mean deviation of 0.13 log unit in thirty-six titrations of PVM. It should be pointed out that their titrations were not simultaneous but were carried out separately at weekly intervals. In the present study an attempt was made to determine the degree of variation of the M.S.50 end point of simultaneous virus titrations in identical groups of mice. Simultaneous duplicate titrations were carried out as described under Methods in mice from each breeder. It will be seen from the results recorded in Table I that the

TABLE I

*Simultaneous Duplicate Titrations of PVM in Mice**

Breeder	Titration	M.S.50 end point (reciprocal of log)	Log difference between titrations
A	1	1.40	0.06
	2	1.34	
B	1	1.07	0.09
	2	1.16	

* All the mice were fed Purina dog chow and given distilled water to drink during the entire 12 day experimental period after inoculation.

M.S.50 end points in simultaneous duplicate titrations differed by less than 0.10 log unit in mice from the same breeder. Hence, it was concluded that M.S.50 end point differences of more than 0.10 log unit in simultaneous titrations in mice nearly identical as to age, sex, weight, and dietary history were probably significant. The difference in susceptibility of mice from the two breeders in this particular experiment may be explained on the basis of differences in age and weight, the mice from breeder B being older and heavier.

Comparison of Milk-Wheat and Purina Dog Chow Diets.—Three experiments comparing the milk-wheat and Purina diets were performed at different times.

In each experiment PVM was titered as described under Methods in two matched groups of mice. Immediately after inoculation one of these groups was given milk-wheat diet and the other Purina dog chow for the entire 12 day period of the experiment. The results of the titrations are shown in Fig. 2.

It will be seen that in all three experiments the M.S.50 end point was higher in mice on the milk-wheat diet than in mice on Purina dog chow. The range

of differences is 0.2 to 0.5 log unit with a geometric mean difference of 0.32 log unit. This difference is considered to be outside the range of experimental error.

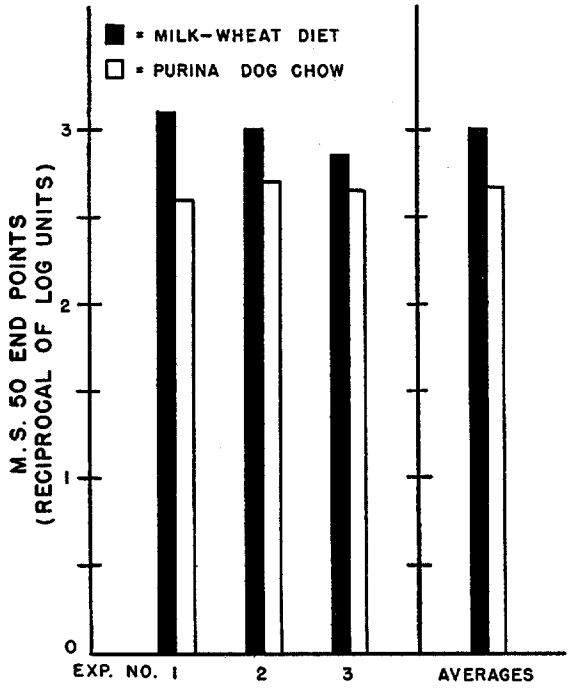

FIG. 2. Infectivity titers of PVM in mice fed the milk-wheat diet or Purina dog chow during the entire 12 day experimental period after inoculation. These diets were started on the day of inoculation when the mice were approximately 3 weeks old. This is a summary of three experiments, each using 48 mice.

Comparison of Milk-Wheat and S-1 Diets.—

Simultaneous pairs of titrations of PVM infectivity were carried out on three different occasions, each in 48 freshly weaned mice. In each experiment, after inoculation with appropriate tenfold dilutions of virus, 24 comparable mice were placed on milk-wheat diet, and 24 on diet S-1 for the 12 day period of the experiment.

The results of these titrations, shown in Fig. 3, suggest that mice on the natural milk-wheat diet were slightly more susceptible to PVM than those on the partially synthetic diet (S-1). However, the geometric mean difference is only 0.14 log unit which is of questionable significance. There was no appreciable difference in the weight gain of infected mice on these two diets during the experimental period.

Effect of Pyridoxine Deficiency.—Pyridoxine has been reported to influence antibody production (32–34). Since PVM is a virus native to mice (1) and mice develop increasing resistance with age, it seemed possible that the presence of antibodies might be an important factor in the resistance of mice to infection

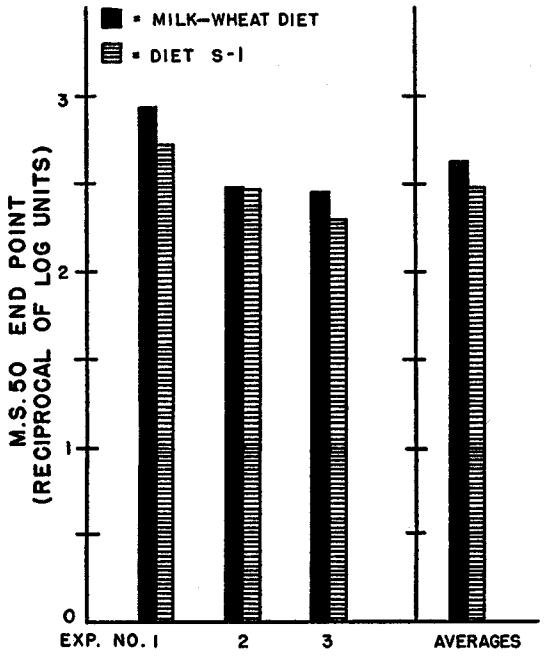

FIG. 3. Infectivity titers of PVM in mice fed a natural milk-wheat diet and a partially synthetic "complete" diet (S-1) during the entire 12 day experimental period after inoculation. The diets were started on the day of inoculation when the mice were approximately 3 weeks old. This is a summary of three experiments, each using 48 mice.

with this virus. Therefore it was decided to investigate the effect of pyridoxine on the mouse's susceptibility.

Nine similar experiments were done each using 48 freshly weaned mice. After inoculation with PVM in the manner described under Methods, one diet group of 24 mice was fed the pyridoxine-deficient diet (S-3) and the other group the same diet plus pyridoxine 1 mg. per 100 cc. of the drinking water (S-1) for the entire 12 day period of the experiment. On the 12th day all surviving mice were sacrificed and the M.S.50 end point was calculated.

In Fig. 4 it is seen that in each experiment the M.S.50 end point was higher in mice receiving pyridoxine than in those fed a similar diet lacking this vitamin. This difference varied from 0.22 log unit to 1.43 units, or from 1.7 to 12.7 times. The geometric mean difference in virus titration end points between

the mice on the "complete" diet and those on the pyridoxine-free diet was 0.67 log unit. This represents a fivefold lower end point in the mice that received no pyridoxine.

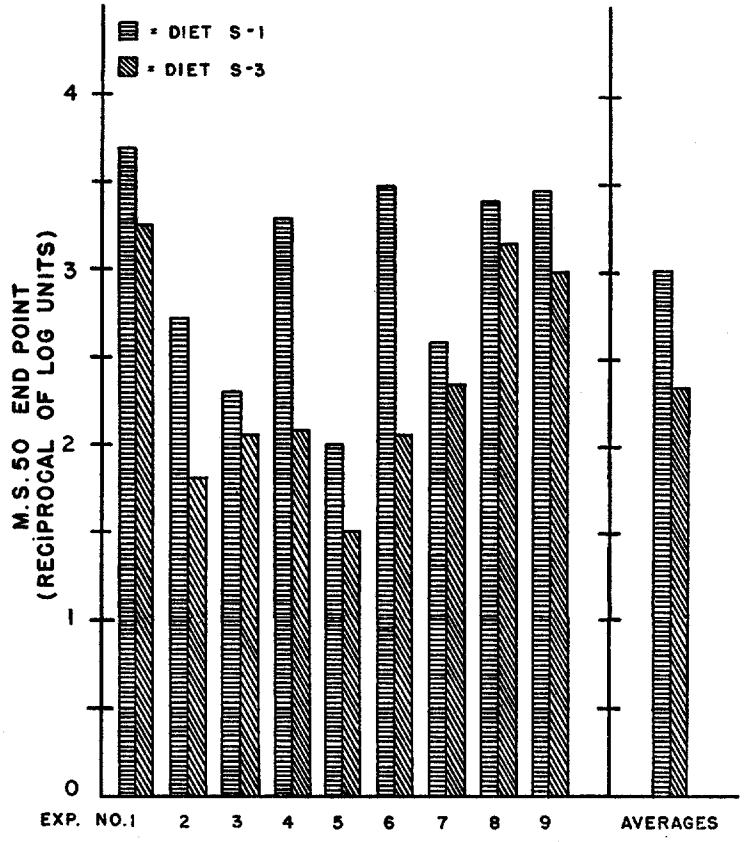

FIG. 4. Infectivity titers of PVM in mice fed a partially synthetic "complete" diet (S-1) and a partially synthetic pyridoxine-deficient diet (S-3) during the entire 12 day experimental period after inoculation. Diets were started on the day of inoculation when the mice were approximately 3 weeks old. This is a summary of nine experiments, each using 48 mice.

Effect of Pyridoxine Added to Purina Dog Chow.—It was shown above that mice fed the partially synthetic diet (S-3) plus pyridoxine were more susceptible to PVM than mice fed a similar diet without pyridoxine, and were almost as susceptible as mice fed the natural milk-wheat diet. Also, mice on the milk-wheat diet were shown to be slightly more susceptible to PVM than mice fed Purina dog chow. It appeared, therefore, that an explanation for this latter phenomenon might be that Purina dog chow was relatively poor in pyri-

doxine. To test this hypothesis two experiments were performed at different times.

Simultaneous virus titrations were done in two groups of 24 mice in each experiment as described under Methods. Immediately after inoculation one group was fed Purina dog chow and given distilled water to drink; the other group was fed Purina dog chow and given pyridoxine 1 mg. per 100 cc. of water to drink. These different regimens were continued throughout the 12 day period of the experiment. At the end of this time the surviving mice were sacrificed, and the M.S.50 end point was determined.

TABLE II

The Effect of Pyridoxine in the Drinking Water of Mice Fed Purina Dog Chow on Their Susceptibility to PVM

Purina dog chow		M.S.50 end point (reciprocal of log)		Log difference
		Concentration of pyridoxine in drinking water, *mg. per 100 cc.**		
No. of experiment	No. of mice	0	1.0	
1	48	3.13	3.56	0.43
2	48	3.15	3.39	0.24
Averages.....		3.14	3.47	0.33

* Pyridoxine 1 mg. per 100 cc. in distilled water to drink was constantly available during the 12 day experimental period after inoculation.

In both experiments, shown in Table II, the M.S. 50 end point was higher in mice given additional pyridoxine, the average difference being 0.33 log unit. This implies that Purina dog chow was, in fact, deficient in pyridoxine, since the addition of pyridoxine in the drinking water rendered mice on that diet more susceptible to PVM.

Effect of Dietary Pyridoxine for Varying Times after Inoculation.—Since dietary pyridoxine throughout the entire 12 day experimental period after inoculation seemed to increase the susceptibility of mice to infection with PVM it was decided to investigate the effect of varying the time of pyridoxine administration within this period. Two separate experiments were done to test this point, each with 96 freshly weaned mice.

All the mice were fed Purina dog chow. In each experiment one group of 24 mice drank distilled water and three other similar groups drank water containing pyridoxine 1 mg. per 100 cc. for varying times. In the first experiment pyridoxine was given for 3, 6, and 12 days beginning immediately after inoculation. In the second experiment the times were 5 and 12 days immediately after inoculation, and the last 8 days of the experimental period only. PVM was titered as described under Methods.

The results are recorded in Table III and Fig. 5. It will be seen that the groups of mice which received no pyridoxine for the first 4 days of the experi-

TABLE III

The Effect of Varying the Time of Administration of Pyridoxine 1 Mg. per 100 Cc. in the Drinking Water of Mice Fed Purina Dog Chow on Their Susceptibility to PVM

No. of experiment	No. of mice	Days of pyridoxine* after inoculation	M.S.50 end point (reciprocal of log)	Log difference‡
1	24	0	3.13	—
1	24	3	3.06	0.07
1	24	6	3.41	0.28
1	24	12	3.56	0.43
2	24	0	3.15	—
2	24	5	3.20	0.05
2	24	8§	3.17	0.02
2	24	12	3.39	0.24

* Pyridoxine in distilled water was given to drink for the number of days indicated after inoculation. For the remainder of the 12 day experimental period the mice drank distilled water alone.

‡ In both experiments the M.S.50 end point in mice receiving no pyridoxine in the water was the reference point.

§ In this titration no pyridoxine was given for the first 4 days after inoculation but was added to the drinking water for the remaining 8 days of the experiment.

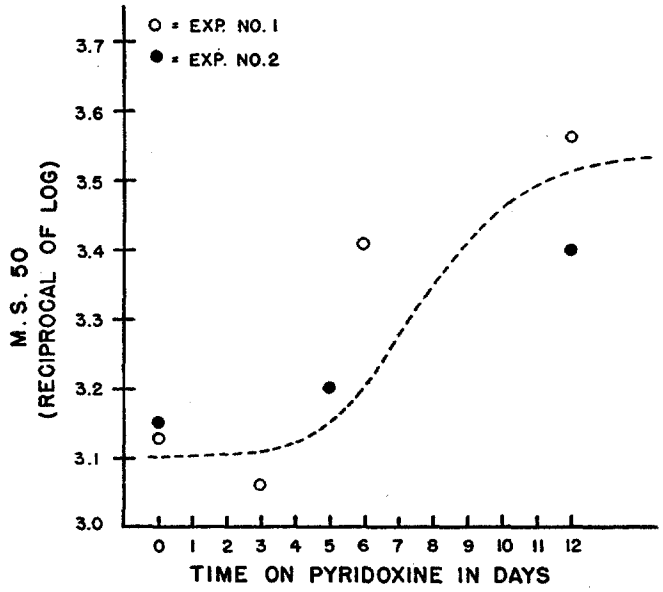

FIG. 5. Relationship between the duration of pyridoxine administration and the infectivity titer of PVM in mice fed Purina dog chow. The pyridoxine was given as a solution, 1 mg. per 100 cc. of drinking water, for the times indicated after virus inoculation.

ment and then the full amount for the remaining 8 days showed a titer similar to those receiving no pyridoxine at all. When pyridoxine was supplied for the first 6 days or more of the experimental period the infectivity titer (M.S.50 end point) increased with the number of days of pyridoxine administration. When supplied for shorter periods no effect was observed. In Fig. 5 the plotted points relating time and infectivity titer seem to fit a sigmoid curve.

Effect of Varying Amounts of Pyridoxine.—In order to determine what dosage of pyridoxine would produce the maximum effect on infectivity titer of PVM, two similar experiments were performed each with 96 weanling mice.

TABLE IV

The Effect of Various Concentrations of Pyridoxine in the Drinking Water of Mice on Their Susceptibility to PVM

Diet S-3		M.S.50 end point (reciprocal of log)			
		Concentration of pyridoxine in drinking water, *mg. per 100 cc.**			
No. of experiment	No. of mice	0	0.1	1.0	10.0
1	96	2.05	2.69	3.48	2.45
2	96	2.69	3.20	3.44	3.12

* Pyridoxine in the various concentrations was constantly available during the 12 day experimental period after inoculation.

The 96 mice were divided into four similar groups of 24 mice, each group being inoculated with varying dilutions of PVM as described under Methods. The mice were all fed diet S-3 during the entire 12 days of the experiment. During this time the four groups were given to drink, beginning immediately after inoculation, the following in distilled water: (*a*) distilled water, alone, (*b*) pyridoxine 0.1 mg. per 100 cc. (*c*) pyridoxine 1.0 mg. per 100 cc., (*d*) pyridoxine 10.0 mg. per 100 cc. This amounted to an average daily pyridoxine intake per mouse of: (*a*) none, (*b*) 5 γ, (*c*) 50 γ, and (*d*) 500 γ, in the respective groups.

The results of these experiments are recorded in Table IV which shows a higher infectivity titer in mice receiving all three dosage levels of pyridoxine than in mice given no pyridoxine at all. Those receiving 0.1 mg. pyridoxine per 100 cc. of water showed a susceptibility intermediate between that of the mice receiving no pyridoxine and those receiving 1.0 mg. per 100 cc. The lower susceptibility of mice receiving 10.0 mg. per 100 cc. in both experiments is unexplained and deserves further investigation.

Effect of Desoxypyridoxine[8].—Since desoxypyridoxine has been found to be a powerful inhibitor of the biological activity of pyridoxine in chicks (35, 36), dogs and monkeys (35), and rats (38), it seemed possible that this vitamin analogue might have some influence on the susceptibility of mice to infections with PVM. In a preliminary experiment weanling mice after inoculation with

[8] Obtained from Merck and Co., Inc.

PVM were fed a diet containing no pyridoxine (S-3) and were given desoxypyridoxine 2.0 mg. per 100 cc. of drinking water for 6 days. This resulted in the death of half of the mice during the initial 8 days of the experiment, without pneumonia and presumably from poisoning with desoxypyridoxine. A similar experiment with both pyridoxine 1 mg. per 100 cc. and desoxypyridoxine 2 or 4 mg. per 100 cc. in the drinking water resulted in an M.S.50 end point about equal to that in control mice on diet S-1. Further PVM titrations in young mice given Purina dog chow and desoxypyridoxine 0.1 mg. per 100 cc., 0.3 mg. per 100 cc., or 1.0 mg. per 100 cc. in the drinking water (or an average of 5, 15, or 50 γ per mouse per day) are summarized in Table V. It will be seen that the M.S.50 end points in two such titrations in mice on 1.0 mg. of desoxypyridoxine per 100 cc. were 0.58 and 0.15 log unit less than in the control

TABLE V

The Effect of Desoxypyridoxine in the Drinking Water of Mice Fed Purina Dog Chow on Their Susceptibility to PVM

Purina dog chow		M.S.50 end point (reciprocal of log)			
		Concentration of desoxypyridoxine in drinking water, *mg. per 100 cc.**			
No. of experiment	No. of mice	0	0.1	0.3	1.0
1	96	3.34	3.19	3.32	2.76
2	48	2.57			2.42

* Desoxypyridoxine in varying concentrations in the drinking water was constantly available during the 12 day experimental period after inoculation.

mice. The smaller concentrations of oral desoxypyridoxine did not seem to influence the infectivity of PVM in mice fed the Purina dog chow. Moreover, the intransal inoculation of 1 mg. of desoxypyridoxine per mouse, either in the suspensions of PVM or on the 3rd day after the virus, did not seem to affect appreciably the observed M.S.50 end point in mice fed Purina dog chow.

The Mechanism of Pyridoxine Effect on Susceptibility.—Several mechanisms for the relative resistance to PVM of mice receiving no dietary pyridoxine after virus inoculation were considered: First, virus multiplication might be less active in mice receiving no pyridoxine. Second, pyridoxine deficiency might interfere with the virulence of virus but not its multiplication. Third, pyridoxine deficiency might affect not the virus but the mouse lung tissue itself resulting in lessened susceptibility to infection. Fourth, pyridoxine deficiency for short periods might stimulate the immune response of the mouse to this virus and thus decrease its susceptibility to infection. Experiments were devised as described below in an attempt to test some of these possibilities.

The Amount of Virus Present in the Lungs of Mice Fed Pyridoxine Compared with the Amount in Mice Fed No Pyridoxine.—

Forty-two young mice were each inoculated intranasally with 0.05 cc. of PVM (10^{-1} dilution). One-half (A) was fed, for the whole experimental period, diet S-1 containing pyridoxine, and the other half (B) was fed diet S-3 which was pyridoxine-free. After 6 days all the mice were sacrificed. The heart blood from each group was pooled separately and its serum was separated and stored at 4°C. for antibody studies. The lungs from each group were collected with aseptic precautions, pooled separately, weighed, ground for exactly 6 minutes in a mortar with alundum, and suspended in 9 parts of trypticase soy broth. After simultaneous light centrifugation the supernates were placed in lusteroid tubes, frozen quickly, and stored at −70°C. in the CO_2 ice chest. A week later these suspensions were thawed and an aliquot of each was saved for hemagglutination tests. Another sample of each pool was titered in the usual fashion in similar groups of 24 young mice fed Purina dog chow. They were sacrificed at 12 days and the M.S.50 end points calculated. The aliquots to be used for hemagglutination were heated at 70°C. for 30 minutes, cleared by centrifugation, and tested as described under Methods.

TABLE VI

Influence of Pyridoxine in Diet on PVM Content of Mouse Lungs

Virus Source Lung suspensions from mice* on various diets after inoculation		Amount of virus—measured by	
Experiment no.	Pyridoxine in diet	Infectivity titer M.S.50 end point (reciprocal of log)	Hemagglutination titer *vs.* mouse RBC (final dilution of heated lung suspension)
1 A	+	3.33	1:640
1 B	0	2.46	1:80
2 A	+	4.25	1:320
2 B	0	3.93	1:160

* The mice were sacrificed on the 6th day after inoculation and the virus suspensions were prepared as described under Methods.

The above experiments were repeated in entirety. The results are shown in Table VI. It will be noted that in each set the infectivity titer (M.S.50 end point) and hemagglutination titer were higher for the lung suspension from mice fed pyridoxine than from pyridoxine-deficient animals. In the first set of experiments this difference was 0.87 log unit, or nearly eightfold for infectivity titer, and eightfold for hemagglutination titer. In the second set these differences were similar but of lesser degree. These results indicate that the amount of PVM, as measured by infectivity or hemagglutination, was greater in each instance in mice fed pyridoxine during the incubation period of the infection. This implies that pyridoxine in the diet of the host during the incubation period is essential for optimal multiplication of this virus.

Virus-Binding Capacity of Mouse Lung Suspensions.—Others (29) have demonstrated the capacity of mouse tissues to combine with free PVM in such a fashion that this virus will no longer cause the agglutinationof mouse eryth-

rocytes. Mouse lung contains this binding substance which may represent the natural receptor for virus. Experiments were devised to test the virus-binding capacity of the lungs of mice fed diets which contained or were free from pyridoxine as described below.

The lung suspension from the PVM inoculated mice in groups A and B described in the preceding section together with similar 10 per cent lung suspensions from two groups, each of 7 uninoculated young mice held on diets S-1 and S-3, were tested. Each of these six lung suspensions was diluted to 2 per cent with 0.85 per cent saline before testing. They were not heated.

A suspension of PVM for hemagglutination was prepared from infected mouse lungs as described under Methods. This was placed in 0.2 cc. amounts into each of six test tubes. Then, to each of these tubes was added 0.2 cc. of one of the six unheated mouse lung suspensions to be tested. A control tube contained 0.2 cc. heated PVM and 0.2 cc. of 0.85 per cent saline. The mixtures were kept for 2 hours at room temperature, and then were centrifuged, together with an aliquot of each of the unheated lung suspensions, for 30 minutes at 3000 R.P.M. The resulting supernates were removed and tested for hemagglutination titer against mouse erythrocytes. The results of these experiments are summarized in Table VII. They show no difference in the virus-binding capacity of lung suspensions from mice which received ample pyridoxine for 6 and 8 days as compared with similar suspensions from mice which received no pyridoxine for that period. This was true whether these lungs were infected with PVM or not.

Antibodies in Mice Fed a Diet Containing Pyridoxine and a Pyridoxine-Free Diet.—Because of the short period involved it seemed unlikely that circulating antibody titers would vary in mice fed different diets for only 6 to 8 days. Nevertheless, two serum pools described above, which were obtained 6 days after inoculation with PVM from similar groups of mice fed diet S-1 or S-3, were titered for PVM antibodies. These two serum pools, together with a pool from uninoculated young mice bled on arrival, were inactivated by heat at 56°C. for 30 minutes. They were tested as described under Methods in dilutions of 1/10, 1/15, and 1/250 for neutralizing antibodies against a low concentration of PVM (the M.S.50 end point was $10^{-1.3}$). All mice used in these neutralization tests were fed Purina dog chow. The results are recorded in Table VIII.

It will be seen that the neutralizing antibody titer in the serum from these groups of young mice was in each instance low and that, as anticipated, there was no difference between the two diet groups and freshly weaned mice.

The Effect of Protein in the Diet.—Evidence has accumulated that pyridoxine is concerned in the metabolism of protein. It may function as a coenzyme in the decarboxylation (39–42) and transamination (43–46) of a number of amino acids. There is evidence that the metabolism of tryptophane (38, 47, 48) in particular is involved. The administration of large amounts of protein (49–53) or of certain amino acids, tryptophane (50) or methionine (54), may accelerate the changes attributable to pyridoxine deficiency in animals. It was decided to test the effect of varying the protein intake of mice given a standard amount of pyridoxine on their susceptibility to infection with PVM.

Four duplicate titrations of PVM in freshly weaned mice were carried out on different occasions. In each experiment one group of mice was fed diet S-1 containing pyridoxine and

30 per cent casein. A similar group was fed this diet but with less casein, which was replaced by an equivalent weight of dextrose. In this manner a 10 per cent casein diet (S-2) was tested

TABLE VII

*The Virus-Binding Capacity of Suspensions of Infected and Uninfected Lungs from Mice on Diet S-3 with and without Added Pyridoxine**

Source of mouse lung suspension (final dilution 1 per cent)			Hemagglutination titer of unheated lung suspension *vs.* mouse RBC	Hemagglutination titer of heat-released PVM after incubation with unheated lung suspension *vs.* mouse RBC	PVM-binding titer‡
Experiment no.	Pyridoxine in diet	Infected with PVM			
1 A	+	+	0§	16	40
1 B	0	+	0	16	40
2 A	+	+	0	32	20
2 B	0	+	0	32	20
3 A	+	0	0	16	40
3 B	0	0	0	16	40
Control (no lung suspension)			—	64	0

* Pyridoxine was given as a 1 mg. per 100 cc. solution in the drinking water throughout the experiment.

‡ The PVM-binding titer is corrected to that of a 10 per cent lung suspension, hence it is determined by multiplying the reduction of agglutination titer by 10.

§ No agglutination was observed when undiluted mouse lung suspension was mixed with an equal volume of the erythrocyte suspension.

TABLE VIII

PVM Neutralization Tests with the Sera of Mice Fed a "Complete" Diet (S-1) or a Pyridoxine-Free Diet (S-3)

Age of mice	Time on diets	Pyridoxine in diet	Infectivity score*			
			Serum dilutions			No serum
			1/10	1/50	1/250	
wks.	*days*					
Control	—	—	—	—	—	12/5
3	0	—	5/5	9/5	8/5	
4	6	+	3/5	4/5	10/5	
4	6	0	3/5	5/5	11/5	

* The mice were inoculated with approximately 1 M.S.50 dose of virus.

three times and a 4 per cent casein diet (S-4) twice. The mice receiving 10 per cent casein failed to gain weight and those on 4 per cent casein lost weight during the experimental period as shown in Fig. 1.

The results of the titrations calculated as M.S.50 end points are shown in Fig. 6. It will be seen that under the experimental conditions described variation of casein intake produced no obvious effect on mouse susceptibility.

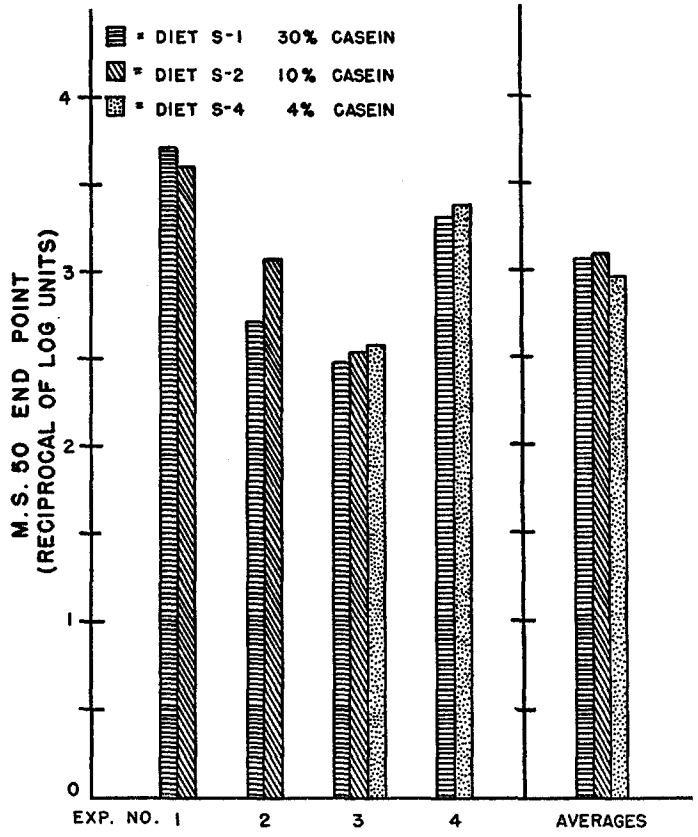

FIG. 6. Infectivity titer of PVM in mice fed partially synthetic "complete" diets containing the same amount of pyridoxine, but with variation in protein (casein), during the entire 12 day experimental period after inoculation. These diets were started on the day of inoculation when the mice were approximately 3 weeks old. This is a summary of four experiments, each using 48 mice.

Similar experiments with different proteins and in pyridoxine-deficient mice are planned.

DISCUSSION

The experimental results reported in this paper indicate that the susceptibility of mice to infection with PVM is affected by the diet they are fed after inoculation.

In 1911 Rous (5) noted that sick or malnourished fowls were more resistant to infection with the virus of fowl sarcoma than healthy, well nourished birds. Olitsky, Traum, and Schoening (6) in 1928 reported similar findings with guinea pigs and the virus of foot-and-mouth disease. Rivers (7) and Sprunt (8) found this to be true in experimental infections with vaccinia. Numerous workers have observed the same phenomenon with the neurotropic viruses of poliomyelitis (9–18), Theiler's encephalomyelitis (15, 19, 21), avian encephalomyelitis (20), and Western equine encephalomyelitis (22). No published reports have been encountered concerning any pneumotropic virus infection.

The effect of a number of specific nutritional factors has been tested including thiamin (9, 11–13, 17, 20, 22), pantothenic acid (14), riboflavin (10, 15), inositol and biotin (16), folic acid (18), minerals (19), and tryptophane (21). Deficiencies in some of these substances, notably thiamin, phosphorous, and tryptophane, may of themselves result in lesions of the nervous system leading to confusion when testing a neurotropic virus.

Only three reports concerning pyridoxine and virus infections have appeared. Lichstein and his associates (16) found this substance by mouth to have little effect on the susceptibility of mice to Theiler's GD VII virus or the Lansing strain of poliomyelitis inoculated intracerebrally. Wooley and Murphy (24) showed that desoxypyridoxine inhibited the multiplication of a bacteriophage of *E. coli*. Bodian (55) described the spontaneous development of poliomyelitis in several monkeys receiving dietary desoxypyridoxine. This finding, suggesting increased susceptibility of a deficient animal to a virus infection, is contrary to usual experience and will be discussed in the accompanying paper.

In none of the published studies has evidence been presented which elucidates the mechanisms involved in the relation between nutrition and the susceptibility of the host to virus infections. The experiments reported in the present paper throw some light on these mechanisms. They indicate that mice fed a complete diet for the first 5 days or more after inoculation were more susceptible to PVM than similar mice denied pyridoxine or fed desoxypyridoxine. This did seem to depend on any differences in the titers of circulating antibodies in mice of the two regimes or in the PVM-binding capacity of their lung tissues. However, more virus was demonstrated by both infectivity and hemagglutination titrations in the lungs of the mice that received pyridoxine. This suggests that during the incubation period of the disease pyridoxine was essential for optimal virus multiplication.

It is obvious that nutritional factors besides pyridoxine may well be concerned in the complex relationship between the mouse and PVM. Additional studies are planned to explore this possibility. The evidence presented in the following paper suggests a dynamic equilibrium between host and virus and indicates that the duration of deficiency may be of the greatest importance in affecting this equilibrium.

CONCLUSIONS

Young mice fed diets deficient in pyridoxine or fed desoxypyridoxine after the inoculation of the pneumonia virus of mice were more resistant to infection than well nourished controls.

The susceptibility of young mice to PVM increased with the duration of pyridoxine administration after inoculation.

Dietary protein restriction when pyridoxine was provided did not affect the susceptibility of mice to PVM.

The PVM-combining capacity of mouse lung and the titer of humoral antibody against PVM were the same in mice fed a complete or pyridoxine-deficient diet for 6 days.

The amount of PVM in mouse lungs 6 days after inoculation was greater by both infectivity and hemagglutination titrations in mice fed pyridoxine than in pyridoxine-deficient controls. This suggests that pyridoxine was essential during the postinoculation period for optimal virus multiplication.

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