Effect of Amino Acid Imbalance on Course of Lansing Poliomyelitis in Mice.* (19917)

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Over a period of years considerable evidence has been obtained indicating that malnutrition results in a decreased susceptibility to some viral infections(1). Jones *et al.*(2) have reported a sparing effect of low protein and low tryptophan diets on mice with Lansing poliomyelitis, and Davies *et al.*(3) have studied the influence of deficiencies of each of the essential amino acids on the course of Lansing poliomyelitis and have found tryptophan, isoleucine, valine and methionine deficiencies especially effective in suppressing the Lansing virus. Rasmussen *et al.*(4) reported that 6-methyl tryptophan exerts similar action on Lansing infections in mice. The experiments reported here were designed to compare the effect of methionine and tryptophan analogues and rations containing excessive amounts of various amino acids on the course of Lansing poliomyelitis in mice.

Methods and results. In all of these experiments, 4-5 week old Webster Swiss mice from our colony were used. Purified rations containing casein as a protein source or mixtures of amino acids replacing protein were fed. The mice were inoculated intracerebrally with a suspension of spinal cords and medullas from mice infected with Lansing poliomyelitis virus (usually 0.03 ml of a 1% suspension, containing 100 LD_{50} of virus). It has been demonstrated that methionine sulfoximine acts as a methionine antagonist for various bacterial and animal species(5-7). After preliminary toxicity studies, 70 mice in each of 2 groups were placed on a 9% casein diet. A week later all of the animals were inoculated intracerebrally with 10 LD_{50} of Lansing virus, and daily intraperitoneal injections of ½ mg of methionine sulfoximine were started. The results of this experiment indicated that methionine sulfoximine had a slight effect in prolonging the incubation time. However, in this experiment methionine sulfoximine was of little real value in altering the course of poliomyelitis in mice. These observations agree with Ainslie's(8) report on the anti-Lansing activity of methionine sulfoximine.

In another series of experiments several tryptophan analogues were tested. These included 6-methyl tryptophan for comparative purposes, 4,6-dimethyl tryptophan, 4-methyl tryptophan, 5-methyl tryptophan, alpha-ethyl tryptamine and alpha-amino (3 indazole) propionic acid. These analogues were incorporated into amino acid rations containing 0.03% and 0.05% DL tryptophan. Of these alpha-ethyl tryptamine and 5-methyl tryptophan appeared to be the most effective tryptophan antagonists so far as the nutrition of the mice was concerned; but alpha-ethyl tryptamine had no significant influence on Lansing infections and 5-methyl tryptophan even when fed at highly toxic levels did not exhibit a protective action comparable to that obtained with 6-methyl tryptophan.

Following this series with methionine and tryptophan deficiencies the effect on mouse poliomyelitis of amino acid imbalances caused by feeding excess methionine and tryptophan was studied. The results of a preliminary experiment suggested that excess methionine but not excess tryptophan was protective.

Consequently, the following experiment was undertaken. Three groups of 28 mice each

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TABLE I. Effects of Excess Methionine on Resistance of Mice to Lansing Virus.

Ration	9% C + 5% DLm*	9% C + 3% DLm	9% C + 1% DLm	9% C
No. of mice†	26	28	27	28
Survivors	0	1	0	0
Avg incubation period, days	14	12.1	7.5	7.1
Avg survival time, days	14.9	13.3	8.5	8.5

* C = Casein; DLm = DL methionine.
† No. of mice in each group alive at time of inoculation.

TABLE II. Effects of 5% DL Methionine Ration on Resistance of Mice to Lansing Virus.

	Group			
	Mice on ration 1 wk prior to inoc.	Mice fed ration 2 days, 9% casein 1 day	Mice on ration 1 day prior to inoc.	9% casein control
No. of mice*	26	27	28	28
Survivors	0	0	1	0
Avg incubation period, days	14	8.4	9.2	7.1
Avg survival time, days	14.9	9.7	11.2	8.5

* No. of mice in each group alive at time of inoculation.

TABLE III. Effect of 6-Methyl Tryptophan + Methionine on Resistance of Mice to Lansing Virus.

Ration	.4% 6-MT + 3% DLm*	.4% 6-MT	3% DLm	Control
No. of mice	14	14	14	14
Survivors	2	0	0	0
Avg incubation time, days	14.3	9.6	9.9	7.9
Avg survival time, days	17.5	11.4	11.1	9.1

* DLm = DL methionine.

were placed on 9% casein rations containing 1, 3, and 5% DL methionine one week before intracerebral inoculation with 100 LD_{50} of Lansing virus. Another group of 28 mice was fed alternately a 9% casein diet for one day followed by 2 days of a ration containing 9% casein + 5% DL methionine. A fifth group was placed on a 9% casein diet for 6 days and then changed to the 9% casein, 5% DL methionine ration the day preceding inoculation. A control group was fed the 9% casein diet but no excess methionine. Uninoculated nutritional controls were also set up for each of these 6 groups. The experiment was terminated 28 days after inoculation, and the results obtained are shown in Tables I and II.

It can be seen from Table I that rations containing 9% casein, when supplemented with 3 or 5% DL methionine, delay the onset of symptoms in mice intracerebrally infected with Lansing virus. Table II indicates that in order fully to obain this effect, a continuous period of high methionine intake is necessary prior to inoculation.

Similar experiments with excess leucine, lysine, histidine, phenylalanine and choline, a methyl donor, were negative.

To determine whether 6-methyl tryptophan and excess methionine were synergists in their protective action, 4 groups of 14 mice each were placed on amino acid mix rations containing 0.05% DL tryptophan one week before being intracerebrally inoculated with 100 LD_{50} of Lansing virus. The ration fed the first group was supplemented with 3% DL methionine. The second group's ration was supplemented with 0.4% 6-methyl tryptophan. The third group had 3% DL methionine and 0.4% 6-methyl tryptophan added to its ration, and the fourth group was used as a control. The results obtained in this experiment are shown in Table III.

These data show that a combination of 0.4% 6-methyl tryptophan and 3% methionine exerts a greater protective effect against Lansing poliomyelitis in mice than was obtained with these levels of either compound alone.

Discussion. The decreased susceptibility of mice to Lansing virus brought about by the feeding of 6-methyl tryptophan and excess methionine is manifested as essentially a delay in the onset of symptoms with a resultant prolonged survival time. In experiments employing 6-methyl tryptophan at higher levels without excess methionine a significant proportion of mice are protected completely(4). A very low survival rate has always been obtained when excess methionine has been fed

alone. This has been due in part to the toxicity and lack of palatability of high methionine rations. Uninoculated nutritional controls fed high methionine rations lose about 40% of their body weight during the test period and deaths in these groups are not infrequent. However, restricted food intake experiments have shown that the protective effect of excess methionine is not directly related to starvation.

Summary. 1. A decreased susceptibility to Lansing poliomyelitis, characterized by prolonged incubation and survival times, has been observed in mice fed excess methionine. To obtain this effect fully, a continuous period of high methionine intake was necessary prior to inoculation. 2. The addition of excess methionine to low tryptophan rations containing 6-methyl tryptophan resulted in a more marked protection against Lansing infection in mice than when the same amounts of methionine or 6-methyl tryptophan were fed alone.

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Studies on Irradiated Animals, III. Effect of Saline on Radiation-Induced Mortality and Weight Changes.* (19918)

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In pharmacological studies it is customary to dissolve chemicals to be injected in saline solution, and also to use saline as control. However, in pharmacological studies on irradiated animals saline solutions may not be inert since the "acute radiation syndrome" includes disturbances of the salt-water metabolism(1). Saline injections might, therefore, alter the course of radiation death. In order to clarify the effect of physiological sodium chloride injections on irradiated animals, the investigations reported in this paper have been undertaken.

Materials and methods. Swiss, male white mice of the Institute strain, 22 g ± 15% of body weight, were used. Irradiation was done as described previously(2,3). The radiation factors were: 200 kv, 15 ma, .25 Cu ± 1.0 mm Al filtration. HVL = .8 mm Cu. X-ray doses of 410 and 385/air, representing the LD_{60} 14 days and $LD_{30}/14$ days were given. Saline was administered daily in amounts of .3 cc of a 0.9% NaCl solution either intramuscularly or intraperitoneally, starting immediately after exposure, for an over-all period of 14 days (with the exception of Sundays) or for 6 consecutive days.† For graphic presentation of results the mortality rate of saline-injected animals is compared with that of the non-medicated irradiation control group. The figures in parentheses indicate the number of animals in the different groups.

Results. 1. *Effect of intramuscular saline injection in its relationship to the time of saline administration.* Fig. 1 summarizes pertinent results obtained in this study. As indicated in Fig. 1, saline injection slowed the death rate at the end of the first week for 2 or 3 days. If injection of saline was continued over the 14-day period (curve B), then a

*The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

† The animals were kept during the 28-day observation period in air conditioned quarters at 75°F.