(Diet 3) showed the least severe changes, while the livers of rats on Diet 2 (DL-methionine) showed the most severe changes. Since fibrosis and various degrees of parenchymal lesions were observed in all rats on 0.032% TA with and without the added lipotropic factors, it cannot be said that any of the lipotropic factors studied were effective in preventing liver damage, although there appears to have been a quantitative alteration in the character of the lesion.

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Failure of Malononitrile in Therapy of Experimental Poliomyelitis.* (17832)

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Recently Szanto and Felsenfeld(1) reported malononitrile to be effective in protecting more than fifty per cent of mice infected with a low concentration of the Lansing strain of poliomyelitis and prolonging the life span of mice infected with a larger dose of the virus. They also reported that mice treated with malononitrile after onset of paralysis showed a markedly prolonged survival time, about one-third of which recovered completely or partially. The purpose of this communication is to report the failure of malononitrile to protect mice against intracerebral inoculation of the Lansing strain of poliomyelitis. It was also found that this drug had no therapeutic effect when administered to mice after the onset of paralysis.

Methods. The Lansing strain of poliomyelitis virus was used in all therapy experiments. The technic of preparation and storage of mouse brain suspensions of this virus for intracerebral inoculation is described elsewhere(2). The identity of the Lansing strain used in this laboratory is periodically verified by production of typical paralysis in rhesus monkeys following intracerebral inoculation, histopathologic findings and neutralization tests with hyper-immune Lansing antiserum. Two strains of Swiss mice (Webster & Beyer) weighing 13 to 15 g were employed in the present studies. Groups of mice were inoculated with either 10 or 100 LD_{50} units of virus intracerebrally in chemotherapy experiments. The LD_{50} titer of the virus was approximately $10^{-3.5}$. Two types of experiments were done. In the first, mice were inoculated daily with malononitrile $CH_2(CN)_2$† beginning 24 hours before virus inoculation and therapy was continued until onset of paralysis. In the second, mice were treated with malononitrile after onset of paralysis, and the survival time was compared with that of untreated, paralyzed mice. The survival time of the paralyzed mice was recorded as the time when the mice were found dead. In most instances the actual time of death was observed.

Results. Mice were inoculated intraperitoneally with 2.5 to 3 mg per kg body weight daily in therapy experiments. The dosage of 3 mg per kg of body weight was given in most experiments, and we also found that this amount of drug was well tolerated by the mice. Combined results obtained in 3 experiments with groups of treated and control mice challenged with approximately 10 and

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2. Milzer, A., Oppenheimer, F., and Levinson, S. O., *J. Immunol.*, 1945, v50, 331.

† Obtained from the Schwarz Laboratories, N.Y.

TABLE I.
Results Obtained in Protection of Infected Mice with Malononitrile.

LD_{50} dose	No. of mice	Drug given	Avg incubation period, days	% mortality
10	32	+	12.0 ± 3.8	80
10	38	0	15.9 ± 5.3	76.4
100	32	+	11.3 ± 2.5	94.6
100	38	0	11.1 ± 3.2	97.7

TABLE II.
Results Obtained in Therapy of Paralyzed Mice with Malononitrile.

LD_{50} dose	No. of mice	Drug given	Avg survival time after onset of paralysis in hr
10	29	+	32.5 ± 14.6
10	30	0	32.1 ± 10.2
100	25	+	29.3 ± 14.5
100	21	0	31 ± 11

100 LD_{50} units of virus intracerebrally are shown in Table I. Malononitrile was started 24 hours before virus inoculation and continued daily until onset of paralysis. About half of the treated mice received the drug daily for at least 10 days prior to onset of paralysis. The results shown in Table I indicate that there was no significant difference in average incubation period or mortality rate in the treated and control mice challenged with 10 or 100 LD_{50} units of virus respectively.

Table II summarizes the results obtained when 3 mg per kg body weight of malononitrile was given after onset of paralysis in mice initially challenged with 10 and 100 LD_{50} units of Lansing virus. Again no significant difference was observed in survival time between the control and treated groups of animals. All mice which developed paralysis uniformly succumbed regardless of malononitrile therapy.

Discussion. The identity of the virus employed in the malononitrile therapy experiments described by Szanto and Felsenfeld(1) is open to question because of the high M.L.D. titer described (3.2×10^{-7}) and the relatively short survival time after onset of paralysis in their control mice. Furthermore, these authors describe no attempts to check the identity of the virus strain that they employed in order to rule out the possible hazard of a spontaneous mouse neurotropic virus. In our experience and that given in the literature(3,4) the LD_{50} titer of the Lansing strain in infected mouse CNS tissue usually ranges from $10^{-3.4}$ to $10^{-4.2}$ in Swiss mice inoculated intracerebrally.

We feel that adequate dosages of malononitrile were given in our experiments because therapy was started 24 hours before virus inoculation and continued daily until onset of paralysis. We also employed the recommended dosage per kg body weight in most of our experiments. On the other hand, Szantos and Felsenfeld gave the drug the day after infection and started daily administration 6 days later for 10 consecutive days in mice inoculated with a low M.L.D. of virus. Mice inoculated with a high M.L.D. of virus were started on therapy the day after infection.

Summary. Malononitrile was found to have no beneficial effect in either the protection or therapy of mice infected with the Lansing strain of poliomyelitis.

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