genated derivatives caused gangrene; in contrast, ergotamine produced gangrene in 80% of the rats. The survival of rats receiving ergot alkaloids or dihydrogenated ergot derivatives was equal to or better than that of the controls.

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Influence of Malononitrile upon Poliomyelitis in Mice.* (17316)

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Recent investigations by histophysical[1] and histochemical[2,3] methods identified the basophilic component of the Nissl bodies in the nerve cells as ribonucleic acid. Chromatolysis of the Nissl substance in the late preparalytic period is the earliest cytologic change in monkeys infected with poliomyelitis virus.[4] While chromatolysis is a reversible process, it may lead to complete cellular necrosis. Recovery is characterized histologically by the reappearance of the Nissl substance in the surviving nerve cells. Malononitrile CH_2 $(CN)_2$ increases the ribonucleic acid content, *i.e.*, the Nissl substance of the nerve cells but not of the liver and pancreas.[5] This prompted us to investigate the influence of malononitrile upon experimental poliomyelitis in mice.

Materials and method. Young, virus free mice, of the average weight of 12 to 15 g,† were infected with the Lansing strain of the poliomyelitis virus. The M.L.D. of this strain showed fluctuation during storage but was easily increased by successive mouse passages. The paralytic period, *i.e.*, the time between the appearance of the paralysis and death of the animal, was short, between a few minutes and 4 hours, even if low concentrations of the virus were used. The virus was inoculated intracerebrally, using 0.03 ml of a 10% mouse brain emulsion with an M.L.D. of 1.3×10^{-3}, 2.7×10^{-5} and 3.2×10^{-7}, respectively. Malononitrile‡ was administered intraperitoneally, using a 0.5 g per L. solution sterilized by passage through a Seitz filter. Since malononitrile is toxic,[6] its effect on normal mice was investigated.

Two types of experiments were set up. In one, the survival rate of the infected mice treated daily with malononitrile before the appearance of paralysis was compared with that of untreated controls. In the second, mice were treated with malononitrile after the onset of the paralysis and the survival time was compared with that in untreated paralyzed mice. Because of the rapid downhill course of the disease in paralyzed animals, relatively few mice could receive treatment after the onset of paralysis. Thus many animals were lost. The survival time of mice was established in such manner that the hour at which the animal was last seen alive was considered as the last hour of its life.

After the death of the animals, brain, spinal cord, liver, heart and kidneys were fixed in Carnoy's or Bouin's fluid. In addition to

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1 Landstrom, M., Casperson, T., and Wohlfart, G., *Z. f. Mikroskop.-Anatom. Forschung*, 1941, **49**, 534.

2 Brachet, J., *Enzymologia*, 1941, **10**, 87 and 96.

3 Gersh, I., and Bodian, D., *J. Cell. Comp. Physiol.*, 1943, **21**, 253.

4 Bodian, D., *Bull. Johns Hopkins Hosp.*, 1948, **83**, 1.

5 Hyden, H., and Hartelius, H., *Acta Psychiatr. et Neurol.*, Suppl., 1948, **48**, 1.

† Purchased from the Carworth Farms, N. Y.

‡ Received from the Schering Corporation, Bloomfield, N. J.

6 Heymans, J. F., and Masoin, P., *Arch. f. Internat. Pharmacodynam. a. Therap.*, 1897, **3**, 77.

TABLE I.
Effect of Malononitrile When Given in Preparalytic Phase.

Virus M.L.D.	No. of mice	Malononitrile given	Avg incubation time, days	Mortality, %
1.3×10^{-3}	24	+	37.1 ± 13.2	41.7
	24	0	10.2 ± 2.8	100
3.2×10^{-7}	84	+	5.3 ± 1.2	97.6
	84	0	4.4 ± 1.3	100

TABLE II.
Effect of Malononitrile Given in the Paralytic Period.

Virus M.L.D.	No. of mice used	Malononitrile given	Avg survival time after onset of paralysis in hr
2.7×10^{-5}	14	+	43.5 ± 8.7
	24	0	1.3 ± 0.4
3.2×10^{-7}	22	+	31.8 ± 6.1
	24	0	0.8 ± 0.2

hematoxylin-eosin, the sections were stained with gallocyanin and thionine (Windle's modification[7]).

Results. The intraperitoneal administration of 6 mg malononitrile per kg body weight to normal mice produced within a few minutes rapid respiration, convulsions and death. Three mg per kg body weight was well tolerated, although a general depression following an excitation state lasted frequently for many hours.

All 24 mice which received the Lansing virus of M.L.D. 1.3 x 10^{-3} died after an incubation period of 10.2 ± 2.8 days. Of 24 mice which received the same amount of the virus and 3 mg malononitrile per kg body weight the day after the infection, and, starting 6 days later, the same amount of the drug daily for 10 consecutive days, only 10, or 41.7%, died within 3 months. The average incubation time of the animals which died was 37.1 ± 13.2 days (Table I).

Of 84 mice injected with a high M.L.D. (3.2 x 10^{-7}) all died. Of 84 animals infected with the same amount of the virus and treated with malononitrile, starting the day after infection, 82 died after an incubation time of 5.3 ± 1.2 days, which does not differ significantly from that of untreated animals (*i.e.*, 4.4 ± 1.3 days). Only 2 of the mice which received malononitrile survived in this group. The period between paralysis and death in the treated animals, however, was 17.8 ± 1.2 hours, as contrased with 0.8 ± 0.2 hour in untreated mice.

Of 38 mice infected with Lansing virus of M.L.D. 2.7 x 10^{-5}, 24 served as controls for the determination of the survival time after paralysis developed, while 14 mice received 3 mg malononitrile per kg body weight after paralysis appeared. The average survival time of the treated animals was 43.5 ± 8.7 hours, while untreated animals lived only 1.3 ±0.4 hours after the onset of paralysis (Table II). Only 3 of the treated animals lived for less than 4 hours, while 2 survived for as long as 14 days. They showed complete recovery but died suddenly when the drug was withdrawn. In some of the other treated animals temporary partial or total disappearance of the paralysis was observed.

Of the 84 mice which received the virus in M.L.D. 3.2 x 10^{-7} concentration, 22 were treated with malononitrile after paralysis developed. Their survival time was 31.8 ± 6.1 hours, as compared with 0.8 ± 0.2 hour in 24 control animals. About one-third of the treated mice showed partial or complete recovery from the paralysis but died later.

The treated animals which survived for three months did not show the usual histological involvement of the central nervous sys-

[7] Windle, W. F., Rhines, R., and Rankin, J., *Stain Technol.*, 1943, **18**, 77.

tem,[8] while those which died despite the treatment showed similar pathologic changes as the untreated mice except that the cytoplasmic basophilic substance of the nerve cells appeared better preserved.

Discussion. The described experiments indicate that malononitrile has a certain value in the treatment of mouse poliomyelitis. If lower concentrations of the virus were used, treatment in the preparalytic stage prevented death in more than one-half of the animals and prolonged the incubation period nearly threefold. While death following huge doses of the virus could not be prevented by the drug, the life span between paralysis and death was prolonged also in these cases.

When malononitrile was given after the appearance of paralysis, in about one-third of the animals significant regression of the clinical symptoms was observed. Complete recovery was observed in solitary cases but the animals died after cessation of the treatment.

There are various ways in which a chemical compound may act against the poliomyelitis virus. It may have a prophylactic effect either due to the action upon the virus before it reaches the nervous system, or it may render the nerve cells unsuitable for the multiplication of the virus. The arrangement of these experiments precludes the possibility of a direct inactivation of the virus before it reached the nervous system. Malononitrile, however, may render the intracellular environment unsuitable for continued development of the virus by its protective effect upon the nucleic acids. It may also help to restore the damaged function by increasing the ribonucleic acid content of the attacked cells. While a direct action upon the virus within the cell is not probable, the possibility that this compound acts by interfering with an enzyme system of the host, essential for the multiplication of the virus, has to be considered.

Summary. Malononitrile treatment preserved the life of more than one-half of the mice infected with a low concentration of the Lansing type poliomyelitis virus, but only prolonged the life span of mice infected with large doses of the virus. Mice treated with malononitrile after the onset of the paralysis showed markedly prolonged survival time. In about one-third of the animals partial or total recovery of the paralytic symptoms was observed; the mice, however, died after cessation of the treatment.

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[8] Lillie, D. R., and Armstrong, C., *Publ. Health Rep.*, 1940, **55**, 718.

Change of Muscular Excitability by Eserine, Acetone and Methyl-alcohol. (17317)

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The potentiation of acetylcholine by eserine, acetone, and methyl alcohol being known, we are interested in finding out their influence on the electrical excitability of the muscle.

The rheobase and chronaxie were determined with condenser discharge. The rectus muscle of the toad was placed in a glass muscle chamber, moistened with Ringer's solution, and observed under dissecting microscope. After the control reading had been taken, the rectus muscle was immersed in a bath of 8 cc of Ringer's solution, bubbled with air, and treated with 10 γ eserine for 30 minutes, or 0.05 cc acetone or methyl-alcohol for 3 minutes, and, in addition, Ringer's solution was alone used without adding any reagent for blank control. Following the treatment, both were redetermined under conditions as before.