THE EFFECT OF VITAMIN B_1 DEFICIENCY AND OF RESTRICTED FOOD INTAKE ON THE RESPONSE OF MICE TO THE LANSING STRAIN OF POLIOMYELITIS VIRUS*

By CLAIRE FOSTER, JAMES H. JONES, PH.D., WERNER HENLE, M.D., AND FRIEDA DORFMAN

(From the Departments of Pediatrics and Physiological Chemistry, The School of Medicine, University of Pennsylvania, and The Children's Hospital of Philadelphia, Philadelphia)

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Little is known concerning the relation of diet to the incidence or progress of poliomyelitis in human beings or in experimental animals. Before Armstrong (2) had produced the disease experimentally in the cotton rat and mouse, Woolpert and Harrison (3) had attempted to infect rats, mice, and guinea pigs on a diet low in vitamins B and C with the virus of this disease. They did not succeed in reproducing any of the symptoms characteristic of poliomyelitis. Several investigators, on the other hand, have claimed that the resistance of human beings and monkeys to the virus of poliomyelitis is decreased by a deficiency of some of the vitamins.

McCormick (4) has reported that in Canada the diets of victims of infantile paralysis are frequently low in vitamin B_1 and that he obtained good results in paralytic cases by administering rather large amounts of thiamine. Ward and associates (5), however, found that in seven cases of human poliomyelitis the excretion of vitamin B_1 in the urine was within normal limits. Jungeblut (6), working with monkeys, has presented evidence to indicate that the incidence of paralysis could be reduced by the administration of rather large amounts of crystalline vitamin C. Sabin (7) attempted to repeat the work of Jungeblut but obtained no protection from vitamin C. Heaslip (8) applied the saturation test for vitamin C to a rather large number of individuals during an epidemic of infantile paralysis in Australia. He concluded that the vitamin stores in the bodies of those with the disease were less than in contacts who remained healthy.

Toomey (9) studied the relation of vitamins A, B complex, C, and D to the susceptibility of monkeys to the virus of poliomyelitis. He found that none of these gave any protection when the virus was administered intracerebrally, and the same was true for vitamins A, B complex, and C, when the virus was injected directly into the lumen of an exposed loop of the intestines. Under these conditions, however, vitamin D gave almost complete protection. Toomey believes that the vitamin acts by preventing the virus from entering the central nervous system along the peripheral nerves. Sabin, Ward, Rapoport, and Guest (10), on the other hand, using the intra-

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sciatic route to inoculate their animals, found that vitamin D afforded no protection and that rachitic animals were no more susceptible than the normal controls.

Relatively little work has been reported on the relation of diet to poliomyelitis in which small experimental animals such as the mouse and cotton rat have been used. Considerable information has been gained recently on the nutrition of the white mouse. This animal is quite similar in its dietary habits to the rat in that it will readily eat a ration composed of purified materials. Owing to its small size the mouse can be used in large numbers with a minimum of food consumption and care. It also reacts in a reproducible manner to the Lansing strain of mouse-adapted virus developed by Armstrong (2). For these reasons the mouse should be a very desirable animal for experiments of this type.

For some time the general problem of the relation of diet to resistance to infection has been studied in this laboratory. In several experiments, the results of which will be published later, the possible relationship between a deficiency of vitamin B_1 in white mice and the susceptibility of these mice to measured doses of Salmonella enteritidis was investigated. For the most part the results were negative.

Realizing that the metabolism of viruses is quite different from that of bacteria, these experiments were extended by keeping the vitamin deficiency the same but using a virus as the infecting agent. For various reasons the virus of poliomyelitis was chosen. The following is a report of the results of these studies and of related additional studies.

Methods and Materials

Mice.—All of the mice were raised in this laboratory on a standard stock diet. The last seven generations of the stock have resulted from a close outcross technique which has been continued and found satisfactory to the present time. This outcross method distributes and redistributes the genetic characteristics so that large numbers of mice with characteristics drawn from a relatively small pool are available, with comparatively little change in the genetic constitution of the colony over a period of years. The young are identified by litters until they are 14 days of age, after which time all data are recorded in relation to individual identity. All litters were reduced to six on the 7th day of age, with as nearly as possible equal numbers of males and females.

The experimental mice were housed in a separate room on raised screens in individual glass jars. The jars were placed on metal racks suspended from the ceiling. The temperature of the colony and experimental rooms was kept at approximately 74°F. and about 45 per cent relative humidity throughout the year. Split litter technique, with consideration for sex and weight of individual animals, was adhered to strictly. Each animal was observed and weighed daily during the experiment.

Diets.—The composition of the diets used in these experiments is given in Table I. The casein was a commercial product (No. 453 edible casein, Casein Company of

America). It was used as received without further purification. Cerelose is a high grade glucose (Corn Products Company). The yeast was powdered dried brewer's yeast (Northwestern Yeast Company). Cerophyl is a mixture of dehydrated powdered grasses (Cerophyl Laboratories).[1] The salt mixture used was salts 12, which has been previously described (11). The linseed oil was a raw product (National Lead Company). The wheat germ oil was obtained from General Mills and the fish liver oil concentrate from National Oil Products Company. At the level used the concentrate furnished 1600 I.U. of vitamin A and 320 I.U. of vitamin D per 100 gm. of diet. Before mixing with the diet it was diluted with corn oil (Corn Products Company). Liver extract E (Wilson)[2] was used as the source of the vitamin B complex other than B_1, and a regenerated cellulose[3] was added to supply roughage. The thiamine was the chloride.[4]

TABLE I

Dietary Ingredients

Diet No.	1	2	3	4	5
Casein, *per cent*	30.0	30.0	67.5	30.0	30.0
Cerelose, *per cent*	59.5	59.5	10.3	59.5	37.5
Yeast, *per cent*	—	—	—	—	6.0
Cerophyl, *per cent*	—	—	—	—	20.0
Salt mixture, *per cent*	4.0	4.0	6.0	4.0	4.0
Linseed oil, *per cent*	1.5	1.5	3.7	1.5	1.5
Wheat germ oil, *per cent*	1.0	1.0	2.5	1.0	1.0
Fish liver oil concentrate, *per cent*	0.008	0.008	0.02	0.008	0.008
Liver extract, *per cent*	2.0	2.0	5.0	2.0	—
Cellulose, *per cent*	2.0	2.0	5.0	2.0	—
Thiamine chloride*	100.0	10.0	250.0	500.0	—

* Thiamine chloride expressed in micrograms per 100 gm. of diet.

Virus.—The mouse-adapted Lansing strain of poliomyelitis virus in the 202nd passage was received through the courtesy of Dr. Charles Armstrong. It was transferred several times in this laboratory and used for the experiments between the 209th and 212th passages. Brains and cords from mice paralyzed 2 to 6 days after inoculation were ground and suspended in sterile saline solution to form a 5 per cent suspension, which was stored in sealed ampules in a dry ice storage cabinet. The virus preparation was thawed just prior to the inoculation and diluted sufficiently with saline solution to yield between 10 and 100 fifty per cent mortality doses per inoculum. In only one experiment more virus was injected (between 100 and 1000 L.D.$_{50}$) as indicated in the text. Suspensions of normal mouse brain were prepared

[1] Kindly supplied by Dr. W. R. Graham, Jr.

[2] Kindly supplied by Dr. C. E. Graham.

[3] Obtained through the courtesy of Dr. C. M. McCay of Cornell University.

[4] Kindly supplied by Merck & Co., Inc.

in an identical manner. The mice were injected intracerebrally under light ether anesthesia with 0.03 ml. of the suspension.

In all of these experiments all animals dying within 48 hours after injection were omitted from the final tabulations, as death may have been due to trauma. The numbers given as the total inoculated are actually those surviving this 2-day period, and percentages have been calculated from these numbers. There were never more than two deaths in an experimental group during this initial period.

EXPERIMENTAL

The Effect of Vitamin B_1 Deficiency.—In the first three experiments, diets 1 and 2 were compared for their influence on the response of mice to intracerebral injection of the poliomyelitis virus. These diets differed only in the amount of thiamine. Diet 1 contained 100 micrograms per 100 gm. of diet, which is sufficient to produce good growth. Diet 2 contained 10 micrograms. On this diet mice show signs of vitamin deficiency in about 2 weeks and were prone to die too soon for satisfactory interpretation of the effects of virus inoculation. The mice were inoculated 20 days after being placed on the experimental diet. In early experiments, at 21 days after inoculation, there was no significant difference in mortality among the groups on diet 1 inoculated with the virus, and those on diet 2 which were inoculated with virus, were injected with normal brain, or were uninjected. There was, however, a considerable difference in the incidence of paralysis between the high thiamine and low thiamine groups inoculated with the standard dose of virus. Of 79 mice which were given diet 1 and inoculated with virus, paralysis was observed in 75 per cent by the 21st day. In contrast to this, of 77 mice which were given diet 2 and virus, only 9 per cent had shown signs of paralysis over the same period. It is possible, but not probable, that paralysis which was not observed preceded death in a few of the animals, inasmuch as the mouse was removed from its cage for examination only at intervals of 24 hours.

In the subsequent experiments an attempt was made to keep the vitamin-deficient animals alive and at approximately constant weight by varying the amount of thiamine in the diet.

In the first of these experiments a total of 82 mice were divided into three groups. One group was given diet 1 and the other two groups were given diet 2. When the animals of the latter two groups showed a moderate vitamin deficiency as indicated by atonia and loss of weight, the amount of thiamine in the diet was increased. Thereafter it was varied either up or down as necessary to prevent much loss or gain in weight. Any dietary changes applied to all animals in these two groups. 26 days after the animals were first placed on the experimental diets the thiamine content of diet 2 was increased to 50 micrograms per 100 gm. of diet. On the 35th day of the experiment, at which time the ages of the mice were from 57 to 65 days, each animal in groups 1 and 2 was injected intracerebrally with 0.03 ml. of the mouse brain suspension infected with the virus, and group 3 was given an equal amount of

suspension of normal brain. Following the injections the amount of thiamine given the animals in groups 2 and 3 varied between 20 and 50 micrograms per 100 gm. of diet until the 54th day after inoculation, when the thiamine was increased to 100 micrograms. No further changes were made during the experiment.

The results of this experiment with respect to deaths and incidence of paralysis are given in Table II, groups 1, 2, and 3. The percentages of deaths and of cases of paralysis by the 12th, 21st, and 46th days are given. By the 12th day 58 per cent of the mice on the complete diet (group 1) had died, in contrast to only 21 per cent of those on the deficient diet (group 2). It is impossible to determine how many of the latter had died from the effects of the

TABLE II

Effect of Vitamin B_1 Deficiency on Response of Mice to Poliomyelitis Virus

Group No.	Diet No.	Amount of thiamine*	Inoculum	No. of mice inoculated‡	12 days		21 days		28 days		46 days	
					Dead	Paralyzed	Dead	Paralyzed	Dead	Paralyzed	Dead	Paralyzed
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	1	100	V	36	58	64	83	75	89	83	92	83
2	2	10+	V	24	21	8	33	17	42	17	58	17
3	2	10+	N	22	14	—	32	—	41	—	45	—
4	1	100	V	55	85	75	95	82	96	82	96	82
5	2	10+	V	166	20	12	42	24	57	36	69	41
6	2	10+	N	57	11	—	14	—	14	—	19	—

V = virus.

N = normal brain suspension.

* Micrograms of thiamine per 100 gm. of diet. 10+ = 10 or more sufficient to keep the mice alive and at approximately constant weight.

‡ The number surviving to 2 days after inoculation.

virus and how many from the vitamin deficiency. However, by this time 14 per cent of the deficient animals injected with the suspension of normal brain had died. At 21 days deaths for the three groups were 83, 33, and 32 per cent, and at 46 days they were 92, 58, and 45 per cent. The differences between the mice on the complete and deficient diets which received the virus (groups 1 and 2) were even greater with respect to paralysis than mortality. At 12 days 64 per cent of the animals in group 1 had been observed in paralysis as compared with only 8 per cent in group 2. Percentages for the two groups were 75 and 17 by the 21st day, and 83 per cent for group 1 as compared with only 17 per cent for group 2 by the 46th day. There was still a significant difference between the normally fed and the vitamin-deficient groups receiving the virus with respect to incidence of paralysis at the 59th day after inoculation, when the experiment was discontinued. By this time the difference in deaths was not statistically significant.

The experiment above described, with only minor changes, was repeated using a larger number of animals.

A total of 278 mice were used, divided into three groups (groups 4, 5, and 6—Table II) similar to the groups 1, 2, and 3 of the previous experiment. There were 55 mice in the group given diet 1 and virus (group 4); 166 animals were given diet 2 and virus (group 5), and 57 animals received diet 2 and suspension of normal brain (group 6). Throughout the experiment the large group 5 was treated as three separate groups, but as there was no significant difference in the results they are presented as a single group. At the start of the experiment groups 5 and 6 were given 10 micrograms of thiamine per 100 gm. of diet and 19 days later the amount was increased to 35 micrograms. 27 days after the institution of the experimental diets the three groups were inoculated as described above. At the time of inoculation the animals were from 47 to 53 days of age. After inoculation the amount of thiamine was again changed either up or down in an attempt to maintain approximately constant weight. On the 20th day after inoculation the amount of thiamine given to one of the subgroups of the large group 5 was increased to 100 micrograms and continued as such for the duration of the experiment.

The results, which are summarized in Table II (groups 4, 5, and 6), again show that the deficiency of vitamin B_1 increased the resistance of the mice to the poliomyelitis virus as measured by the percentage of deaths and the incidence of paralysis. There was a statistically significant difference in both of these factors at the termination of the experiment, which was on the 46th day after inoculation. It is to be noted that in both experiments there was a greater difference with respect to deaths and paralysis between the adequately fed and deficient animals at the 12th day following the inoculation than there was later in the experimental period. This would indicate that the effect of the deficiency was more pronounced in delaying the action of the virus than in actually preventing it.

The Effect of Restricted Food Intake.—One of the outstanding characteristics of vitamin B_1 deficiency is a loss of appetite with a concomitant decrease in consumption of food. It appeared possible that the results described above were due to the decreased intake of food and not directly to the vitamin deficiency. The effect of simply restricting the daily food allowance on the response of mice to inoculation with the virus of poliomyelitis was studied in several experiments. Different diets and variation in time and degree of food restriction were used.

In the first experiment diet 1 was employed and the animals were divided into 5 groups. One group received an *ad libitum* supply of the diet and was given virus. The other four groups were given a restricted amount of the diet. No food was given to two of these groups for 60 hours immediately following the inoculation, after which the amount given was between 1.0 and 1.5 gm. of diet per animal per day. At certain levels of food intake it is possible to observe a difference in gain or loss of

weight in mice when the intake is varied by as little as 0.1 gm. per day. Owing to this fact the amount of food given was not varied by more than 0.2 gm. on consecutive days, and changes were usually made at intervals of not less than 1 week. The amount of food given on any day was the same for all animals in these two groups. One of these groups was injected with virus and the other with normal brain. The two remaining groups, one of which received virus and the other, normal brain, were fasted for 96 hours beginning 8 days before the inoculation. After the period of fasting the animals were each given from 1.0 to 1.5 gm. of the diet per day and again the daily allowance was uniform for all animals for these two groups.

Although the numbers in the various groups were too small to be statistically significant, restricting the amount of diet apparently increased the resistance of these animals to the virus. This resistance was shown in both the incidence of deaths and paralysis. By the 12th day following inoculation the percentages of deaths of the animals given the virus were 74, 25, and 17 respectively for the normally fed group, the group fasted after the inoculation, and the group fasted before the inoculation. The incidence of paralysis at the same time in these groups was 58, 6, and 25 per cent. At 21 days deaths were 100, 56, and 33 per cent, and the incidence of paralysis was 79, 50, and 41 per cent respectively in the same three groups. There were a few deaths in the two groups which were given a restricted amount of food but which were injected with a suspension of normal brain.

Following these preliminary observations an experiment was conducted with a larger number of animals.

A total of 276 mice were divided into 12 groups as indicated in Table III (groups 7 to 18). The mice were from 24 to 28 days of age at the time they were placed on the experimental diets. 7 days later the animals were all injected intracerebrally with either the virus or a suspension of normal brain, as indicated in Table III. In all groups in which the amount of food was restricted no food was given for 48 hours following the inoculation. Thereafter, each animal in these groups was restricted to 1 gm. of food per day, which is about 40 per cent of the normal food consumption. The first group in this experiment (group 7) was given diet 1 *ad libitum* plus virus. The next two groups were given the restricted amount of the same diet, with and without virus.

In this experiment there was a significant difference at 12 and 21 days after inoculation between groups 7 and 8 (restricted and unrestricted with virus) with respect to deaths and paralysis (Table III), and at 46 days there was a significant difference in the incidence of paralysis, but the significance with respect to deaths was lost by the 28th day. As seen from the table there were several deaths due to restriction alone (group 9).

When the complete diet was restricted there was, presumably, a deficiency of several dietary components. There is no indication as to what specific deficiency might have been responsible for the observed results. To study the

effect of simply feeding an insufficient amount of calories, two groups of animals (groups 10 and 11, Table III) were given diet 3 (Table I). At first an attempt was made to increase all the constituents of the diet except carbohydrate by $2\frac{1}{2}$ times, because in the restricted groups about 40 per cent of the average food consumption was allowed. It was found, however, that the mice would not

TABLE III

Effect of Restriction of Food Intake on Response of Mice to Poliomyelitis Virus

Group No.	Diet No.	Amount of diet	Inoculum	No. of mice inoculated*	12 days		21 days		28 days		46 days	
					Dead	Paralyzed	Dead	Paralyzed	Dead	Paralyzed	Dead	Paralyzed
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
7	1	*Ad libitum*	V	40	80	83	98	85	98	85	98	85
8	1	Restricted	V	38	58	26	74	45	87	50	97	58
9	1	Restricted	N	18	22	—	28	—	28	—	28	—
10	3	Restricted	V	36	39	33	75	58	81	64	94	69
11	3	Restricted	N	29	17	—	21	—	21	—	21	—
12	1S	Restricted	V	17	65	24	82	47	100	47	100	47
13	1S	Restricted	N	11	18	—	27	—	27	—	27	—
14	4	Restricted	V	19	53	16	84	53	95	53	100	58
15	4	Restricted	N	17	18	—	18	—	18	—	18	—
16	5	*Ad libitum*	V	20	75	55	95	60	100	65	100	65
17	5	Restricted	V	17	24	29	82	71	88	76	100	82
18	5	Restricted	N	14	29	—	36	—	36	—	36	—
19	1	*Ad libitum*	V	16	100	88	100	88	100	88	100	88
20	3	*Ad libitum*	V	23	100	96	100	96	100	96	100	96
21	3	Restricted	V	25	28	28	68	52	92	60	100	64
22	3	Restricted	N	23	26	—	43	—	43	—	48	—
23	5	*Ad libitum*	V	35	100	80	100	80	100	80	100	80
24	5	Restricted	V	39	18	15	67	59	85	72	100	79

V = virus.
N = normal brain suspension.
1S = diet 1 plus 0.3 per cent saline solution by stomach tube.
* The number surviving to 2 days after inoculation.

tolerate such a high concentration of salt mixture. Consequently it was reduced to 6 per cent, with all other ingredients except carbohydrate remaining at $2\frac{1}{2}$ times that in diet 1. On 1 gm. per day of this diet the only deficiency was calories. Of the two groups receiving this diet one was injected with the virus and the other with a suspension of normal brain. From the data given in Table III it can be seen that restriction of calories (group 11) had approximately the same effect as restriction of the complete diet.

Occasionally animals which are deprived of sufficient food voluntarily restrict their water intake. If this had been the case with the mice in these experiments

the increased resistance might have been due to dehydration. Groups 12 and 13 were given the restricted amount of diet 1. In addition, for 8 days following the inoculation, a 0.3 per cent solution of sodium chloride was administered by stomach tube. For the first 5 days 1.0 ml. of the solution was given to each mouse twice daily, but due to the rather high death rate the amount was reduced to 1.0 ml. once daily for 3 days, and then was discontinued. These results are presented in Table III (groups 12 and 13), and again show that a definite amount of protection, especially with regard to paralysis (compare group 12 with group 7), resulted from the dietary restriction in spite of the probably greater intake of fluid. Again there were some deaths in the restricted groups injected with the suspension of normal brain (group 13).

In the first series of experiments reported above the diet was deficient only in vitamin B_1. In the next series the intake of the complete diet was restricted, making a partial deficiency of all the ingredients. It is conceivable, but not probable, that the deficiency which was responsible for the results in the latter case was also vitamin B_1. The experiment employing the diet in which all of the ingredients except the carbohydrate were increased would indicate that this is not the case. But to test this possibility further, groups 14 and 15 were given a diet similar to diet 1 (diet 4, Table I) except that it contained 500 micrograms of thiamine per 100 gm. of diet. Group 14 received this diet at the restricted level of 1 gm. per animal per day, with virus, and group 15, the same diet without virus. Comparing group 14 with group 7 it is seen that the restriction delayed the action of the virus, even though the amount of vitamin B_1 received by the animals in group 14 was greater than that received by the animals on the unrestricted intake.

The last three groups in this experiment (groups 16, 17, and 18) were given diet 5, the composition of which is given in Table I. This has been used for some time as the regular stock diet in this laboratory, with very satisfactory results. Group 16, which was inoculated with virus, received an *ad libitum* amount of this diet. Group 17, also inoculated with virus, was given the restricted amount of this diet, while group 18 was similarly restricted but was injected with a suspension of normal brain. As before, restricting the amount of diet delayed the action of the virus.

As a final experiment, 161 mice were divided into six groups (groups 19 to 24, Table III). At the time of inoculation these animals varied in age from 39 to 51 days. Group 19 was given an unrestricted amount of diet 1 plus virus; group 20, an unrestricted amount of diet 3 plus virus. Groups 21 and 22 were fed the restricted amount of diet 3. The first of these two groups was injected with the virus and the latter with a suspension of normal brain. Group 23 was given an *ad libitum* amount of diet 5 with virus and group 24 was treated in the same way except that the diet was restricted. The amount of virus used was about ten times that of the former experiments. As the data in Table III

show, restriction of the amount of diet given the animals to about 40 per cent of their normal intake, beginning 3 days before inoculation, delayed the action of the virus with respect to both death rate and incidence of paralysis.

It is impossible to compare directly the results obtained in the vitamin-deficient experiments with those obtained in the experiments in which the food intake was restricted. In the first place, the experiments were conducted at different times and on animals of different litters. Furthermore, the amount of virus used in one of the experiments on the restriction of food intake was greater than in any of the vitamin-deficient experiments. As obtained, the data, although not conclusive, suggest that the vitamin-deficient animals were somewhat more resistant than were the animals which received the limited amount of food. In the latter case especially, the effect appears to have been more in delaying the action of the virus than in preventing it. For example in groups 12 and 14 all of the animals were dead by the 33rd day although each animal was given but 1 gm. of food per day. A smaller percentage of these animals was observed in paralysis than in group 7, which received an *ad libitum* amount of the diet. In other groups, such as groups 17 and 24, by the 38th day there were not only as many deaths as on the corresponding control groups (groups 16 and 23) but the incidence of paralysis was also as high, and in one case, slightly higher (group 17 compared with group 16). Earlier in the experiment there had been a difference with respect to both deaths and cases of paralysis among the same groups.

In contrast to this delaying action of restriction of food intake, the vitamin deficiency appeared actually to prevent paralysis in many cases. Even at the end of the experiment there was still a statistically significant difference in deaths and incidence of paralysis between the vitamin-deficient and adequately fed animals. Again, however, the delaying effect on the action of the virus is evident, for the greatest difference came at about the 12th day after inoculation.

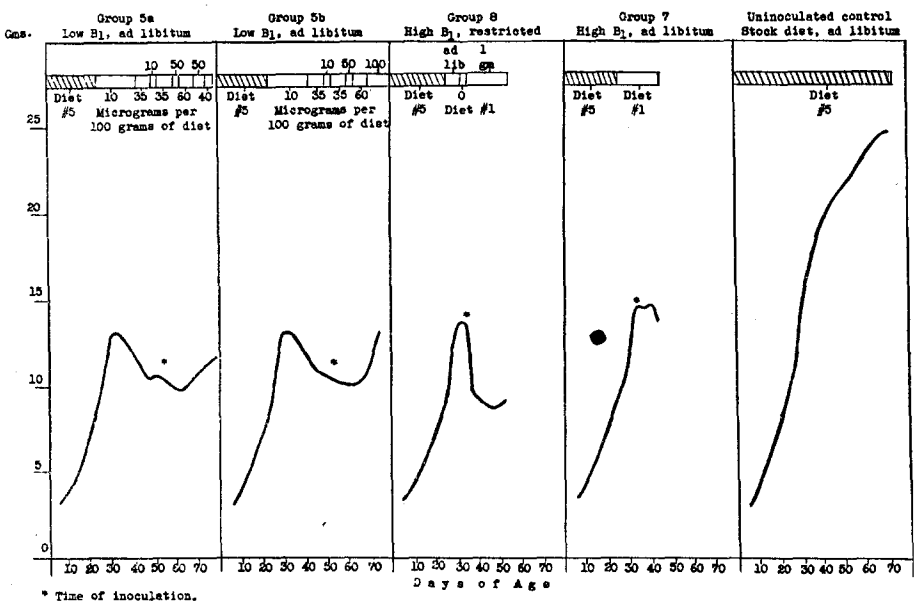
The general manifestations were similar in the animals on the low thiamine diet and those on restricted food intake. Evidence of infection with poliomyelitis in these deficient animals was usually limited to paralysis. Until shortly before death they appeared normally active and were not generally ill. In the well fed mice, on the other hand, the first signs of paralysis of the legs were usually accompanied or preceded by marked loss of weight, general debility, swelling of the eyelids, and at least partial closure of the eyes with a viscous exudate.

The changes in average weight of some of the groups of animals during the experiments are shown in Chart 1. The growth curves are averages for all the males in the groups indicated. As weight curves for the females were very similar to those for the males, except that the weights of the females were somewhat less, only the curves for the males are presented. The plotted values are the average weight of the animals still alive on any given day. As indi-

cated by the curves for groups 5*a* and 5*b*, the weights of the animals on the vitamin-deficient experiments were fairly constant at the time of inoculation. In the experiments in which the amount of food was restricted (group 7, Chart 1) there was naturally a very rapid drop in weight during the 2 day period of complete starvation immediately following the inoculation. Thereafter, when 1 gm. of food per day was being given, the average weight remained rather constant. At any point in the experiment the deficient animals showed a

CHART 1

EFFECT OF B_1 DEFICIENCY AND RESTRICTED FOOD INTAKE ON GROWTH OF MICE INOCULATED WITH POLIOMYELITIS VIRUS



quantitative response to increased thiamine or increased total food intake. The thiamine in the diet of the animals in group 5*a* (Chart 1) was increased to 50 micrograms per 100 gm. of diet at 71 days of age and at the same time the thiamine in the diet of the animals in group 5*b* was increased to 100 micrograms. The mice on an unrestricted amount of high thiamine diet continued to gain until the onset of paralysis, when they showed a rapid loss of weight (group 7, Chart 1). A normal growth curve on the stock diet is also shown, for comparison.

DISCUSSION

About the time of the preliminary publications from this laboratory (1), Rasmussen, Waismann, Elvehjem, and Clark (12) reported in a short note that the resistance of mice to the virus of poliomyelitis and to Theiler's virus is

increased by a deficiency of vitamin B_1. These authors did not report any observations on restricting the food intake. Pinkerton and Moragues (13) have also found that the Lansing strain of poliomyelitis virus produced an apparently higher mortality rate and somewhat earlier death in normal than in riboflavin-deficient animals.

Aside from these experiments on the relation of diet to poliomyelitis in mice, numerous observations have been made indicating that it is not always the healthy, well nourished individual or animal that is most resistant to disease. As early as 1911 Rous (14) noted that unhealthy chickens were not as susceptible to sarcoma virus as healthy animals. Tannenbaum (15) has reported that diets restricted in such essential components as proteins, vitamins, minerals, and fats inhibited the formation of both spontaneous and induced tumors in mice. Similar results were obtained by simply restricting the caloric intake (16). Bischoff, Ingraham, and Rupp (17) have recently reported that a deficiency of vitamin B_6 produced a marked and significant decrease in tumor growth rate in Marsh-Buffalo mice, which was corrected by the addition of vitamin B_6 without significantly changing caloric intake. Miner, Miller, Baumann, and Rusch (18) also found that a diet low in pyridoxine (B_6) affords considerable protection in rats against liver tumors produced by feeding *p*-dimethylaminoazobenzene. Rivers (19) has also stated that it is a common observation in his laboratory that unhealthy or malnourished rabbits show less reaction to vaccinia virus and exhibit a lower titer to the active agent than do perfectly healthy animals, and Sprunt (20) has reported that rabbits on a starvation diet are more resistant to vaccinia virus than are regularly fed animals. Bloomfield and Lew (21) also found that a deficiency of the vitamin B complex protected their animals against spontaneous ulcerative cecitis which was rather prevalent among animals on their regular stock diet.

Several possibilities come to mind to explain the results obtained. It might be that by reducing the food intake the cell is being deprived of some nutrient necessary for the growth and development of the virus. That is, the cell might be looked upon as simply furnishing the medium in which the virus can develop quite similarly to bacteria growing in culture media. However, it is known that viruses exhibit an extreme type of parasitism and it may well be that the virus not only depends upon the nutrients furnished by the cell, but it depends also on the vital or life processes of the cell. Any disturbance which would interfere with the normal physiological processes taking place within the cell could then likewise interfere with the development and multiplication of the virus within the cell.

It is also possible, as Sprunt (20) has suggested, that the restriction of the food in some manner interferes with the spread of the virus. It is conceivable that the deficiency may give rise to some substance which can counteract the virus. The authors have no data to indicate if any of these possible explana-

tions of the manner in which a deficiency of vitamin B_1 or a decreased food intake inhibits the virus is correct. Work is being continued along the lines suggested by the studies conducted thus far.

SUMMARY

In several experiments it was shown that a deficiency of vitamin B_1 in the diet increased the resistance of mice to the Lansing strain of poliomyelitis. The source of the virus was a suspension of infected mouse brain in saline, which was injected intracerebrally. Both the mortality rate and the incidence of paralysis were lower in the deficient animals than in the normally fed controls. The protection was more pronounced with respect to paralysis than with respect to the number of deaths. Some deaths in the deficient groups were undoubtedly due to the vitamin deficiency, as indicated by numerous deaths among groups of animals which were given the deficient diet but injected with a suspension of normal brain. An attempt was made to maintain a state of chronic vitamin deficiency by giving small amounts of the vitamin. The results also seem to indicate that the effect of the deficiency was more in delaying the action of the virus than in preventing it. The greatest difference between normally fed and deficient animals receiving the virus came at about the 12th day after inoculation.

Comparable results were obtained by restricting the intake of the complete diet to 1 gm. per mouse per day, which is about 40 per cent of the intake of the normally fed mice. Restriction of the caloric intake alone gave similar results. Restriction of food intake was effective in experiments in which extra vitamin B_1 was given in the diet and also when a diluted saline solution was given by stomach tube to assure a sufficient intake of fluid.

Other data are necessary before an explanation can be given for the manner in which these deficiencies increase the resistance of the mice to the virus of poliomyelitis.

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BIBLIOGRAPHY

1. Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *Proc. Soc. Exp. Biol. and Med.*, 1942, **51,** 215; *Science*, 1943, **97,** 207; *Am. J. Med. Sc.*, 1943, **205,** 465.
2. Armstrong, C., *Pub. Health Rep., U. S. P. H. S.*, 1939, **54,** 1719, 2302.
3. Woolpert, O. C., and Harrison, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1430.
4. McCormick, W. J., *Med. Rec.*, New York, 1939, **150,** 303.
5. Ward, R., Sabin, A. B., Najjar, V. A., and Holt, L. E., Jr., *J. Bact.*, 1943, **45,** 86.
6. Jungeblut, C. W., *J. Exp. Med.*, 1937, **66,** 459.

7. Sabin, A. B., *J. Exp. Med.*, 1939, **69,** 507.
8. Heaslip, W. G., *Australian J. Exp. Biol. and Med. Sc.*, 1938, **16,** 287.
9. Toomey, J. A., *Am. J. Dis. Child.*, 1937, **53,** 1202.
10. Sabin, A. B., Ward, R., Rapoport, S., and Guest, G. M., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48,** 451.
11. Jones, J. H., and Foster, C., *J. Nutrition*, 1942, **24,** 245.
12. Rasmussen, A. F., Jr., Waismann, H. A., Elvehjem, C. A., and Clark, P. F., *J. Bact.*, 1943, **45,** 85.
13. Pinkerton, H., and Moragues, V., cited as unpublished observations by Pinkerton, H., *Bact. Rev.*, 1942, **6,** 37.
14. Rous, P., *J. Exp. Med.*, 1911, **13,** 397.
15. Tannenbaum, A., *Am. J. Cancer*, 1940, **38,** 335.
16. Tannenbaum, A., *Cancer Research*, 1942, **2,** 460.
17. Bischoff, F., Ingraham, L. P., and Rupp, J. J., *Arch. Path.*, 1943, **35,** 713.
18. Miner, D. L., Miller, J. A., Baumann, C. A., and Rusch, H. P., *Cancer Research*, 1943, **3,** 296.
19. Rivers, T. M., Infantile paralysis, New York, The National Foundation for Infantile Paralysis, Inc., 1941, **57.**
20. Sprunt, D. H., *J. Exp. Med.*, 1942, **75,** 297.
21. Bloomfield, A. L., and Lew, W., *J. Nutrition*, 1943, **25,** 427.