THE INFLUENCE OF VARIED PROTEIN INTAKE AND OF TRYPTOPHANE DEFICIENCY ON THEILER'S ENCEPHALOMYELITIS OF MICE[1]

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Received for publication October 3, 1947

From a series of studies on the effect of nutrition on poliomyelitis in various animals, we have reported some of the results obtained with vitamin deficiencies, inanition, and mineral deficiencies. Of the vitamins studied, thiamine has been found to have the greatest influence, since a deficiency of this vitamin resulted in decreased susceptibility of mice to the Lansing strain of poliomyelitis virus, as evidenced by fewer paralyses and a lower death rate. Restriction of the total food intake or simple caloric restriction did not reproduce these results, although both the development of paralyses and death were delayed (Rasmussen *et al.*, 1944*a*). Similar observations have been reported by the Philadelphia group (Foster *et al.*, 1944). Thiamine deficiency also resulted in a marked reduction in infections with Theiler's GDVII (Waisman *et al.*, 1945) and Theiler's TO (unpublished results) viruses. With the latter virus the results are less clear, since the incubation period may be long, and no attempt was made to maintain the deficient mice beyond the period when deaths due to deficiency appeared. With Theiler's FA virus the results were equivocal (Rasmussen *et al.*, 1944*a*). Restrictions of carbohydrate or of the diet as a whole with GDVII virus resulted in clear evidence of infection in about 50 per cent of the animals, and death in the rest, without paralysis or encephalitic signs, although many of the mice manifested early signs of the disease before dying. Deaths were not delayed and the incidence was not reduced. Restriction of fat content had no effect (unpublished results). A deficiency of pantothenic acid in mice produced no change in resistance to the Lansing strain but resulted in decreased susceptibility to Theiler's GDVII virus (Lichstein *et al.*, 1944). Riboflavin deficiency, on the other hand, had a slight effect in increasing resistance to the Lansing virus, but no effect against the Theiler's GDVII or FA viruses (Rasmussen *et al.*, 1944*b*). Pyridoxine, inositol, and biotin deficiencies were found to be inactive against infection with the Lansing and GDVII viruses (Lichstein *et al.*, 1945). *Para*-aminobenzoic acid, which has striking antirickettsial activity, had no influence on infection with the GDVII virus at concentrations in the diet ranging from none to 10 per cent (unpublished results).

In the studies with minerals, deficiencies of calcium, magnesium, chlorine, and sodium (Lichstein *et al.*, 1946*b*) and a high intake of fluorine (unpublished results)

[1] These studies were aided in part by a grant from the National Foundation for Infantile Paralysis, Inc., New York.

had no effect, whereas deficiencies of potassium or phosphorus resulted in a greatly reduced incidence of paralysis with Theiler's GDVII virus (Lichstein *et al.*, 1946*b*). In the last two deficiencies the number of paralyzed animals was increased as the amounts of phosphorus or potassium in the diets were increased. It should be noted, however, that although the incidence of paralysis varied, deaths without signs of infection invariably brought the total fatality rate in these mice to 100 per cent.

Attempts to improve the resistance of mice by improving the diet have not been successful to date. The addition of liver preparations to the mouse diet and higher fat content (15 or 25 per cent corn oil or butter fat) produced no change in resistance to the GDVII virus, although excellent weight gains were obtained on some of the rations (unpublished results).

In poliomyelitis of monkeys vitamin deficiencies have been relatively ineffective. Deficiencies of thiamine (Clark *et al.*, 1945), biotin, and ascorbic acid (unpublished results) produced no change in susceptibility. Folic acid deficiency when acute had no effect, but when chronic a reduced incidence of poliomyelitis resulted (Lichstein *et al.*, 1946*a*). Potassium deficiency, likewise, has no effect on poliomyelitis in the monkey (unpublished results). When monkeys were fed our so-called "super" diet, in which extra vitamins, whole liver substance, plasma globulin, and yeast were included, the results with poliomyelitis were suggestive of increased resistance in one experiment, but this could not be repeated (unpublished results).

Some of these studies have been extended to other viruses—avian encephalomyelitis in chicks and Western equine encephalomyelitis in mice. The incidence of avian encephalomyelitis depended on the age of the chick, the previous state of nutrition, and the amount of thiamine in the diet (Cooperman *et al.*, 1946). When the chicks were inoculated at the age of 1 day, those receiving an optimum level of thiamine were protected to a greater extent than those receiving low or suboptimum levels. When the chicks were inoculated after receiving an optimum diet for 2 weeks, thereafter receiving the experimental diets, those receiving the lowest level of thiamine were protected to the greatest degree.

Thiamine deficiency in animals infected with Western equine encephalomyelitis virus resulted in a marked modification of the course of infection, since most of the mice died without showing typical signs of infection. Titrations of the rains of these mice showed, however, that the virus had multiplied in these animals as well as in the mice on an optimum diet (Kearney *et al.*, in manuscript). Inanition and potassium deficiency (unpublished results) had no effect in this virus disease.

In view of the importance of amino acids in the building of proteins, be it virus or host, we have been studying deficiencies of protein and of amino acids. Although preliminary experiments with lysine, valine, and methionine deficiencies are suggestive of interesting consequences in infection with the GDVII virus, only the work with protein and tryptophane deficiencies is complete enough to report at this time. Jones *et al.* (1946) have recently reported the effect of low protein and low tryptophane diets in mice with the Lansing strain of poliomyelitis

virus. They found that low protein diets (5 per cent casein) produced a slight delay and the low tryptophane diet a pronounced delay in the onset of poliomyelitis. Paralysis was, however, observed in all mice before death.

MATERIALS AND METHODS

Mice. Swiss mice raised in our laboratory were used in all of these experiments.[2] Mice were placed on the experimental diets when they were from 20 to 26 days old, and a split litter technique with consideration for weight and sex of the individual animals was employed throughout. The mice were kept, as described previously (Rasmussen *et al.*, 1944*a*), individually in screen-bottom cages with food and water available at all times.

Diets. The composition of the various diets used in these experiments is given in table 1. Diet no. 1 is our regular synthetic optimum diet. Mice receiving this ration appeared sleek and healthy and gained an average of 4 to 5 g during the first week, and continued to gain throughout the experimental period, the average weight gain for 1 month being about 10 g. Diet no. 2 was the same except for the casein content, which was raised to 36 per cent. When dietary components were increased or decreased, the amount of sucrose was either decreased or increased accordingly. Mice receiving the 36 per cent casein diet gained at a faster rate than those on the 18 per cent casein diet, but reached a peak of about 9 g gain in 2 weeks, after which they leveled off. On diet no. 3, containing 15 per cent casein, the average weight gain was 3.5 g the first week; the mice continued to gain throughout the experimental period and averaged a total weight gain of 9 g in 3 ½ weeks. Mice fed diet no. 4, 9 per cent casein, gained 2 to 3 g the first week and 7 to 9 g during a 3- to 4-week period. Growth was not so good on this ration as on those with higher levels of casein, but the mice appeared healthy. Since the amount of cystine is low when casein is fed at the 9 per cent level, this diet was supplemented in some of the rations with cystine. Rats fed 9 per cent casein diets plus 6 per cent gelatin show a retardation of growth, which can be overcome either by the addition of tryptophane or niacin; these factors were included, therefore, in some of the diets. Mice on these rations (diets 5 to 8) gained at about the same rate as those on the unsupplemented 9 per cent casein diet and appeared healthy. Diet no. 9 contained the ten essential amino acids in pure form, but no protein, the weight difference in the diet being made up by extra sucrose. The amino acids were present as 10.9 per cent of the diet, or 8.4 per cent as active isomers.[3] Sodium acid phosphate is included in the amino acid diets to replace the phosphorus present in 18 per cent casein. On this diet mice maintained their weights or lost 0.5 to 1.0 g during the first 4 or 5 days, after which small weight gains brought the weights up to the original values. During a 3- or 4-week period weights were maintained within 1 g with occasional gains of 2 g above the original weights. Diet no. 10 is the same diet as no. 9 except for 2 per cent casein, which was added to supply the peptide linkage. Response to this diet was similar to that described

[2] Original source was the laboratory of Dr. Webster.

[3] Abbreviated from now on to 8.4 per cent EAA diet.

for diet no. 9. Diet no. 11 consisted of the same ingredients as no. 9, except that tryptophane was absent. Mice fed this ration lost an average of 2.0 to 2.5 g

TABLE 1

Composition of diets

INGREDIENTS	DIET 1	DIET 2	DIET 3	DIETS* 4, 5	DIET 6	DIET 7	DIET 8	DIETS* 9, 10	DIETS* 11, 12	DIET 13	DIET 14	DIET 15
Sucrose	73.2	54.2	75.2	81.2 (81.0)	75.2	75.2	74.9	78.67 (76.67)	78.97 (76.97)	78.17	74.2	73.9
Casein	18.0	36.0	15.0	9.0	9.0	9.0	9.0	— (2.0)	— (2.0)	—	—	—
Gelatin	—	—	—	—	6.0	6.0	6.0	—	—	—	—	—
Salts IV†	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin mixture‡	0.5	0.5	0.5	0.5	—	0.5	—	0.5	0.5	1.0	1.0	1.0
Niacin-deficient vitamin mixture§	—	—	—	—	0.5	—	0.5	—	—	—	—	—
Choline	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.6
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cystine	—	—	—	— (0.2)	—	—	—	—	—	—	0.2	0.2
Tryptophane	—	—	—	—	—	—	0.3	—	—	—	—	0.3
Amino acid mixture‖	—	—	—	—	—	—	—	10.9	10.6	10.6	—	—
Acid-hydrolyzed casein	—	—	—	—	—	—	—	—	—	—	15.0	15.0
NaH_2PO_4	—	—	—	—	—	—	—	0.63	0.63	0.63	—	—

No. 1 = 18% casein regular optimum diet; no. 2 = 36% casein diet; no. 3 = 15% casein diet; no. 4 = 9% casein diet; no. 5 = 9% casein diet + cystine; no. 6 = 9% casein + 6% gelatin, niacin deficient; no. 7 = 9% casein + 6% gelatin, niacin present; no. 8 = 9% casein + 6% gelatin, niacin deficient, tryptophane supplemented; no. 9 = 8.4% essential amino acid (EAA) diet; no. 10 = 8.4% EAA diet + 2% casein; no. 11 = 8.4% EAA diet minus tryptophane; no. 12 = 8.4% EAA diet minus tryptophane + 2% casein; no. 13 = 8.4% EAA diet minus tryptophane + double portions of vitamins; no. 14 = acid-hydrolyzed casein diet + double vitamins + cystine, tryptophane deficient; no. 15 = acid-hydrolyzed casein diet + double vitamins + cystine + tryptophane.

* Diets 4 and 5 differ only in cystine and sucrose content. The numbers in parentheses refer to no. 5. Diets 9 and 11 differ from 10 and 12, respectively, only in casein and sucrose content. The numbers in parentheses refer to nos. 10 and 12 in the respective columns.

† Phillips and Hart salts (Phillips and Hart, 1935).

‡ Added as a dry mixture containing the following parts per 100 g of diet: thiamine 300 μg, riboflavin 300 μg, pyridoxine 300 μg, niacin 500 μg, Ca-pantothenate 2.0 mg, *i*-inositol 100 mg, *p*-amino-benzoic acid 100 mg, biotin 10 μg, folic acid 25 μg. In addition, adequate amounts (2 drops) of oleum percomorphum were fed by mouth each week.

§ Contained no niacin, otherwise the same as that given above.

‖ Amino acid mixture contains the following parts per 100 g of diet: *l*(+)-lysine HCl·H_2O 1.5, *dl*-tryptophane 0.3, *l*(+)-histidine HCl·H_2O 0.6, *dl*-phenylalanine 1.0, *l*(−)-leucine 1.2, *dl*-isoleucine 1.5, *dl*-threonine 1.5, *dl*-methionine 0.9, *dl*-valine 2.1, *l*(+)-arginine HCl 0.3. In the tryptophane-deficient diets (nos. 11, 12, 13) this mixture contained no tryptophane. Amino acids were purchased from Merck and Company.

the first week, leveled off in weight somewhat the second week, and then lost weight gradually, the total weight loss during a 4-week period being about 4 g.

When 2 per cent casein was added (diet no. 12), the weight losses were similar. Diet no. 13 contained double portions of all vitamins, but other than this was identical with no. 11. In diet no. 14, acid-hydrolyzed casein at 15 per cent of the ration was used as the nitrogen source. At this level the ration is low in cystine; this amino acid was therefore added to the ration in 0.2 per cent concentration, and tryptophane, which was destroyed during the hydrolysis, was left out, making this diet tryptophane-deficient, but supplying the other essential and the nonessential amino acids. Double portions of vitamins were also given in this ration. Weight losses occurred of the same order of magnitude as with the other tryptophane-deficient diets. In diet no. 15 the no. 14 ration was completed by the addition of tryptophane at 0.3 per cent. Mice fed this diet lost about 0.5 g during the first week and then gained about 2 g during the second week. The initial weight loss was slightly less and the following gain more than in mice fed the 8.4 per cent EAA ration (diet no. 9).

Mice maintained on the tryptophane-deficient rations, diets no. 11 to 14, showed the following signs of deficiency: Loss of weight began immediately and continued at a slower rate after the first week. Slight irritability, weakness—especially of the hind legs—and tremors were among the early signs; these became more apparent in about 2 weeks. The hind legs were spread to the side, almost at right angles to the body (abduction), and the hip region became low, simulating in appearance a pelvic paralysis, although the muscles were merely weak, not paralyzed. Such signs were more noticeable after the mice were twirled by the tail and dropped on the table; at times the mice would react violently to such treatment—hopping and jumping about with the head thrown back as in opisthotonus, or they would appear to "scramble" along in marked ataxia, with all legs moving rapidly, but only the front legs at all effective in propelling the mouse. Tremors and some revolving were evident when the animals were held by the tail and spun. As the deficiency became more severe, all of these signs became more marked. The mice sat with their heads in the food cups; their backs were humped with both the head and hips close to the table, and they appeared to be mainly head and shoulders. Tonic convulsions with the hind legs completely extended and the fore limbs flexed were frequently observed. During such convulsions the mice became rigid and cyanotic and appeared dead. Death sometimes occurred during these seizures, but more often the mouse would gradually relax after a few seconds and commence breathing. At times the convulsions seemed incomplete in the sense that the hind legs and lower region of the body were stiff and extended but the front legs were moving convulsively. Rapid movements of all legs were often observed just before the extension and flexion of the convulsion. Tonic convulsions were seen more readily after vigorous spinning but also occurred spontaneously. Many of the mice would undergo such convulsions 4 and 5 times during a period of a few days before death supervened. Weakness increased to the point of prostration in the deficient mice, and death occurred after from 18 to 35, or more, days on the diet.

Mice receiving tryptophane evidenced no signs such as these except for a

tendency to spread the hind legs in one or two mice that did not respond well to the 8.4 per cent EAA diet.

Virus. The virus used in these experiments and in many of our previous series was received by us as Theiler's GDVII strain of mouse encephalomyelitis virus from the Rockefeller Institute in 1940 and has since been maintained in this laboratory by intracerebral passage in mice. In our hands, however, this virus has not produced in mice the disease described by Olitsky (1945) as characteristic for the GDVII virus. In view of the recent criticism of the use of the GDVII virus as a model for poliomyelitis (Olitsky, 1945; Schneider, 1946) we feel that further clarification is needed of the type of infection produced by the virus with which we have been working. The first signs of infection following intracerebral inoculation of this virus into mice occur in about 5 to 10 days, the average being 7 to 8 days. The onset of the disease is characterized by hyperirritability, circling, spasmodic and convulsive movements, and, occasionally, tonic-clonic convulsions. Encephalitic signs are more marked in young mice (21 to 28 days old) and less marked in older animals (35 to 40 days old). These signs are rarely so severe as in an infection such as Western equine encephalomyelitis in mice and may not be seen without the stimulation of spinning by the tail. The encephalitic stage may last for 3 or 4 days but usually is followed in 24 hours by a poliomyelitic stage. Cord involvement is apparent first by a tendency to save or favor one leg, and this progresses through partial to complete flaccid paralysis of the limb. The paralysis then progresses during the course of another day to involvement of other muscle groups, resulting at times in quadriplegia, shoulder, hip, back, and side paralyses. Death soon follows severe paralysis. The mice may die at any stage of the infection, but more often the disease runs its characteristic course for 3 or 4 days from the onset of symptoms to death. Definite flaccid paralysis is seen in almost 100 per cent of the mice, and death has been invariable. On intraperitoneal injection paralysis follows in 9 to 10 days and encephalitic signs are less noticeable.

The signs of this disease are in contrast to those described by Olitsky (1945) for the GDVII virus, who states that there is only occasional weakness or paralysis of one or more limbs and that signs invariably precede death by only a few hours. It is in general agreement, however, with the early description by Theiler and Gard (1940), who described the cardinal symptoms of this infection as a flaccid paralysis of the limbs. The behavior of Theiler's TO virus in our mice is similar to that described both by Theiler (1937) and by Olitsky (1945). There can be no doubt that the virus we have been using is not TO, but whether it is the GDVII strain or a spontaneous virus remains to be decided.

In the experiments reported here the source of virus was infected mouse brains preserved in 50 per cent glycerol-saline solution. The mouse brains were washed with normal saline, ground with alundum, and suspended in normal saline at 10 per cent concentration; the 10 per cent suspension was allowed to settle or was centrifuged lightly and the supernatant diluted with normal saline to 1.0 per cent or 0.1 per cent of the original weight of brains. Mice were routinely injected intracerebrally under light ether anesthesia with 0.03 ml of the diluted suspension. When normal brain material was injected, the source was normal

mouse brains preserved in the 50 per cent glycerol-saline solution. These were prepared in the same manner and injected in the same concentration as the virus-containing brains in a given experiment. The mice were observed twice daily for signs of infection or deficiency, beginning 4 days after inoculation, for a 28-day period, at which time the experiments were usually terminated. During the observation the mice were spun vigorously for a few seconds while held by the tail to aid in eliciting encephalitic signs and were allowed to walk about and down the cage to test for paralysis and strength of grip.

Mice dying within 3 days after inoculation were excluded from the final tabulations to eliminate those which may have died from trauma or those which did not respond to the synthetic diets.

EXPERIMENTAL PROCEDURES AND RESULTS

Low protein diets. Prior to the series here reported we had conducted a number of experiments on the influence of the kind and level of protein in the diet on infection with Theiler's GDVII virus. In these experiments mice were fed diets containing casein at 9, 13, 15, 18, and 21 per cent levels. In some cases cystine, niacin, and tryptophane supplements were made, and in others the protein was supplemented with gelatin or zein. The results in all cases in which the level of protein was 15 per cent or less were the same.

The incidence of paralysis was low compared to that in mice receiving 18 or 21 per cent casein, but most of the animals died before paralysis or encephalitic signs were observed. Control mice that were not inoculated or were inoculated with normal brain suspensions did not die but showed poor growth. Since other workers in our laboratories had obtained good growth in mice fed diets containing 13 or 15 per cent casein, we suspected the adequacy of our basal ration. When these studies were initiated, the vitamins were added to the ration by dissolving them in an alcohol solution, adding the solution to a given amount of casein, drying, and using measured amounts of this fortified casein in the final ration. When the vitamins were added as a dry mixture, better growth was obtained, and when a mixture of the vitamins was given to the mice showing growth failure, significant growth responses occurred. Similar results were obtained when thiamine alone was added, indicating a destruction of this vitamin during the preparation of the fortified casein. Since we had used similar procedures without difficulty in other work, it appears that the casein must have contained small amounts of sulfite, which is highly destructive to thiamine. Inasmuch as neither the deficiency signs nor the response of these mice to the virus was characteristic of thiamine deficiency, other nutritional complications may have arisen from a combination of the low protein diet and the anorexia associated with the vitamin deficiency.

These results have not been reported in detail since they are not based on diets low only in protein; they are mentioned only because they demonstrate the difficulties that may be encountered in nutrition studies, in spite of utmost care in preparing rations, and because they offer still another example of the influence nutrition may have on a virus infection.

The low protein diets were then restudied, with the dry vitamin mixture used,

in three experiments, series 81, 84, and 87. In series 81, 140 mice, 23 days old, were divided into eight groups. Group 1, 7 mice, received 36 per cent casein (diet no. 2) and were not inoculated. Group 2, 28 mice, received the same diet and virus inoculation. Groups 3 and 4, with 7 and 28 mice, respectively, received the 18 per cent casein diet (diet no. 1), group 3 remaining uninoculated and group 4 receiving the virus. Groups 5 and 6 were fed the 9 per cent casein diet (diet no. 4), group 5, with 7 mice, remaining uninoculated and group 6, 28 mice, being inoculated with virus. Groups 7 and 8 received the 9 per cent casein diet with a supplement of cystine (diet no. 5). Group 7, with 7 mice, was the uninoculated control group, and group 8, 28 mice, received virus inoculation. The

TABLE 2

Effect of low protein diets on incidence of virus infection

DAYS AFTER INOCULATION	INCIDENCE OF VIRUS INFECTION EXPRESSED IN CUMULATIVE PERCENTAGES OF TOTAL MICE IN EACH GROUP													
	Series 81				Series 84							Series 87		
	Group 2 (28)* Diet 2	Group 4 (28) Diet 1	Group 6 (27) Diet 4	Group 8 (27) Diet 5	Group 1 (6) Diet 1	Group 3 (20) Diet 3	Group 5 (24) Diet 4	Group 7 (26) Diet 5	Group 9 (22) Diet 7	Group 11 (21) Diet 6	Group 13 (24) Diet 8	Group 2 (33) Diet 4	Group 4 (33) Diet 5	Group 6 (32) Diet 1
6	0	7	4	0	33	5	4	4	19	19	8	0	0	3
8	64	68	41	41	50	25	46	43	54	48	62	21	9	6
10	100	96	55	89	100	70	83	92	82	95	92	51	36	31
12	100	100	63	96	100	85	92	100	95	100	92	94	73	72
14	100	100	74	96	100	100	100	100	95	100	92	97	82	87
16	100	100	74	100	100	100	100	100	95	100	92	97	88	91
18	100	100	74	100	100	100	100	100	95	100	92	100	88	91
Total														
% pvi†	100	100	74	100	100	100	100	100	95	100	92	100	88	91
% par‡	93	96	67	89	100	95	96	100	77	95	88	85	79	75
% f§	7	4	4	0	0	5	4	0	18	5	4	6	0	0

Days = day when definite paralysis occurred, or day of death if no paralysis.

* Number of mice surviving through third day after inoculation.

† pvi = positive virus infection, i.e., typical signs of infection; includes paralysis.

‡ par = paralysis, included in table as pvi.

§ f = death without typical signs of infection, included in the table as pvi.

groups were inoculated after 11 days on the rations with a 1.0 per cent suspension of the virus. The results of this series are summarized in table 2. In all groups the majority of the inoculated mice showed definite paralysis. In group 6 the incidence of infection is 26 per cent less than in the other groups, the rest of the mice surviving. In each of groups 2, 4, and 6, one mouse died without showing signs of infection. In group 8 frank paralyses occurred in 89 per cent of the mice, with the remaining 11 per cent (3 mice) dying after signs of encephalitis but before paralysis was observed. No deaths occurred in the control groups.

In series 84 a total of 210 mice, 20 to 23 days old, were placed on the following rations: group 1, 18 per cent casein (diet no. 1); groups 2 and 3, 15 per cent casein (diet no. 3); groups 4 and 5, 9 per cent casein (diet no. 4); groups 6 and 7,

9 per cent casein with cystine supplement (diet no. 5); groups 8 and 9, 9 per cent casein plus 6 per cent gelatin, with niacin supplement (diet no. 7); groups 10 and 11, 9 per cent casein, 6 per cent gelatin, no supplements (diet no. 6); groups 12 and 13, 9 per cent casein, 6 per cent gelatin, with tryptophane supplement (diet no. 8). The even-numbered groups were the uninoculated control groups with 7 mice in each group. The odd-numbered groups were all inoculated with a 1.0 per cent suspension of the virus after 13 days on the diets. Group 1 contained 7 mice, group 3, 21 mice, and the others contained 28 mice each. The results, presented in table 2, show no significant differences in any of the groups. In each of groups 3, 5, 11, and 13 one died without signs of infection. In group 9, 4 mice died in this manner,making the totals in this group 77 per cent paralyzed, 18 per cent died without signs of infection, or 95 per cent total deaths.

In series 87 the 9 per cent casein diets were again studied. A total of 126 mice, 23 to 26 days old, were divided into groups as follows: groups 1 and 2 received the 9 per cent casein ration (diet no. 4); groups 3 and 4 received this diet supplemented with cystine (diet no. 5); and groups 5 and 6 the 18 per cent casein diet (diet no. 1). Groups 1, 3, and 5, consisting of 7 mice each, were the uninoculated controls. Groups 2, 4, and 6, with 35 mice each, were inoculated with virus. These groups were injected after 11 days on the rations with a 0.1 per cent suspension. The results, given in table 2, show an incidence of infection essentially the same regardless of the diet.

The findings of the low protein experiments indicate that reduction of the protein intake of mice to 9 per cent of the diet, when the protein source is casein, has no influence on infection with Theiler's GDVII virus.

Tryptophane-deficient diets. Three experiments were conducted on the effect of tryptophane deficiency on infection with Theiler's GDVII virus. The first of these, series 88, was a preliminary experiment with various amino acid deficiencies, but only the results with tryptophane will be here presented. Four groups of 14 mice, 24 to 26 days old, were placed on the following diets: group 1, tryptophane-deficient (diet no. 11); group 2, tryptophane-deficient plus 2 per cent casein to supply the peptide linkage, or "strepogenin" factor (diet no. 12); group 3, the ten essential amino acids supplied in pure form, but no nonessential amino acids, the final concentration of the active isomers being 8.4 per cent of the diet (diet no. 9); group 4, the same as in group 3 but with 2 per cent casein added (diet no. 10). All groups were inoculated after 5 days on the rations with a 0.1 per cent suspension of the virus. The results, summarized in table 3, even with such small numbers of animals were suggestive enough to warrant further study. In group 1 none of the mice showed evidence of infection, the only signs seen being those referable to the deficiency, such as hind leg weakness, a tendency to spread the hind legs, tremors, and tonic convulsions. In group 2, three mice gave evidence of virus infection. Two of these showed only an indication of encephalitis—a tendency to paw the air and face spasmodically, with death on the twelfth and fourteenth days after inoculation. The third mouse appeared to have a partial paralysis of the right front leg on the twenty-sixth day. For simplicity these are included in the table as fatalities. The two groups

receiving the full complement of the essential amino acids responded well to the virus, showing signs of the disease in its characteristic course in most cases; when paralysis was not observed, definite encephalitic signs were evident. The final death rates in all four groups were approximately the same.

The second experiment, series 93, was a more effective study of tryptophane deficiency. A total of 147 mice, 21 to 23 days old, were placed on the rations as follows: group 1, 14 mice, received the 8.4 per cent EAA diet (diet no. 9); group 2, 14 mice, received the same diet and were inoculated with a 0.1 per cent suspension of normal brains. Group 3, 28 mice, received the same diet and were inoculated with a 0.1 per cent suspension of virus. Group 4, 14 mice, comprised the uninoculated tryptophane-deficient control group (diet no. 11). Group 5, 21 mice, received the tryptophane-deficient diet and an injection of normal brain suspension. Group 6, 28 mice, received the tryptophane-deficient diet and were inoculated with virus. Group 7 consisted of 28 mice, 14 of which received the 18 per cent casein optimum diet (diet no. 1) and 14 this diet minus the corn oil; these are included together as one group since no differences were found between them either in response to the diets or to the virus. Inoculations of normal brain suspension and virus were performed after 7 days on the diets. The results of this experiment are presented in table 4. All mice in group 7 exhibited positive signs of infection, and only one mouse failed to show paralysis. These results are characteristic of the

TABLE 3

Results of series 88

DAYS AFTER INOCULATION	GROUP 1 (12),* DIET NO. 11, TRYPTOPHANE DEFICIENT	GROUP 2 (13), DIET NO. 12, TRYPTOPHANE DEFICIENT + 2% CASEIN	GROUP 3 (11), DIET NO. 9, 8.4% EAA	GROUP 4 (14), DIET NO. 10, 8.4% EAA + 2% CASEIN
	% f	*% f*	*% pvi*	*% pvi*
11	17	15	18	14
13	42	38	45	36
15	42	54	55	43
17	42	54	55	57
19	42	54	73	57
21	42	62	73	64
27	58	69	73	64
Total				
% pvi†	0	23	73	57
% par‡	0	0	55	36
% f§	58	46	0	7
% F‖	58	69	73	64

* Numbers in parentheses = numbers of mice surviving through the third day after inoculation.

† pvi = positive virus infection, i.e., typical signs of infection; includes paralysis.

‡ par = paralysis, included in table as pvi.

§ f = death without typical signs of infection.

‖ F = total fatality regardless of signs shown.

many times we have used mice on the optimum ration and this virus. The group receiving tryptophane as part of the 8.4 per cent EAA diet (group 3) also showed a high percentage of virus infection, with unequivocal encephalitic signs and paralysis. The incidence of paralysis, however, was lower than in the optimum group, with 17 of the 25 mice (68 per cent) showing paralysis and 7 (28 per cent) dying before paralysis was observed. No deaths occurred in the control groups on this diet.

Mice receiving no tryptophane presented a markedly contrasting picture. Only one mouse of the 24 in this group (group 6) showed signs of infection and it developed a partial paralysis of the left front leg after a history of encephalitis. The rest of the animals in this group manifested only signs of deficiency—weakness, tremors, a tendency to spread the hind legs, especially after the mouse was

TABLE 4

Incidence of infection in series 93

DAYS AFTER INOCULATION	GROUP 3 (25), DIET NO. 9, 8.4% EAA + VIRUS	GROUP 4 (12), DIET NO. 11, TRYPTOPHANE DEFICIENT, UNINOCULATED	GROUP 5 (19), DIET NO. 11, TRYPTOPHANE DEFICIENT + NORMAL BRAIN	GROUP 6 (24), DIET NO. 11, TRYPTOPHANE DEFICIENT + VIRUS	GROUP 7 (25), DIET NO. 1, 18% CASEIN OPTIMUM + VIRUS
	% *pvi*	% *F*	% *F*	% *F*	% *pvi*
7	12	0	0	0	16
9	32	8	0	4	60
11	60	8	0	13	88
13	80	8	0	46	96
15	88	8	11	58	100
17	96	17	21	71	100
19	96	17	21	83	100
21	96	50	26	83	100
29	96	67	58	96	100
Total					
% pvi.....	96	—	—	4	100
% par.....	64	—	—	4	96
% f.....	0	—	—	92	0
% F.....	96	67	58	96	100

See table 3 for explanation of symbols.

spun by the tail, hunched posture, and tonic convulsions. The hind legs often appeared to be stiff, and in most cases quite weak, but the mice were able to walk, grip the cage, and walk down the cage, and were therefore considered not paralyzed. These signs of deficiency appeared earlier in many of the deficient mice receiving virus than in the two groups of controls and progressed to severe deficiency about a week earlier, considering the groups as a whole. The controls presented the same signs as the deficiency progressed. Death also occurred earlier in the virus-inoculated deficient mice than in the controls (table 4), reaching 71 per cent on the twenty-fourth day on the diet, or 17 days after inoculation, at which time the deaths totaled 17 per cent and 21 per cent in the uninoculated and normal brain-inoculated control groups. This suggests that these mice had died from virus infection, although no clinical signs were evident.

After 24 days on the diet, deaths in the control groups occurred more frequently, reaching 67 per cent and 58 per cent by the thirty-sixth day; it is therefore possible that the deaths occurring in the virus-inoculated group after 24 days (17 days after inoculation) may have been due to deficiency. If the deaths up to this time are considered to be due to virus infection, and the death rate of the deficient mice is compared to the two groups receiving tryptophane, there are still real differences in incidence up to the seventeenth day after inoculation, the percentages at this time totaling 96 in the group receiving the 8.4 per cent EAA diet, 100 per cent in the group receiving the 18 per cent casein, and 71 per cent in the tryptophane-deficient mice. From the time of inoculation until this time the delay of death in the deficient animals is consistent. Tonic convulsions were recognized in this series as a part of the deficiency syndrome, since mice in both control groups manifested this sign in a manner identical with the virus-inoculated groups. In summary of this experiment then, it appears that tryptophane deficiency hindered and possibly prevented virus infection in many of the mice. Further, this deficiency radically altered the course of the disease, only one of the 24 mice showing signs of infection. Since many of these animals developed severe signs of deficiency and died earlier than the control deficient mice, it is possible that the virus infection increased the severity of the deficiency.

The third experiment, series 97, was conducted in repetition and extension of the previous experiments. The mice were 19 to 24 days old when placed on the rations, and many of the younger ones died. The number of mice given for each group is therefore the number surviving until 3 days after inoculation. Groups 1, 2, and 3 were fed the tryptophane-deficient diet as such (diet no. 11), group 1, 12 mice, remaining uninoculated, group 2, 15 mice, being inoculated with a suspension of normal brains, and group 3, 26 mice, being inoculated with a suspension of the virus. Groups 4 and 5 were fed the tryptophane-deficient diet, but with double portions of vitamins (diet no. 13) in an attempt to rule out vitamin-deficiency complications that might have arisen from the pronounced inanition associated with tryptophane deficiency. Group 4, 15 mice, was injected with normal brain suspension, and group 5, 26 mice, with the virus. In groups 6 and 7 a deficiency of tryptophane was produced by feeding as the nitrogen source acid-hydrolyzed casein, supplemented with extra cystine and double portions of vitamins (diet no. 14). It was hoped that this diet would eliminate complications due to the lack of nonessential amino acids and total nitrogen, as well as vitamins. Group 6, 13 mice, received an inoculation of normal brain material and group 7, with 17 mice, the virus. Group 8, 19 mice, received the same diet as groups 6 and 7 but with tryptophane added (diet no. 15). Group 9, 13 mice, received the 8.4 per cent EAA diet (diet no. 9) and group 10, 18 mice, the 18 per cent casein optimum diet (diet no. 1). The last three groups were all injected with virus. Both the normal brain and virus suspensions were inoculated in 0.1 per cent concentration after 8 days on the diets. Beginning on the fourth day after inoculation, the mice were thoroughly examined every hour from 8:00 A.M. until 10:00 P.M. daily for signs of infection and deficiency. These examinations were made of control animals as well as of virus-

inoculated groups each time. Following death, the brains and spinal cords, including the cervical region, from mice in all groups were removed and placed in 10 per cent formalin or 50 per cent glycerol-saline solution for histological study or titration of virus content. The results of this experiment are presented in table 5. With three different tryptophane-deficient diets, the results were the same. In each case most of the mice evidenced only signs of deficiency and none referable to the virus. In group 3 only 6 of the 26 mice (23 per cent) showed signs of infection. In 5 of these, encephalitis of a mild sort was the only sign; in the sixth, there was no encephalitis but a questionable hind leg paralysis on the thirteenth day after inoculation. In group 5 the extra vitamins had no apparent effect, since the deficiency signs and the response to the virus were

TABLE 5

Incidence of infection in series 97

DAYS AFTER INOCULATION	GROUP 3 (26), DIET NO. 11, TRYPTOPHANE DEFICIENT		GROUP 5 (26), DIET NO. 13, TRYPTOPHANE DEFICIENT + DOUBLE VITAMINS		GROUP 7 (17), DIET NO. 14, ACID HYDROLYZED CASEIN		GROUP 8 (19), DIET NO. 15, ACID-HYDROLYZED CASEIN + TRYPTOPHANE	GROUP 9 (13), DIET NO. 9, 8.4% EAA	GROUP 10 (18), DIET NO. 1, 18% CASEIN OPTIMUM
	% f	*% pvi*	*% f*	*% pvi*	*% f*	*% pvi*	*% pvi*	*% pvi*	*% pvi*
5	4	0	4	0	6	0	0	8	6
7	23	0	19	0	35	0	26	16	50
9	35	4	35	8	47	0	84	92	78
11	50	12	42	12	59	6	100	92	100
13	62	19	58	23	76	12	100	100	100
15	62	23	58	31	76	18	100	100	100
17	69	23	62	35	76	18	100	100	100
19	69	23	62	35	82	18	100	100	100
21	69	23	65	35	82	18	100	100	100
23	73	23	65	35	82	18	100	100	100
27	77	23	65	35	82	18	100	100	100
Total									
% pvi.....	23		35		18		100	92	100
% par.....	4		15		18		84	77	100
% f.....	77		65		82		0	8	0
% F.....	100		100		100		100	100	100

See table 3 for explanations of symbols.

about the same as in group 3. There were four cases of paralysis in this group, but the total positive virus infection is again low. Mice receiving the acid-hydrolyzed casein diet (group 7) appeared similar in every way to those in group 3, and no effect could be seen from the extra nitrogen present in this diet. Of the 17 mice in this group, only 3 showed signs of infection, and each of these showed paralysis of the left front leg but no encephalitis. On this same diet plus tryptophane (group 8), 84 per cent of the mice became paralyzed and 100 per cent showed clear-cut signs of infection. In group 9, which received the same diet as group 3 but with tryptophane, 77 per cent of the mice became paralyzed, and all but one mouse showed signs of infection. Mice receiving the 18 per cent casein diet, as usual, responded with unmistakable paralysis.

A comparison of the death rates of the controls and virus-inoculated deficient mice (figure 1) again suggests that the mice may have died from virus infection, since very few of the controls had died when the inoculated groups had reached 90 to 100 per cent mortality. If this series is compared with series 93, it is evident that all virus-inoculated groups in series 97 died faster than in series 93,

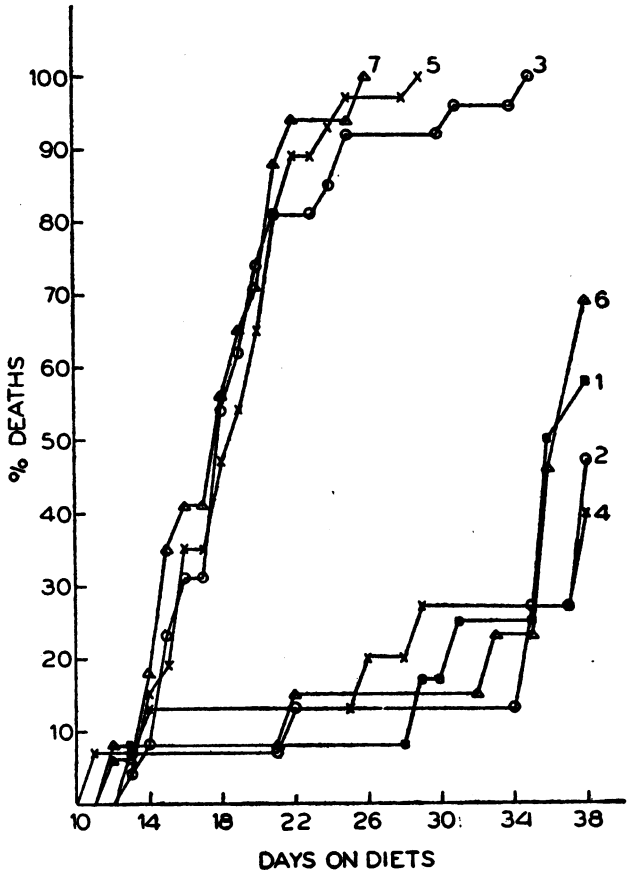

FIG. 1. DEATH RATES OF TRYPTOPHANE-DEFICIENT MICE (Series 97)

Groups 1, 2, and 3 were tryptophane-deficient groups (diet no. 11): group 1 was not inoculated; group 2 was inoculated with normal brain material; group 3 with virus. Groups 4 and 5 were tryptophane-deficient plus double vitamins (diet no. 13): group 4 was inoculated with normal brain material and group 5 with virus. Groups 6 and 7 had an acid-hydrolyzed casein diet (diet no. 14): group 6 was inoculated with normal brain material; group 7 with virus.

including those on the rations containing tryptophane, although control mice died less rapidly than in series 93. This supports the view that the mice died from the effects of the virus, the faster death rate probably being due to a more potent virus inoculum in this series. Again a delay is apparent in deaths in deficient groups compared to those receiving tryptophane, though not so marked as in series 93. Sizable differences in incidence exist, however, through the

fourteenth day after inoculation in group 3, the thirteenth in group 5, and the twelfth day in group 7, compared to the optimum mice.

The deficiency signs in this experiment were similar to those seen in previous series. In the virus-inoculated groups deficiency signs appeared earlier and became severe sooner than in the control deficients, as evidenced especially by the time of appearance of tonic convulsions. It was increasingly apparent in this experiment that when tonic convulsions made their appearance, the deficiency was acute and the mouse would die within several days. Many of the

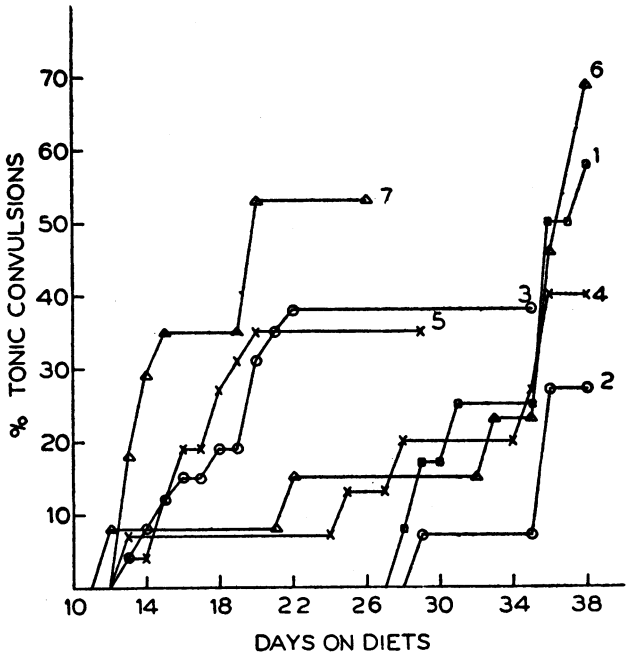

FIG. 2. PERCENTAGE OF TONIC CONVULSIONS IN TRYPTOPHANE-DEFICIENT MICE PLOTTED AGAINST TIME (Series 97)

Groups 1, 2, and 3 were tryptophane-deficient groups (diet no. 11): group 1 was not inoculated; group 2 was inoculated with normal brain material; group 3 with virus. Groups 4 and 5 were tryptophane-deficient plus double vitamins (diet no. 13): group 4 was inoculated with normal brain material; group 5 with virus. Groups 6 and 7 had an acid-hydrolyzed casein diet (diet no. 14): group 6 was inoculated with normal brain material; group 7 with virus.

deaths seemed to be direct results of the convulsions, since mice which had undergone several of these were often found dead in this position. Figure 2 indicates the percentage of mice in the various deficient groups experiencing tonic convulsions, the time referring to the day of the first such convulsion in a given mouse. These convulsions made their appearance as early as the thirteenth day on the diet, or 5 days after inoculation, and reached a maximum around the twenty-first day in group 3. By this time most of these mice were dead and the percentage did not increase further. Tonic convulsions began in the control groups after about 24 to 28 days on the diets and steadily increased in frequency

until the experiment was terminated on the thirty-eighth day. It should be noted that no table or figure can tell the whole story. Although we have succeeded in eliciting convulsions in many of the mice and have examined them as frequently and carefully as possible, a few of the mice have been observed in convulsions without the stimulation of spinning, and several have been found dead in the position of the convulsion without previous history of such signs. For this reason the final percentages indicated on figure 2 cannot be taken as the total number of mice undergoing tonic convulsions, and the curves merely indicate a trend in the time period in which convulsions are seen. This trend, however, is definite, and there is no question in our minds that severe signs of deficiency occurred earlier in the mice that received virus inoculations. The general picture presented in these experiments can best be described as a precipitation of the tryptophane deficiency by virus infection, with resultant death of the mice without signs of infection. The direct cause of death then may be either the virus or the deficiency or both, and the indirect cause of death from this point of view would be the virus.

In order to investigate this concept, titrations for virus content were performed using brains and cords from mice in all of the virus-inoculated groups. For these titrations brains and cords were removed from the mice as soon after death as possible and stored in 50 per cent glycerol-saline solution until all the necessary brains were collected and could be titrated simultaneously. To determine the amount of virus which would have been present from the inoculation alone, before multiplication could take place, a "blank" titration was performed, using three brains and cords from the deficient mice, 1 from each group, which were sacrificed about 2 hours after inoculation. These were also preserved in glycerol-saline solution until they could be titrated with the others. Material from the deficient mice was grouped according to the time of death and the signs and symptoms shown, several brains and cords being pooled for each titration. Each pool of brains and cords was ground with alundum and diluted with broth to made 10 per cent suspensions of the original weight of tissue. These were then centrifuged lightly to remove the tissue particles, and 10-fold dilutions of the supernatants were made in broth as indicated for each titration. Three mice were injected intracerebrally under light ether anesthesia with 0.03 ml for each dilution. The mice used were from our own colony and were placed on this experiment as for one of the nutritional series, using the split litter technique. They were fed the 18 per cent casein optimum diet (diet no. 1) and inoculated with the various suspensions when 26 to 31 days old, according to the date of birth. The materials and dilutions used in the titrations are listed below.

(1) "Blank titration." Three brains and cords from deficient mice as described above were pooled and injected at 5 per cent, 1 per cent, and 0.1 per cent dilutions.

(2) Brains and cords were pooled from five optimum mice in group 10 that had become paralyzed in 5 to 7 days and were allowed to die (6 to 8 days) before the brains were removed. The dilutions injected were 10^{-2} through 10^{-6}.

(3) Brains and cords were pooled from four deficient mice in group 3 that had

died 6 to 9 days after inoculation, with only deficiency signs and no signs of infection. The dilutions injected in this and the rest of the titrations were 10^{-2} through 10^{-5}.

(4) Brains and cords were pooled from four deficient mice in group 5 that had died without signs of infection in 6 to 9 days.

(5) Brains and cords were pooled from three deficient mice in group 7 (acid-hydrolyzed casein diet) that had died without signs of infection in 6 to 9 days.

(6) Brains and cords were pooled from two deficient mice in group 3 that had died without signs of infection in 10 to 13 days after inoculation.

(7) Brains and cords were pooled from three deficient mice in group 7 that had died without signs of infection in 10 to 13 days.

(8) Brains and cords were pooled from three deficient mice, one from group 3 and two from group 5, that had died without signs of infection in 10 to 13 days.

(9) Brains and cords were pooled from three deficient mice, two from group 3 and one from group 5, that were listed as positive virus infection and died in 14 to 17 days.

(10) Brains and cords were pooled from three deficient mice in group 3; two had died without signs of infection, one was listed as positive virus infection, and all three had tonic convulsions with death occurring in 10 to 13 days.

(11) Titration from group 8, acid-hydrolyzed casein diet, with tryptophane added. Four brains were pooled from mice that had become paralyzed and died in 6 to 8 days.

(12) Titration from group 9, 8.4 per cent EAA diet (containing tryptophane). Three brains were pooled from mice that had died in 6 to 8 days; one had been paralyzed, one obviously had a virus infection without paralysis, and one had died without signs of infection.

The results of these titrations are presented in table 6. Unfortunately, not enough mice were available to carry out these titrations further, and the end points cannot be stated with certainty. So far as the optimum titration is concerned, it is reasonably certain that the end point was nearly reached. This virus has seldom produced infection at dilutions of 10^{-7} and 10^{-8} in even one of three mice. For the titrations of deficient brains we have, of course, no previous basis. If the incubation periods are taken as an indication, then the end points were nearly reached, since in all titrations the time of paralysis lengthened from 5 to 6 days at the 10^{-2} dilution to 11 and 12 days with the 10^{-5} dilution. With these points and the fact that blank titration produced negative results in mind, it is apparent that the virus multiplied in the deficient mice to approximately the same degree as in optimum mice, regardless of the signs shown by the mice. The course of the disease in all mice in these titrations was typical.

Controls for the presence of other viruses. In general, irritability, tonic convulsions, and similar signs are referable to lesions of the brain, the causes of which may differ widely. Since other viruses that produce central nervous system lesions can also produce the signs and symptoms seen in our deficient mice, the possibility of a latent virus activated by the deficiency had to be considered, although we have had no suggestion of such in our mouse colony. Pinkerton

and Swank (1940) reported one instance in which thiamine deficiency allowed a latent psittacosis virus to multiply and produce signs of infection in pigeons. Particularly, the virus of lymphocytic choriomeningitis (LCM) and Theiler's FA virus can produce tonic convulsions of the sort we have described for mice on the tryptophane-deficient diets. In fact, the original paper of Theiler and Gard (1940) on the FA virus describes tonic convulsions caused by this virus in complete resemblance to those we have seen, with the sole exception that in tryptophane deficiency such convulsions are not accompanied by the other encephalitic signs prominent in FA infection. We attempted to test for a latent virus in two ways. First, two guinea pigs weighing 350 to 400 g were inoculated with the brain and cord suspension used in titration no. 10, described above. One pig received 0.1 ml intracerebrally and the other 1.0 ml subcutaneously. These animals have remained healthy, indicating that LCM virus was probably

TABLE 6

Titrations of brains and cords of mice from series 97

TITRATION NO.	DILUTIONS INJECTED, RESULTS*					
	5%	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
1	0/3	0/3	0/3	—	—	—
2	—	3/3	3/3	3/3	3/3	2/3
3	—	3/3	3/3	3/3	3/3	—
4	—	3/3	3/3	3/3	2/3	—
5	—	3/3	3/3	3/3	2/3	—
6	—	3/3	3/3	3/3	3/3	—
7	—	3/3	3/3	3/3	2/3	—
8	—	3/3	3/3	3/3	0/3	—
9	—	3/3	3/3	2/3	0/3	—
10	—	3/3	3/3	3/3	3/3	—
11	—	3/3	3/3	3/3	3/3	—
12	—	3/3	3/3	3/3	3/3	—

* Numerator = no. of mice developing infection.
Denominator = no. of mice injected.

absent from this preparation. A third pig, injected with known LCM virus, succumbed to this infection.

The second procedure was the passage of brains from uninoculated tryptophane-deficient mice into young (24-day-old) mice from another stock.[4] The deficient mice used as the source of brains were part of a more recent experiment to be published at a later date. They were, however, our own stock of mice and were fed the same diet (diet no. 11); and the results with these mice did not differ from those reported in the present communication, in both the control and virus-inoculated groups. Three groups of brains and cords were injected into normal mice. One group consisted of three tryptophane-deficient mice, un-

[4] Webster Swiss mice supplied by the Department of Veterinary Science, University of Wisconsin, through the courtesy of Dr. G. K. L. Underbjerg.

inoculated, that had developed signs of severe deficiency and had undergone two or more tonic convulsions. One died on the twentieth day on the ration and the other two were sacrificed. The brains and cords of the three were removed, ground with alundum in the fresh, unglycerinated state, and a 5 per cent suspension was made in broth. Five mice were then injected intracerebrally with 0.03 ml of the 5 per cent suspension. The second group consisted of two mice in an advanced stage of deficiency that had shown tonic convulsions on the twenty-sixth and twenty-ninth days on the diet. These were sacrificed on the thirtieth day and the brains and cords prepared as described for the first group. The 5 per cent suspension was injected intracerebrally into five mice. The third group consisted of two mice that were markedly deficient but in which tonic convulsions had not been observed. One died after 29 days on the diet and was kept until the next day in the refrigerator. The second was sacrificed on the thirtieth day, and the two brains and cords were pooled and prepared as in the other groups. Five mice were then injected with the 5 per cent suspension. In addition, bacteriological cultures were made of the heart blood of these seven mice and of the three brain suspensions in thioglycolate broth and on agar plates containing 5 per cent defibrinated sheep's blood. No microorganisms were found after 1 week's incubation, and the cultures were therefore considered bacteriologically negative. Although no bacteriological studies were conducted on mice in the series reported here, the dead mice in series 97 were autopsied routinely, and no gross pathology was noted.

The mice injected with brains and cords from these uninoculated deficient mice remained healthy for 28 days, at which time they were sacrificed, and it appears unlikely that a latent virus was responsible for the signs we have described for tryptophane deficiency.

Histological studies. Histological studies (formalin fixation, gallocyanin stained) of a small number of the mice showed no direct correlation between the severity of the lesions and the observed signs. Encephalitic, but not poliomyelitic, lesions were seen, however, in some of the animals that died without showing typical signs of infection.

DISCUSSION

Although there are now many reports in the literature of the various effects aberrant nutrition can have on virus diseases, we have not found any report in which the virus infection affects nutrition in any specific fashion. The results we have described here seem to depend both on the effect nutrition may have on the virus disease and on the effect the virus may have on nutrition. Tryptophane deficiency has resulted, in our experience, in a modification of the course of the disease, to the extent that the usual signs of infection are completely depressed in the majority of animals. The infection can scarcely be considered more fulminating in the deficient mice, in which death supervened without signs, since the time of death was later than in mice receiving tryptophane. Although the disease was inapparent up to the time of death, the virus had none the less multiplied, as demonstrated by titrations of the brain and spinal cords. No

attempt was made to compare the rates of multiplication of the virus in mice lacking or receiving tryptophane, but at the time of death the virus had multiplied to about the same degree in deficient mice as in mice on diets adequate in tryptophane, regardless of whether signs of infection or only signs of deficiency had been observed. Histological studies also suggested virus multiplication, since encephalitic lesions were present in brain sections of some of the mice that had not shown signs of infection. Histological examinations extensive enough to determine how frequently such lesions might be present were not possible under our experimental conditions.

The virus, on the other hand, seemed to precipitate the deficiency of tryptophane insofar as the described deficiency signs can be taken as evidence. Signs of advanced deficiency commonly appeared earlier in mice that had received virus, but these same signs appeared later in mice that were not inoculated or that had received injections of normal brain suspensions. Extra vitamin supplements and the presence of the nonessential amino acids apparently had no effect. When tryptophane was present, however, even in a concentration as low as 0.3 per cent of the diet, the results were reversed; deficiency signs were not evident and the majority of mice exhibited the characteristic signs of infection in its usual course, with death following encephalitis and paralysis. The fact of virus multiplication, the presence of encephalitic lesions in some of the mice, the lack of paralysis or clear encephalitic signs, and the early appearance of severe deficiency signs suggest the possibility that death in these mice may have resulted from a combination of deficiency and viral effects, before more characteristic encephalitic signs and paralysis could develop.

Since the virus had multiplied in the deficient mice, and it seems not unlikely that tryptophane is a constituent of the virus, it is possible that virus multiplication rendered residual tryptophane unavailable to the host, thus making the deficiency signs more acute.

Preliminary experiments indicate that a similar situation obtains in methionine and valine deficiencies with this same virus. Tonic convulsions have also been observed in methionine deficiency, but not as yet in a deficiency of valine. It may be that some necessary metabolic component cannot be synthesized when certain of the essential amino acids are not available in sufficient quantity.

The importance of inanition in the results we have obtained is difficult to assess. It is true that mice on these rations eat very little. We have studied the effects of inanition on this virus disease and have ascertained that reduction of the total food intake to 1 g per day, or caloric restriction to 1 g of the fats, proteins, salts, and vitamin mixture, plus only 0.5 g of sucrose does not duplicate the results with tryptophane deficiency. Typical signs of infection were somewhat suppressed in about half of the mice, but there was no delay or reduction in incidence. Many of the deaths occurred before paralysis began in control groups fed the complete diet ad libitum and might be considered more fulminating infections. Attempts to reduce the food intake further have resulted in the death of too many of the mice to conduct an effective study. The inanition studies were conducted with the 18 per cent casein diet. This has not been

repeated with the essential amino acid diet since those mice that were fed this ration, including 0.3 per cent tryptophane, also ate very little. The weights are barely maintained and there is often an initial weight loss, not so marked as on the deficient diet. Despite the low food intake of mice on this ration, the signs of infection were clear; uninoculated controls showed no signs of deficiency and survived throughout the experiment.

The observations reported here are seemingly at variance with those of Jones *et al.* (1946) in their study of low tryptophane diets and poliomyelitis. They found that diets low in tryptophane produced a marked delay in onset but no other modification of the disease. They were using, however, a different virus, the Lansing strain of poliomyelitis, and tryptophane was supplied at 0.02 per cent level in an otherwise deficient diet containing zein as the protein source. This amount was supplied in order to prevent an undue number of animals from dying of deficiency, a necessary precaution when dealing with a virus that may have a long incubation period. Weight curves given for their mice indicated that this amount of the amino acid was enough to maintain the animals at fairly constant weight. Inasmuch as our deficient diet contained no tryptophane and steady weight losses occurred, the two series of experiments are not strictly comparable.

Since in our experiments the deficient diet seemed to suppress paralysis more effectively than encephalitis, both of which occur in this virus disease, it has appeared worth while to study this deficiency with WEE virus, which produces mainly encephalitis with little cord involvement, and with Theiler's original (TO) virus, which produces poliomyelitis with almost no encephalitic signs. Experiments along these lines are under way and will be reported at a later date. It also appears important to approach this problem from another point of view—the use of amino acid analogues, which may be metabolic antagonists. The study of bacterial viruses has been notably successful with this approach. Tryptophane is apparently necessary for the multiplication of the T2 bacteriophage in *Escherichia coli*, since the analogue 5-methyl tryptophane inhibits the synthesis of virus without affecting the respiratory activity of the host cell, and this inhibition can be reversed specifically by the addition of tryptophane (Cohen and Fowler, 1947). Amino acid analogues have also inhibited the growth of vaccinia virus in Maitland type tissue cultures (Thompson, 1947). The amino acids for which analogues were used in this case were glycine, valine, phenylalanine, and methionine.

SUMMARY

Low protein (9 per cent casein) and high protein (36 per cent casein) diets have, in our hands, exerted no influence on infection of mice with Theiler's GDVII virus.

A marked effect on this disease has, however, been produced with diets deficient in tryptophane. The deficiency has been induced by feeding pure amino acid diets (containing only the essential amino acids) minus tryptophane and by feeding acid-hydrolyzed casein; in one series double amounts of vitamins were also

given in an attempt to rule out possible complications in the deficiency. Regardless of the diet used to produce the deficiency, or of the presence of extra vitamins and the nonessential amino acids, the results have been an accelerated death rate compared to control deficients, a delayed death rate compared to those mice receiving the same diets plus 0.3 per cent tryptophane or the 18 per cent casein optimum diet, and a lack of the characteristic signs of infection in the majority of deficient mice. The signs shown by these animals are, however, characteristic of the deficiency but appear earlier than in uninoculated controls, the effect seeming to be a precipitation of the deficiency by virus infection. That the virus had multiplied in the deficient mice in which no signs of infection could be observed was demonstrated by titration of their brains and cords in young normal mice. Histological studies confirmed this, since evidence of encephalitis was found in such animals. Mice receiving tryptophane, added to the purified amino acid or acid-hydrolyzed casein diets, or those on the 18 per cent casein diet, showed typical encephalitis and progressive paralysis. On the amino acid diet paralyses were frequently fewer than on the 18 per cent casein diet, but infection was indicated by encephalitis when para'ysis was absent.

Tryptophane deficiency alone has produced definite signs. Among these is a convulsion of the tonic type, during which the hind legs of the mouse are completely extended, the forelegs flexed, and the whole mouse is extremely rigid and cyanotic. At times the hind legs are extended and the forelegs move convulsively. These convulsions occur in late stages of the deficiency, since death usually follows within several days. Death may occur in one of these seizures, but often the mice undergo such convulsions 3 to 5 times on as many days before dying. The possibility that a latent virus is responsible for these signs has been tested by passage of the brains and spinal cords of these mice intracerebrally into young normal mice and intracerebrally or subcutaneously into guinea pigs. No evidence of an infectious agent was found.

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