Effect of Acetylcholine on St. Louis Encephalitis Infection in the Mouse.* (19671)

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It is generally assumed that viruses which multiply only within cells of a particular type must find within these cells a peculiarly favorable metabolic milieu. The most striking examples of viral cytotropism are probably those neurotropic viruses which multiply within neurons. It is not improbable that the enzymatic peculiarities which are responsible for cytotropism may in some instances be related to the physiological function of the host cells involved. In the case of the neurons, a metabolic entity of unique functional importance is the acetylcholine-acetylcholinesterase-choline acetylase system(1,2). For these reasons, it seemed possible that among the many chemical agents which have been shown to modify acetylcholine (ACh) metabolism, some might be found which would alter the course of neurotropic viral infections. In this report the effect of acetylcholine itself on St. Louis encephalitis in mice will be presented.

Material and methods. The strain of St. Louis encephalitis used had been maintained for several years in the laboratory of Dr. G. O. Broun, of the Department of Internal Medicine. The mice, which were of the "Swiss" strain, were obtained from a commercial breeder, and maintained on the Rockland diet, with water *ad lib.* They were of fairly uniform size; those used in the first experiment averaged approximately 20 g in weight, those in the second experiment about 16 g, and those in the third and fourth about 18 g. It had been determined previously that ACh, given intraperitoneally at dosage levels of about 100 mg/kg was well tolerated, resulting only in apparent discomfort (increased salivation and decreased activity) for a period of 5 minutes or less. When doses of 150 mg/kg were given, however, occasional fatalities occurred within a few minutes.

Experimental. In the first experiment, 40 female mice were inoculated intranasally, without anesthesia, with 0.03 cc of a 1% aqueous suspension of brain tissue from a mouse dying of St. Louis encephalitis. The inoculum was given from a suitable calibrated syringe fitted with a 26-gauge needle, and was divided equally between the 2 nares. Five to 10 minutes before inoculation, the 20 mice in the treated group were injected intraperitoneally with 2 mg of ACh in 0.1 cc of distilled water. Similar injections were given daily until death occurred, or for 15 days in surviving animals. The control animals were not treated in any way, since it seemed improbable that the minute amount of distilled water given intraperitoneally could influence the course of the infection. (On the third day after inoculation, one control mouse was accidentally killed in closing a cage door). The second experiment was a repetition of the first with the following changes: The number of treated animals was 40 and the number of controls was 41. The dosage of ACh was 2 mg twice a day for the first 3 days; thereafter it was 1.6 mg/mouse daily. The third experiment was a repetition of the second except for the number of animals involved and the fact that the mice were of mixed sexes. The fourth experiment was a repetition of the second with the following difference: The first injection of ACh was given 18 hours before virus inoculation and the second injection 18 hours after virus inoculation.

Results. The figures given in Table I show that in all 4 experiments the survival rate in the treated animals was significantly higher than that in the controls. The probability that the figures representing totals for control and treated animals are significant, as determined by the χ^2 method(3) is approximately 6000:1. With rare exceptions, animals which lived did not at any time show evidence of

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TABLE I. Effect of Acetylcholine on St. Louis Encephalitis in Mice.

	No. of mice	Died	Lived	% survival
Control	19	10	9	47
ACh	20	4	16	80
Control	41	29	12	29
ACh	40	16	24	58
Control	55	40	15	27
ACh	50	27	23	46
Control	48	34	14	29
ACh	42	21	21	50
Total controls	163	113	50	31
Total ACh	152	68	84	55

illness, and animals which became ill died within 24-48 hours. The great majority of deaths occurred on the 6th and 7th days after inoculation; rarely an animal became sick as late as the 11th day and died on the 12th or 13th day. The average survival time of the treated mice which died was about 24 hours longer than that of the control mice which died.

Discussion. In the first 3 experiments, although virus was never injected until the period of increased salivation had apparently ended, the possibility existed that persistent changes in the nasal mucosa might have resulted in increased local inactivation of the virus. For this reason, in the fourth experiment the first and second intraperitoneal injections of ACh were given 18 hours before and 18 hours after the introduction of the virus into the nares. Since the increase in survival rate of the treated mice in this experiment was comparable to that in the other experiments, it is believed that the possible importance of local changes has been fairly well excluded, particularly since it has been shown (4) that no virus can be recovered from minced nasal mucosa 4 hours after the intranasal instillation of 10 times the virus dosage used in our experiments.

The mechanism of action of ACh in reducing the mortality from this viral infection can be determined only by further work. There is evidence that ACh, when present in high concentrations, may increase the rate of synthesis of ACh *in vitro*(5). The ACh-cholinesterase system is apparently an important factor in determining the permeability of certain cells(2). Cholinesterase inhibitors have been shown to influence dehydrogenase systems in the brain(6), suggesting that cholinesterase may have important effects on the general metabolism of neurons.

It is fortunate that a large group of carefully studied agents capable of inhibiting cholinesterase are available for further study. These include physostigmine, prostigmine, certain bacterial exotoxins, cobra venom, curare, caffeine, acetyl-B-methylcholine, the alkyl fluorophosphonates and many others. The choline acetylase system also has been extensively studied from the viewpoint of inhibition and stimulation(2). It seems possible that the use of these agents may make it possible to relate the multiplication of certain neurotropic viruses to specific metabolic entities within the neurons which they invade.

Summary and conclusions. Mice inoculated intranasally with the virus of St. Louis encephalitis were treated by the intraperitoneal injection of acetylcholine in large but well tolerated dosages. The survival rate of the treated animals was 55%, as compared to 31% for the control animals. Reasons are given for believing that the observed effect may be the result of alterations in cerebral metabolism.

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