hibitor concentration. It was shown that the kinetics of eserine-inhibition may be analyzed by classical methods which ignore enzyme concentration but the cases of the other two inhibitors may not be so treated. Cholinesterase inhibition by the fluoro- and pyrophosphates was shown to be irreversible and to depend upon enzyme concentration and time of incubation of the enzyme with the inhibitor before the addition of substrate. The upper limit of cholinesterase concentration in rat brain was estimated to be 1×10^{-6} molar if structural restrictions are not assumed.

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Some Effects of Large Doses of Ergot Products on Rats.* (17315)

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An examination of the literature reveals much interest in various ergot products, particularly the dihydrogenated derivatives, because of their possible usefulness in the treatment of migraine,[1,2] in the prevention of cyclopropane arrhythmias,[3] and in the treatment of hypertension.[4-6]

Toxicity of the dihydrogenated derivatives is less than that of the natural alkaloids;[7] Orth and others[8] show that dihydroergocornine did not produce gangrene in the tails of rats, whereas ergotamine routinely produced gangrene. They also show that pregnant female rats receiving dihydroergocornine delivered normal litters and raised them to maturity, whereas similar rats receiving ergotamine tartrate lacked maternal instincts.

No accounts were found in the literature of experiments in which ergot products were injected over extended periods of time. Orth and others[8] made semiweekly injections during the gestation period of rats in doses up to 35 mg/kg of dihydroergocornine. Observations over extended periods were thought to be desirable since many patients would take the ergot product more or less regularly for years.

Methods. A total of 73 rats were injected subcutaneously 6 times a week for periods up to 17 weeks. This rigorous treatment contrasts with that of human therapy in which ergot products are usually injected only twice a week.

Control rats were injected 6 times a week with physiological saline.

All rats were weighed once a week.

Preliminary experiments show that doses comparable to therapeutic doses in man (for those products whose therapeutic doses have been established) produced no measurable effect on weight of rats. Therefore, it was decided to make the experiments still more rigorous by using doses comparable, on a weight basis, to those of ergotamine tartrate that produce gangrenous tails in rats. Preliminary experiments showed that a dose of about 0.5 mg/kg of ergotamine tartrate adminis-

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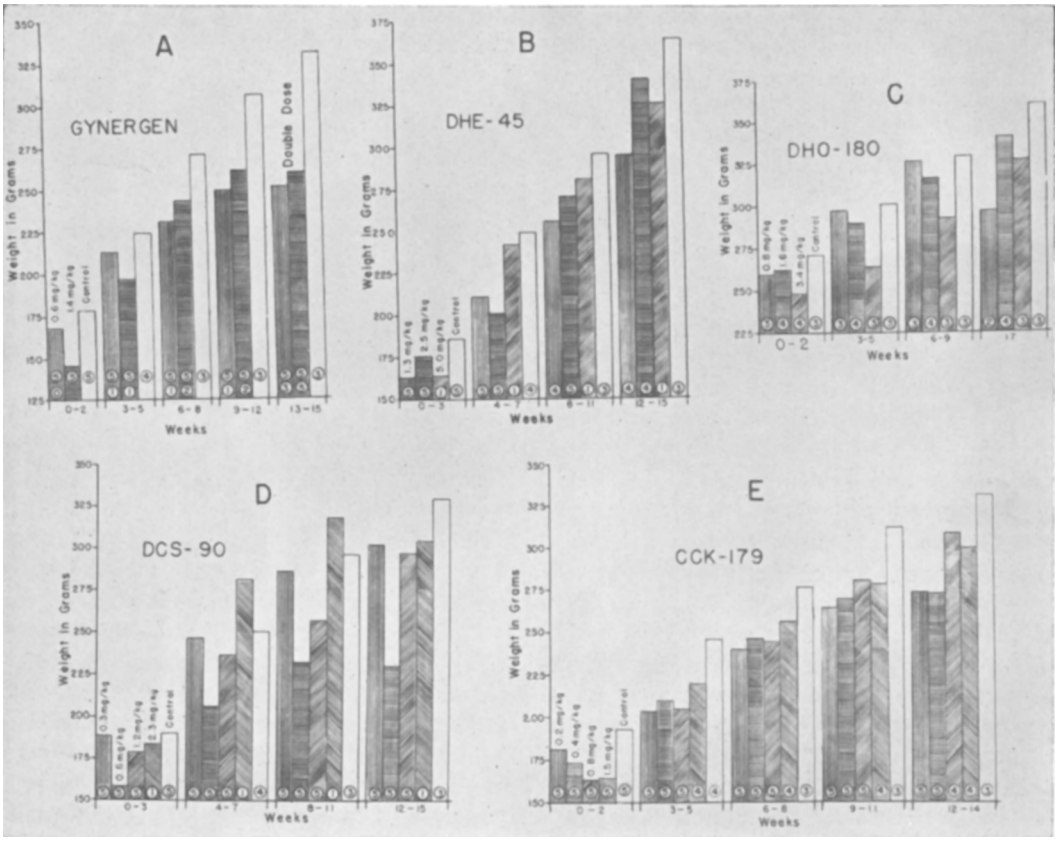

FIG. 1.

Effects of ergot products on growth rates, survival and production of gangrenous tails in rats.

A. Upper circle: Number of rats surviving the period. Lower circle: Number of rats with gangrenous tails at end of the period.

B to E, inclusive. Number encircled: Number of rats surviving the period.

tered by daily subcutaneous injection produced gangrene in about 4 weeks in about 20% of the rats. This is about 200 times the therapeutic dose in man.

The results of several weeks' weighings were averaged for simplicity in graphing. It was felt that this procedure was justified since the rate of growth of control rats was essentially linear. The results are shown in the figure.

Results. Ergotamine tartrate (Fig. 1, A) caused a decrease in the rate of growth in the doses employed. With doses as low as 0.6 mg/kg, one rat (20% of the rats) developed a gangrenous tail in 2 weeks. This dose was near the minimum for production of gangrene since doubling the dose caused an increase in the number of rats with gangrenous tails within 2 days. Survival was equal to or better than controls.

Dihydroergotamine (DHE 45), dihydroergocornine (DHO 180), dihydroergocristine (DCS 90), and a mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine (CCK 179) fail to produce a consistent inhibition of growth. These materials did not produce gangrene, and the survival of rats was equal to or better than controls.

Summary. The effect of several dihydrogenated derivatives of ergot were studied on rate of growth, production of gangrene and survival, as compared with ergotamine tartrate. None of these materials inhibited growth consistently; none inhibited growth as much as ergotamine tartrate. None of the dihydro-

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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