THE COMPARATIVE EFFECTS OF VITAMIN B_1 DEFICIENCY AND RESTRICTION OF FOOD INTAKE ON THE RESPONSE OF MICE TO THE LANSING STRAIN OF POLIOMYELITIS VIRUS, AS DETERMINED BY THE PAIRED FEEDING TECHNIQUE*

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In a previous publication from this laboratory (1) it was shown that the resistance of mice to the Lansing strain of poliomyelitis virus was increased by a diet deficient in vitamin B_1. This increased resistance was indicated by a prolonged incubation period and a decrease in the incidence of paralysis and death following intracerebral inoculation with the virus. The prolongation of the incubation period was more pronounced than the decrease in the incidence of paralysis and death. Subsequent to the experiments on vitamin B_1 deficiency it was found that restriction of food intake (complete diet) or restriction only of caloric intake from carbohydrate while maintaining the intake of other dietary components, augmented the resistance of the mice, especially as manifested by the increased incubation period. Rasmussen, Waisman, Elvehjem, and Clark (2) have reported similar findings in respect to vitamin B_1 deficiency and to restriction of calories. In our earlier work no direct comparison was made to determine the relative effectiveness of B_1 deficiency *versus* food restriction. The data suggested, however, that the deficiency of B_1 produced a greater protection than did either type of restriction of food intake. To determine if an insufficient supply of vitamin B_1, aside from its effect on food intake, exerts a direct influence on the development of the poliomyelitic infection, a careful comparison of B_1 deficiency and simple food restriction has been made by use of the paired feeding technique. The results are reported below.

EXPERIMENTAL

As usual, the mice were housed in individual glass cages (Fig. 1), and the general care of animals and procedures were the same as previously described (1). Two different experiments were conducted. Since the first was of a preliminary nature, only the latter will be described.

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Two hundred mice from fifty litters (four from each litter) were divided into four groups. All four animals from any one litter were of the same sex. Ten days prior to inoculation, groups I and III were placed on diet 2 (1), which contains 10 micrograms of thiamine per 100 gm. of diet, and groups II and IV were given diet 1 (1), which contains 100 micrograms of thiamine per 100 gm. of diet. In dividing the animals into groups particular care was taken

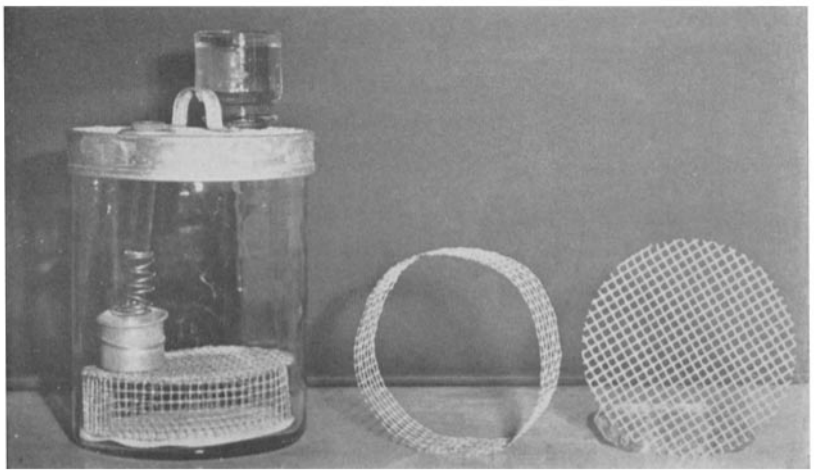

FIG. 1. Individual mouse cage. This mouse cage consists of a battery jar (Gaynor Glass Works) approximately 21.5 cm. high by 15 cm. inside diameter, into which is placed a raised screen. The screen is made of a circular piece of 16 gauge, 6 mm. mesh galvanized wire, 14.5 cm. in diameter, which is wired at three points to a strip of the same mesh, 38 mm. wide by 42 cm. long, the ends of which are fastened together to form a circle. A factory-made top of wire mesh bound with steel bands, containing an 18 mm. opening on one side and a 9 mm. opening on the other side, completes the cage. Into the larger cage-top opening is placed a test tube 16 mm. in diameter by 14.5 cm. long, with its open end up and fitted with a red rubber serum bottle stopper. This tube fits into a spring steel coil holding a 43 mm. diameter aluminum food cup. The smaller opening in the cage top is used for a water bottle assembled from a 100 ml. specimen bottle fitted with a size 9, one-hole rubber stopper through which a glass tube 8 mm. in diameter and 14 cm. long is inserted.

A piece of No. 5269-A filter paper 150 mm. in diameter (Arthur H. Thomas Company) is placed under the raised screen, and has been found very satisfactory for collecting the spilled food in the cages.

to match the weights of the litter mate pairs in groups I and II as nearly as possible. There was a maximum difference of only 0.3 gm.

Group I served as a basis for the amount of food given. The food fed each animal in this group was weighed daily. Any food spilled during the subsequent 24 hours was collected from the bottom of the cage, separated from the feces, and weighed, together with the food remaining in the food cup. The amount of food consumed was calculated and each litter mate in groups II, III, and IV was then given the same amount of diet as was consumed by the litter mate in group I in the preceding 24 hours. Any food spilled by animals in groups II, III, or IV was collected and weighed. For the following day an amount of food equal to the amount eaten by the paired litter mate in group I plus an amount equal to that spilled by the

particular animal in group II, III, or IV was given. In this way the amounts consumed by all members of a given litter were equalized and the error due to spilling was negligible. In some cases individual animals in group III refused to eat as much as the paired litter mate in group I. In those cases groups I and III cannot be considered as strictly paired. As an additional control, another group of animals (group V) was given an unlimited amount of the diet containing 100 micrograms of thiamine (diet 1). These animals were of about the same age and closely related to but not litter mates of the animals of the first four groups.

For the greater part of the experiment, the majority of the mice on the unrestricted low thiamine diet (group I) consumed from 1.2 to 1.5 gm. daily. Those given an unrestricted amount of the high thiamine diet (group V) consumed from 2.0 to 3.5 gm. daily.

As in the earlier experiments, the amount of thiamine given to the animals in groups I and III was gradually increased in an attempt to maintain these animals at approximately constant weight. Thirteen days after the start of the experiment the mice of groups I, II, and V were inoculated with 15 L.D.$_{50}$ of virus (0.3 per cent suspension of mouse brain infected with the Lansing strain of poliomyelitis), and groups III and IV were injected with normal brain suspension. The diet and inoculum given each group are summarized as follows:

Group I. 10 to 60 micrograms of thiamine per 100 gm. of diet. Fed *ad libitum*. Inoculated with virus.

Group II. 100 micrograms of thiamine per 100 gm. of diet. Paired with group I. Inoculated with virus.

Group III. 10 to 60 micrograms of thiamine per 100 gm. of diet. Paired with group I. Inoculated with normal brain.

Group IV. 100 micrograms of thiamine per 100 gm. of diet. Paired with group I. Inoculated with normal brain.

Group V. 100 micrograms of thiamine per 100 gm. of diet. Fed *ad libitum*. Inoculated with virus.

Starting on the 3rd day after inoculation, the mice were observed hourly throughout the day and night. The animals which were injected with the normal brain were also disturbed at hourly intervals in order to make all groups comparable in this respect. The various signs and symptoms for which hourly examinations were made and the code used in recording any abnormalities observed are listed in Table I.

RESULTS

As there were only two deaths in each of groups III and IV these groups are not included in the following consideration of the data. The results with respect to number and time of deaths in groups I, II, and V are shown in Table II as daily cumulative deaths and in Fig. 2 as percentage of each group dead. Of all the inoculated animals which died in these three groups, only two were not observed in paralysis before death. These two deaths were in the group receiving an unrestricted amount of the high thiamine diet and occurred previous to the 3rd day after inoculation, at which time the hourly observations were started. In this experiment it is, therefore, unnecessary to present the incidence of paralysis and death separately.

The various types of paralyses observed and the incidence of abnormalities of the eyes are summarized in Table III. No difference in the character or duration of the manifestations before death could be demonstrated on comparing the three groups I, II, and V. The same is true when these three groups

TABLE I

Various Symptoms for Which Examinations Were Made at Hourly Intervals, and Code for Recording Observed Abnormalities

I. Neuromuscular

a. Paralysis of left front leg
b. Paralysis of right front leg
c. Paralysis of left hind leg
d. Paralysis of right hind leg
4. Paralysis of 4 legs
5. Paralysis of head and trunk
6. Prodromal signs of paralysis (some muscle groups affected but not enough to produce disuse of the part)
7. Rapid breathing
8. Labored breathing
9. Tongue out and bloody

II. Neuromuscular

1. Tremor
2. Spinning without being rotated by operator
3. Twitching movements
4. Mild convulsions—head forward
5. Severe convulsions—head forward
6. Mild convulsions—head backward
7. Severe convulsions—head backward
8. Priapism

III. Eye Conditions

R = right eye
L = left eye

1. Edema of lids
2. Hyperemia of lids
3. Edema and hyperemia of lids
4. Serous, colorless exudate
5. Serous, reddish exudate
6. Viscous exudate
9. Staring, fixed look

IV. Gait

0. Awkward gait, slightly hunched
1. Whole back arched, hind legs straightened—appears to walk on toes of hind feet
2. Dorsal spine arched; lumbar spine and sacrum lowered; walks with hind legs flattened; waddles slightly
3. Exaggeration of preceding gait
4. Body flattened; shuffling gait; appears to use head to aid locomotion
5. Tilts to one side (L = left; R = right; C = moving in circles)
6. Loss of proprioceptive sense with respect to one leg
7. Loss of proprioceptive sense with respect to more than one leg
8. Unable to distinguish edge of table
9. General slight atonia, not sufficient to affect gait

V. Activity

0. Hyperirritable
1. Normal activity reduced
2. Lethargic
3. Moves only when stimulated
4. Stands on all legs and well balanced; good grip in paws and legs but refuses to walk
6. Lies on one side and kicks feebly; rights itself with difficulty
7. Moribund
8. Does not eat
9. Dead

are compared for the time elapsing between the first appearance of any eye changes and death. Paralysis was first noted from 1 to 77 hours before death, the median duration being 27 hours. In most cases the first prodromal signs of

TABLE II

Comparative Effects of Thiamine Deficiency, Food Restriction, and Normal Diet on Death Rate of Mice Following Inoculation with Poliomyelitis Virus

Group No. / No. of mice 2 days after inoculation		I 50	II 50	V 48
Thiamine per 100 Gm. diet in group I	Time after inoculation	Daily deaths, cumulative:		
micrograms	*days*			
30	0	.	.	.
30	1	.	.	.
40	2	.	.	1
40	3	.	.	2
40	4	.	1	2
40	5	.	1	2
45	6	.	1	4
45	7	.	1	4
45	8	.	1	6
45	9	.	1	9
50	10	.	4	11
50	11	1	5	18
50	12	2	7	23
50	13	4	8	28
50	14	7	11	31
50	15	8	17	35
50	16	10	19	38
50	17	11	21	38
50	18	14	23	38
55	19	16	24	38
55	20	18	25	38
55	21	21	27	40
60	22	25	28	41
60	23	34	28	41
60	24	34	31	41
60	25	35	31	41
60	26	36	33	41
60	27	36	33	41
60	28	37	33	41

paralysis preceded any detectable abnormalities of the eyes, but when compared to frank paralysis, there was no consistent difference in the time of the first appearance of these two groups of signs.

Immediately after death was noted, the central nervous system was removed and fixed according to the histological technique standard in this laboratory

(3). The brains and cords of animals surviving to the 28th day after inoculation were similarly prepared. Micro blood sugar determinations were also

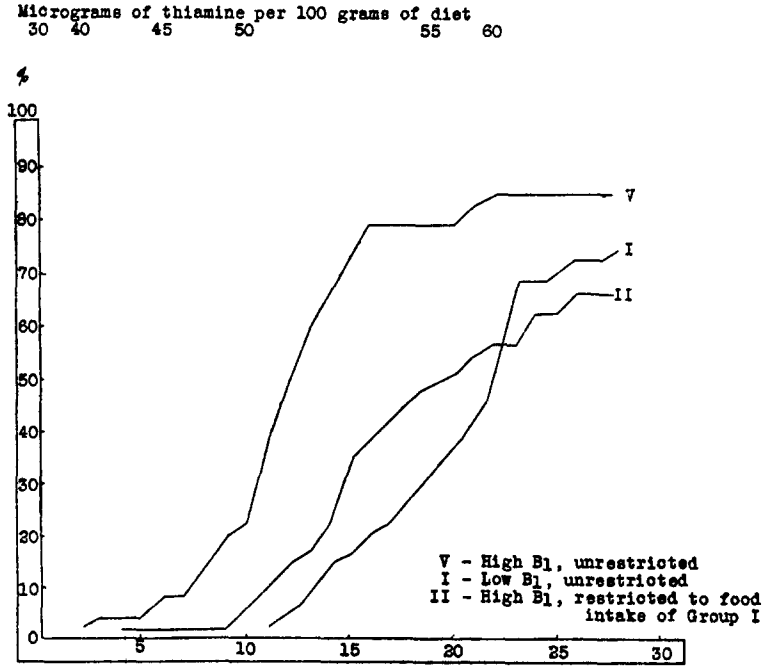

FIG. 2. Percentage deaths of animals of groups I, II, and V plotted against time in days after inoculation.

TABLE III

Number of Animals in Each Virus-Inoculated Group Showing Various Types of Clearly Defined Signs of Poliomyelitis

Group No.	Total No. of deaths	Paralysis*						No. of animals in which abnormalities of eyes were observed
		a	b	c	d	5	9	
I	40	29	20	12	13	3	2	30
II	35	29	24	8	5	6	1	27
V	40	34	31	7	6	9	2	25

* See Table I for explanation of code numbers and letters.

carried out on these animals as well as on their controls which had been injected with normal brain suspension. The results will be reported later.

It is very clear that during the first part of the experiment the death rate among the animals of group V was much higher than in either group I or group

II. The maximum difference came at about the 15th day after inoculation, when 73 per cent of the animals in group V were dead as compared to 34 per cent in group II and only 16 per cent in group I. There was a difference between groups I and II on the one hand and group V on the other throughout the experimental period of 28 days. However, statistically this difference was not significant after about the 22nd day.

There was also a difference in the death rate in groups I and II during the early part of the experiment. This difference reached a maximum at the 17th

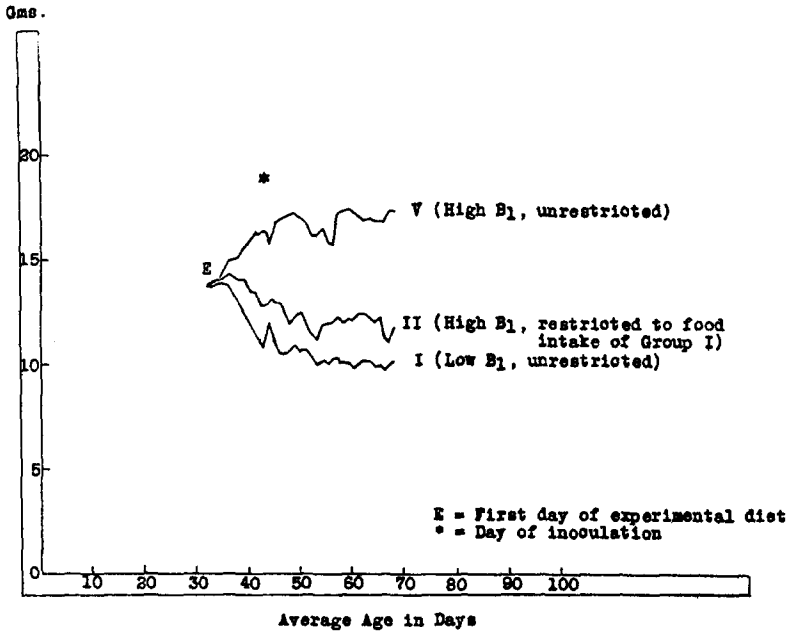

FIG. 3. Growth curves of the male mice of groups I, II, and V.

day, after which the rate of death among the animals on the low thiamine diet increased until there was no difference between the animals on the low thiamine diet (group I) and their paired controls on the high thiamine diet (group II). At the end of the experiment the number of deaths on the low thiamine diet was actually a little greater than on the high thiamine diet. When examined by the Chi square method there was a statistically significant difference between groups I and II on the 17th day and it may be suspected that the difference from the 15th to the 20th day was not due to chance. There had been suggestive but inconclusive evidence pointing in the same direction in the preliminary experiment.

The changes in weight of the animals of groups I, II, and V are presented as

the average for the males in each group in the form of growth curves in Fig. 3. As expected, the animals receiving an unrestricted supply of the high thiamine diet showed good growth before inoculation and maintained their weights well after inoculation. The animals of groups I and II lost weight rather rapidly for a few days after being placed on the experimental diet and then remained fairly constant for the rest of the experimental period. It is to be noted that throughout the experiment the average weight of the animals of group II was above that of group I. This confirms work done by Sure, Kik, and Smith (4) several years ago, in which they showed that rats on a complete diet but receiving the same amount of food as litter mates on a vitamin B_1-deficient diet grew more than did the animals on the incomplete ration. It is interesting to note that the diet which is more efficient in producing growth in an animal appears also to be more suitable for producing conditions necessary for the development of poliomyelitic infection in the same animal.

SUMMARY

In a paired feeding experiment the effects of vitamin B_1 deficiency and of restriction of food intake have been compared. In both groups of animals the number of cases of paralysis and the number of deaths were less than in a control group on an unrestricted amount of the complete diet. The maximum difference occurred on the 15th day after inoculation. The incidence of paralysis and death in the vitamin-deficient group was also less than in the paired restricted group. The maximum difference occurred on the 17th day following inoculation, after which the difference gradually became less. At the end of the experiment (28 days) there was a slightly greater number of deaths in the restricted group than in the vitamin-deficient group. Apparently the effect of vitamin B_1 deficiency on the action of the virus of poliomyelitis in the mouse is not due solely to the resulting anorexia.

From the 3rd to the 25th day after inoculation the animals were examined at hourly intervals throughout the day and night. On the 26th and 27th days they were examined every 3 hours. Except for two mice in the unrestricted group dying before the hourly examinations were begun, peripheral paralysis was observed in every animal which died.

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