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

II. Influence of Pyridoxine Administered in the Period before as Well as after the Inoculation of Virus*

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In the preceding paper (1) it was shown that young mice fed diets deficient in pyridoxine during the period after inoculation with pneumonia virus of mice (PVM) were less susceptible to infection with this virus than animals fed a complete natural diet, or partially synthetic diets containing pyridoxine. These findings substantiated the observations of most workers that deficient animals are relatively insusceptible to virus infections. However, certain noteworthy exceptions to this general rule have been reported by others.

Kuczynski (2) found that monkeys or mice fed restricted diets seemed to be more rather than less susceptible to infection with yellow fever virus. Pinkerton and Swank (3) noted that certain pigeons died of spontaneous infection with a psittacoid virus 6 to 10 days after their diet had been restricted in thiamin and other vitamins of the B complex. They postulated a causal relationship between the deficiency and the infection.

Sabin and Duffy (4), and Sabin (5) published extensive experiments with mice and the virus of vesicular stomatitis of horses. Mice are normally 100 per cent susceptible to a fatal ascending paralysis after intramuscular inoculation of this virus at 2 weeks of age, and 100 per cent resistant at 6 weeks or older. Sabin and Duffy showed that this "maturation resistance" could be delayed by dietary deficiency. Young mice which were denied certain vitamins of the B complex, notably thiamin or riboflavin, were more susceptible to the infection at 4, 5, and 6 weeks than control mice fed a full diet.

MacCallum and Miles (6) inoculated material containing the virus of infectious hepatitis into rats fed diets that contained normal or slightly deficient amounts of protein. They described the initiation of a transmissible disease in the deficient but not in the well nourished animals.

Cooperman *et al.* (7) showed that under certain conditions thiamin-deficient chicks were more susceptible than controls to infection with avian encephalomyelitis virus.

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Wilson and his coworkers (8) reported that 5 of 7 undernourished monkeys died after the intratracheal inoculation of influenza A virus (strain PR8) whereas 10 well nourished controls developed only slight signs of infection.

Bodian (9) noted the development of paralytic poliomyelitis in 1 uninoculated and 2 orally inoculated monkeys that previously had been fed desoxypyridoxine for 10 or more days. Normal monkeys fed a complete diet were never infected by oral inoculation with that strain of virus. This observation, together with the others just described, implies that under certain conditions animals fed diets deficient in a variety of factors may show a lowered resistance to some virus infections. Experiments will be reported in the present paper which suggest that the duration of dietary deficiency may have a potent influence on the host's ultimate resistance to a virus infection. The object of the present study was to test the effect of prolonged dietary deficiency on the host's susceptibility to a virus infection. The effects of pyridoxine deficiency of mice for varying periods before PVM inoculation, as well as after, will be described.

Materials and Methods

The mice, diets, and techniques used in the experiments reported below are identical with those described in detail in the preceding paper (1).

EXPERIMENTAL

Effect of Purina Dog Chow and Milk-Wheat Diet Fed for Varying Times before Inoculation with PVM.—In the preceding paper it was shown that young mice fed Purina dog chow for the 12 day experimental period after inoculation with PVM were slightly less susceptible to this infection than similar mice fed a natural milk-wheat diet. Two experiments were carried out to test the effect of longer periods on these two diets.

Forty-eight freshly weaned mice were divided into two equal groups. One group of 24 was fed Purina dog chow and water and the other group milk-wheat diet and water for 8 days. At that time simultaneous duplicate titrations of PVM were done in both groups. The two dietary regimes were continued during the next 12 days at which time all surviving mice were sacrificed and the infectivity titer (M.S.50 end point) was calculated. In a similar experiment the mice were held for 15 days on these two diets before inoculation. The results of these two experiments and of three others in which two diets were fed only during the post-inoculation period are recorded in Table I.

It will be seen that the infectivity titer in the short experiments was in each instance lower in mice fed the Purina dog chow than in mice fed the natural diet. When longer periods were studied, however, the reverse was true. The infectivity titer in the mice fed Purina dog chow for 8 days before inoculation was greater by 0.37 log unit than in similar mice fed the milk-wheat diet for that time. When the preinoculation period on the two diets was 15 days a similar but more marked difference of 0.86 log unit was found. These results are recorded graphically in Fig. 1. In this figure the susceptibility of mice on the natural milk-wheat diet was arbitrarily chosen as the reference line, and the

log differences in duplicate titrations were plotted in relation to this line so that negative values indicate lesser and positive values greater susceptibility to infection.

TABLE I

The Effect of the Length of Time Prior to Inoculation That Mice Were Fed Purina Dog Chow or Milk-Wheat Diet on Their Susceptibility to PVM

No. of experiment	No. of mice	No. of days on diets before inoculation	M.S.50 end points (log units)		Difference* (log units)
			Diet		
			Milk-wheat	Purina dog chow	
1	48	0	−3.09	−2.64	−0.45
2	48	0	−3.00	−2.72	−0.28
3	48	0	−2.84	−2.64	−0.20
Average.....		0	−2.98	−2.66	−0.31
4	48	8	−2.37	−2.74	+0.37
5	48	15	−1.70	−2.56	+0.86

* In each experiment the difference between titrations was calculated by subtracting the M.S.50 end point (log units) of PVM in mice fed Purina dog chow from the end point in mice fed milk-wheat diet.

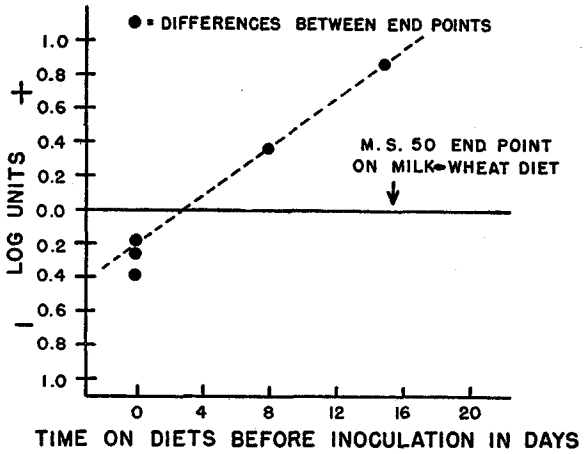

FIG. 1. The differences in log units between the M.S.50 end points of PVM in mice fed the milk-wheat diet or Purina dog chow for varying lengths of time before inoculation as well as after inoculation.

Effect of Diet S-1 and Diet S-3 Fed for Varying Times before Inoculation with PVM.—In the preceding paper (1) it was shown that young mice fed a synthetic diet deficient in pyridoxine (S-3) for the 12 day experimental period after

inoculation with PVM were less susceptible to infection with this virus than similar mice fed a diet containing pyridoxine (S-1). Seven experiments were done to test the effect of longer periods of deficiency before virus inoculation.

In each experiment 48 freshly weaned mice were divided into two comparable groups on the day of their arrival from the breeder. One group was fed the pyridoxine-free diet (S-3) and the other group the "complete" diet (S-1), or in some cases diet S-3 with the addition of

TABLE II

The Effect of the Length of Time Prior to Inoculation That Mice Were Fed a Diet Containing Pyridoxine (S-1) or a Pyridoxine-Deficient Diet (S-3) on Their Susceptibility to PVM

No. of experiment	No. of mice	No. of days on diets before inoculation	M.S.50 end points (log units)		Difference* (log units)
			Diet		
			S-1	S-3	
1	48	0	−3.69	−3.24	−0.45
2	48	0	−2.71	−1.80	−0.91
3	48	0	−2.29	−2.04	−0.25
4	48	0	−3.29	−2.08	−1.21
5	48	0	−2.00	−1.50	−0.50
6	48	0	−3.48	−2.05	−1.43
7	48	0	−2.56	−2.34	−0.22
8	48	0	−3.39	−3.15	−0.24
9	48	0	−3.44	−2.69	−0.75
Average.....			−2.99	−2.32	−0.67
10	48	8	−1.51	−0.70	−0.81
11	48	8	−2.75	−2.95	+0.20
12	48	11	−0.90	−1.25	+0.35
13	48	14	−0.75	−1.04	+0.30
14	48	16	−2.14	−2.75	+0.61
15	48	19	−2.00	−2.47	+0.47
16	48	19	−1.49	−2.35	+0.86

* In each experiment the difference between titrations was calculated by subtracting the M.S.50 end point of PVM (log units) in mice fed diet S-3 from the end point in mice fed diet S-1.

pyridoxine 1 mg. per 100 cc. of drinking water. Mice on the latter regime were considered to be comparable to mice which received diet S-1 which contained pyridoxine. After varying lengths of time on the two regimes, all the mice in each group were inoculated with simultaneous duplicate titrations of PVM. The diets were continued for the 12 day period following inoculation when all surviving mice were sacrificed and the M.S.50 end point of infectivity titer was calculated.

In this way the effects of dietary differences for preinoculation periods of 8, 8, 11, 14, 16, 19, and 19 days were tested. The results of these experiments

together with nine others where the two diets were fed only during the post-inoculation period are recorded in Table II and Fig. 2. It will be seen that whereas the infectivity score was less in each case in the mice fed the pyridoxine-deficient diet during the postinoculation period only, the reverse was true in six of the seven experiments where the deficiency was of longer duration. Moreover, within the periods studied, the relative increase in susceptibility of pyridoxine-deficient mice was roughly proportional to the time they were fed the deficient diet before inoculation.

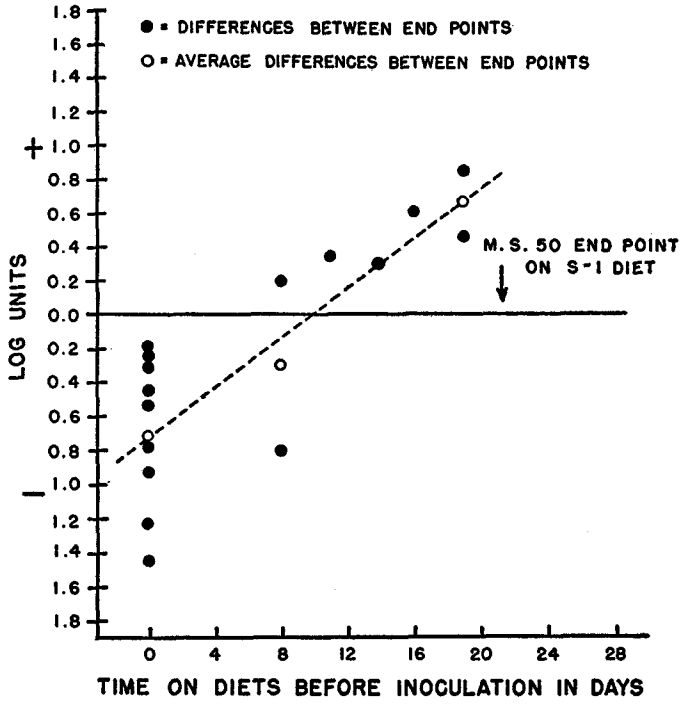

FIG. 2. The differences in log units between the M.S.50 end points of PVM in mice fed a partially synthetic "complete" diet (S-1) or a partially synthetic pyridoxine-free diet (S-3) for varying lengths of time before inoculation as well as after inoculation.

Effect of Pyrodoxine Deficiency on the Growth and the Lymphoid Tissues of Mice.—

The effect of pyridoxine on the growth of young mice was tested. Five freshly weaned mice with an average weight of about 8 gm. were fed a diet which contained pyridoxine (S-1), and a similar group of mice were fed the pyridoxine-free diet (S-3) for 6 weeks. The two groups were weighed at intervals. The average weights are plotted in Fig. 3. It will be seen that the two groups gained equally for 5 weeks but the deficient mice lost weight in the 6th week.

All the mice were sacrificed at the end of 6 weeks. The thymus glands and spleens were removed and weighed. These organs were then fixed, together with selected lymph nodes in 10 per cent formalin, and sections were made by the usual pathological techniques for histological examination. The results concerning body and organ weights are recorded in Table III. It will be seen that the mice fed the pyridoxine-deficient diet for 6 weeks weighed an average of 15.4 gm. and were lighter by 3.5 gm. than similar mice fed this same diet plus pyridoxine. The relative differences in the weights of the thymuses and spleens between the two groups were more marked. Both of these organs were smaller in the pyridoxine-deficient animals. This is immediately ap-

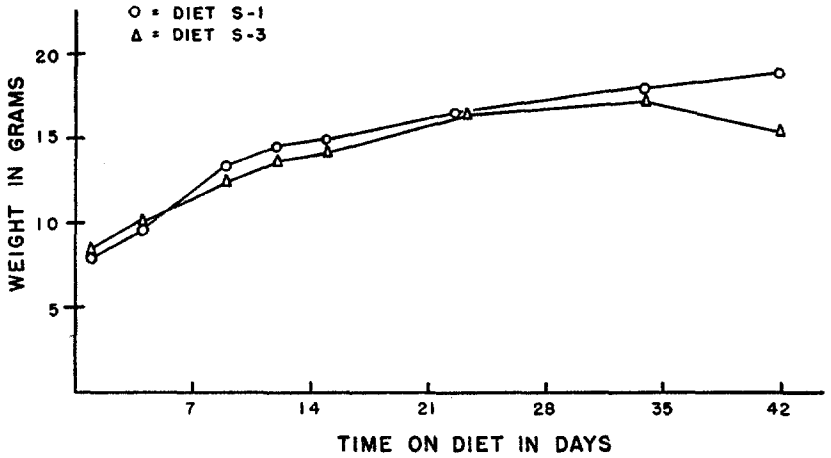

FIG. 3. Weight curves of mice fed the partially synthetic "complete" diet (S-1) or the partially synthetic pyridoxine-free diet (S-3) for 6 weeks. Each point represents the average weight of 5 mice. The mice were weanlings, approximately 3 weeks old at the beginning of the experiment. Diets were supplied in excess at all times.

parent on comparing the ratios of thymus or spleen weight to total body weight in the two groups.

On histological examination the spleens of the pyridoxine-deficient mice showed hypoplasia of the lymphoid elements and hemosiderosis. The thymuses and lymph nodes of these deficient mice were also hypoplastic.

Effect of Pyridoxine Deficiency for 6 Weeks on Humoral Antibodies against PVM in Uninoculated Mice.—Stoerk (10, 11) and Axelrod (12) and their coworkers reported that young rats fed pyridoxine-deficient diets for 5 or 7 weeks respectively responded to the injection of heterologous erythrocytes with lower titer antibodies than control rats that received pyridoxine.

It seemed reasonable that the increased susceptibility of mice to infection with PVM when fed a pyridoxine-deficient diet for 8 or more days before in-

oculation might be explained on the basis of impaired synthesis of circulating antibodies against this latent virus.

To test this hypothesis a group of 25 freshly weaned mice about 3 weeks old was fed a partially synthetic diet containing pyridoxine (S-1). A comparable group of mice was fed a

TABLE III

*The Effect of Pyridoxine Deficiency for 6 Weeks on Spleen, Thymus, and Body Weights of Mice**

Diet	Pyridoxine	Average initial body weight	Average weights after 6 wks. on diets				
			Body	Thymus	Spleen	Ratio × 10^4	
						Thymus/body	Spleen/body
		gm.	*gm.*	*gm.*	*gm.*		
S-1	+	8.1	18.9	0.068	0.124	36	66
S-3	0	8.3	15.4	0.038	0.051	25	33

* Five mice were fed each diet for the 6 week experimental period.

TABLE IV

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

Age of mice	Time on diets	Pyridoxine in diet	Infectivity score*			No serum
			Serum dilution			
			1/10	1/50	1/250	
Control	—	—	—	—	—	12/5
3 wks.	0	—	5/5	9/5	8/5	—
4 "	1 wk.	+	3/5	4/5	10/5	—
4 "	1 "	0	3/5	5/5	11/5	—
7 "	4 "	+	3/5	4/5	8/5	—
7 "	4 "	0	2.5/5	5/5	3/5	—
9 "	6 "	+	1/5	4/5	4/5	—
9 "	6 "	0	0.5/5	2/5	2.5/5	—

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

similar but pyridoxine-free diet (S-3). At the end of 1 week, 4 weeks, and 6 weeks, 7 or 8 members of each group were exsanguinated and the specimens of blood from the mice on each regime were pooled separately. The serum was separated and stored with sterile precautions at 4°C. The resulting six serum pools together with a similar pool from normal mice just weaned, were inactivated by heating to 56°C. for 30 minutes and tested for neutralizing antibodies against PVM as described in the preceding paper. Approximately 1 M.S.50 dose of virus was used with serum dilutions of 1:10, 1:50, and 1:250.

The results of these tests are shown in Table IV. It will be seen that the sera from the older mice contained slightly more neutralizing antibody than those from younger mice. However, no difference was apparent between mice that received or were denied pyridoxine during the experimental period.

Further experiments concerning antibody production in mice inoculated with sublethal doses of virus are anticipated.

DISCUSSION

The observations reported in this and the preceding paper indicate that an animal fed a deficient diet may be either more or less susceptible to a virus infection than completely nourished controls, depending upon the duration of the deficiency. Attention was directed to one dietary component, pyridoxine, and to one disease, the respiratory infection caused by PVM in mice. There is no reason to assume, however, that the changing effect of diet with time may not apply to other essential factors in the diet besides pyridoxine, and to other virus diseases besides PVM infection.

When the mice were fed the deficient diet only during the period following virus inoculation the results agreed with those of most other observers, since the deficient animals were then less susceptible to infection than well nourished controls. However, when pyridoxine deficiency was extended to include several weeks of the preinoculation period the reverse situation occurred and deficient animals were then more susceptible to infection than controls.

At first glance the present data suggest that the susceptibility of mice fed the deficient diets actually remained constant and, in the long term experiments, was greater than the susceptibility of control mice fed complete diets only because the latter mice became relatively resistant. It is generally recognized that mice may develop increased resistance to PVM with maturity. However, the data do not lend themselves to this kind of analysis since no attempt was made to standardize the infectivity titer of the virus in different experiments. It is obvious that the inoculation of low titer virus in the long term experiments might equally well explain the experimental results.

One is nevertheless reminded of the observations of Sabin and Duffy (4) that the "maturation resistance" of mice to infection by the virus of vesicular stomatitis developed more slowly when the animals were fed diets deficient in certain components of the B complex of vitamins. Vesicular stomatitis virus is a neurotropic agent to which mice are not naturally exposed. Therefore, it is not surprising that the ordinary mechanisms for specific acquired immunity were apparently not involved in the "maturation resistance" of mice to that agent. On the other hand, PVM is a pneumotropic virus naturally occurring, in latent form, in many strains of mice and causing manifest infection only in that species unless adapted to others. For this reason it has seemed reasonable to suppose that the relative resistance of older mice to infection with PVM

probably reflected some degree of acquired immunity resulting from previous exposure to the latent virus. Horsfall and Hahn (13) were able to demonstrate circulating antibodies against PVM in the sera of 8-week-old mice. However, these authors were unable to correlate the antibody titer observed in various strains of mice with the presence of infectious virus in the strain.

Since pyridoxine deficiency may interfere with antibody production (10–12) it was anticipated that antibody titers against PVM would be lower in mice fed a pyridoxine-deficient diet for 6 weeks. Such a finding would explain the greater susceptibility of chronically deficient mice to PVM infection. It was therefore surprising to find no difference in the titer of anti-PVM antibodies in uninoculated mice fed complete or pyridoxine-deficient diet for 6 weeks. Similar experiments in mice inoculated with sublethal doses of virus are anticipated.

It is possible, of course, that specific acquired immunity of the mouse's lung tissues may be involved in resistance to PVM rather than humoral antibodies. We have not yet done experiments to test this possibility or its relation to pyridoxine deficiency.

Cooperman, Lichstein, Clark, and Elvehjem (7) have reported that the susceptibility of thiamin-deficient chicks to infection following the intracerebral inoculation of avian encephalomyelitis virus may be either more or less than in well fed controls depending on the duration of thiamin deficiency before inoculation. These authors noted that one-day-old chicks fed a deficient diet for 1 week or less before inoculation, as well as after, were more susceptible to infection than control chicks fed complete diets. Older chicks fed the deficient diet for 2 weeks before inoculation as well as after were less susceptible to infection than well fed controls. These results appear to be the direct opposite of those reported in this paper. However, they confirm the thesis that a dynamic equilibrium between host and virus may exist. Since different virus, host, mode of inoculation, and dietary deficiency were tested, it is not surprising that different time relationships were apparent.

Because of the many variables it seems impossible to analyze the data of others in terms of dynamic equilibrium. Whatever the mechanisms involved, however, the observations here described may help to explain the occasional reports by others (2–9) that the poorly nourished rather than the well nourished host is more susceptible to a virus infection.

CONCLUSIONS

Young mice fed diets deficient in pyridoxine for 8 days or longer before the inoculation of PVM, as well as after inoculation, were more susceptible to infection than control mice fed complete diets.

Young mice fed a pyridoxine-deficient diet gained weight as well as controls fed a complete diet for 5 weeks, but they lost weight in the 6th week.

The ratio of thymus or spleen weight to body weight was less in mice fed a pyridoxine-deficient diet for 6 weeks than in controls fed a complete diet. Histologically the thymuses and spleens showed hypoplasia.

No measurable difference in antibodies against PVM was found in the sera of uninoculated mice fed complete or pyridoxine-deficient diets for 6 weeks.

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