skin test antigens or preselect animals of equivalent reactivity. In the latter case, 3 toxoplasmin preparations, each derived from a heterologous cell source, would be needed; one to be used to sensitize the animals, a second to select animals and a third to be standardized.

Whether the nature of the toxoplasmin suppression is due to the dissipation of specifically sensitized cells or to the dissipation of nonspecific cellular elements or both has not been resolved.

Suppression of the tuberculin skin test reaction by tuberculin (unpublished observations of Baer, H. and Kolb, R. W.) and unrelated agents, such as viruses, has been demonstrated(4,5,6). Whether or not the basis for suppression of reactivity of toxoplasmin and the other agents is analogous is not known.

Summary. Suppression of the skin reactivity to toxoplasmin by simultaneous injection of larger amounts of the antigen in the same sensitized guinea pigs was demonstrated by fewer animals reacting to the lesser amount of toxoplasmin and a decrease in the mean skin reaction as compared with the reaction when the toxoplasmin was injected alone.

The technical assistance of Mr. Jack C. Jenkins is gratefully acknowledged.

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Received October 6, 1966. P.S.E.B.M., 1967, v124.

Local Electrographic Responses to Intrahippocampal d-Tubocurarine and Related Neuromuscular Blocking Agents.* (31803)

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When injected into the cerebral ventricles, d-tubocurarine (dTC) markedly excited the central nervous system, causing extensive autonomic changes and convulsions(1). By selective perfusion of the individual ventricles, the actions of dTC were restricted more specifically to structures immediately lining the ventricle to produce tremors, myoclonic jerks or EEG discharges(2,3). On further analysis, the abnormal electrographic activities were shown to originate primarily from an action on the hippocampus(4,5). Comparable spike discharges were more readily developed by microinjecting the drug directly into the hippocampus(6,7). Our research was primarily structured to analyze the local electrographic paterns of excitation induced by dTC microinjected into the hippocampus. To characterize this activity further, the study was expanded to include other neuromuscular blocking agents. We hoped also to characterize the local mechanisms associated with hippocampal effects of dTC and if possible, to relate these to the cholinergic receptor mechanisms which have been defined pharmacologically at the neuromuscular junction.

Materials and methods. Acute experiments were carried out on 41 male adult cats (2.4-4.0 kg) while they were lightly anesthetized with dial-urethane, immobilized with either decamethonium or gallamine triethiodide (Flaxedil) and respirated. These animals had been prepared previously under ether anesthesia during which bipolar recording electrodes were placed stereotaxically in several key brain areas. Also, at this time, a microinjection cannula-electrode assembly ("Injec-

* Investigation supported by USPHS Grant MH 08833 from Nat. Inst. Mental Health.

trode") was lowered into the dorsal hippocampus. A detailed account of the construction of the "Injectrode" and its utilization for microinjecting drugs intrahippocampally and for recording local electrographic activities had been reported previously(8,9). However, at this time, a brief description of the assembly and the procedures as related to the current study seem in order.

The injection-recording unit consisted of metal tubing (cannula) to which were cemented two insulated recording electrodes; a finer micropipette was inserted into the cannula for accurately delivering predetermined small volumes of the drugs at its tip. Thus, chemically-induced changes in electrical activity in the surrounding hippocampal area can be recorded in the general vicinity of the microinjections. This spontaneous and evoked hippocampal activity was recorded on the electroencephalograph concurrently with bipolar electrograms from ipsilateral neurophysiologically-related cortical and subcortical sites along with the contralateral hippocampus. The local hippocampal activities also were monitored continuously on an oscilloscope. As a reflection of autonomic changes produced by the microinjected drugs, the EKG was recorded simultaneously with the electrograms.

Microinjections were made into the right dorsal hippocampus (R.Hipp.), Horsley-Clark coordinates, A_2, R8, D+5(10). In the series, electrograms were recorded from the following brain areas: right frontal cortex (R.F.C.), right suprasylvian gyrus (R.S.G.), right reticular formation (R.R.F.), right septum (R.Sept.), right posterior hypothalamus (R.P.Hypo.) and from the contralateral left hippocampus (L.Hipp.). Supplemental microinjections into the dorsal hippocampus were made at the same primary focal site.

At the end of the experiment the brain was lesioned electrolytically at the tips of the cannula and electrodes, fixed in formalin and subsequently stereotaxic placements confirmed. In some experiments methylene blue was microinjected and its intrahippocampal distribution was taken as an index of the diffusion of the microinjected drugs.

The agents microinjected into the hippocampus included: d-tubocurarine hydrochloride (dTC), gallamine triethiodide (Flaxedil), dihydro-β-erythroidine hydrobromide, decamethonium bromide or succinylcholine chloride. Concentrations of d-tubocurarine (0.05-0.1%) and of the other neuromuscular blocking agents were adjusted so that the volumes microinjected into the dorsal hippocampus were usually under 0.01 ml. All doses indicated in the text refer to the weight of the corresponding salts.

Results. Local electrographic discharges produced in the hippocampus by microinjected d-tubocurarine (dTC) were dependent on the cumulative doses administered. In a series involving 30 experiments, the average dose of dTC required for establishing a focus was calculated to be 4 μg; the usual latency for developing this focal activity was 10-20 minutes. In the upper tracings of Fig. 1, the graded focal responses to slight increases in cumulative doses of dTC from 6-8 μg are illustrated. A relatively simple focus was established at the lower dose after an interval of 14 minutes (Fig. 1B). This hypersynchronous discharge consisted of a spike-wave complex in the hippocampus which was projected to other ipsilateral limbic areas and the contralateral hippocampus, but was not significantly represented in the recordings from the two cortical areas. A few minutes later the focal discharge acquired an afterdischarge interposed between the spike and wave (Fig. 1C). A few more micrograms of dTC microinjected at the site further increased the amplitude and the duration of the afterdischarge (Fig. 1D); the waveform of the discharge stabilized and persisted in this form for several hours (Fig. 1E). These results were typical for the series.

In the lower tracings selected from another experiment a somewhat higher initial dose (10 μg) of dTC produced a characteristic focus but rapidly advanced into a brief period of seizure discharges (Fig. 1, B'-D'). During the post-ictal period, the focus was refractory for a few minutes (Fig. 1E') and then settled down, firing at a consistent rate (3-5/min) without any further

seizures (Fig. 1F'). The waveform and characteristics of the discharges and the doses required to establish the focus varied little from one experiment to another; however, the threshold for initiating the discharge could be markedly elevated by deepening the anesthetic state of the animal.

For comparative purposes the dTC study was expanded to include a series of pharmacologically related neuromuscular blocking agents. The dosage ranges explored along with the profiles of the local hippocampal

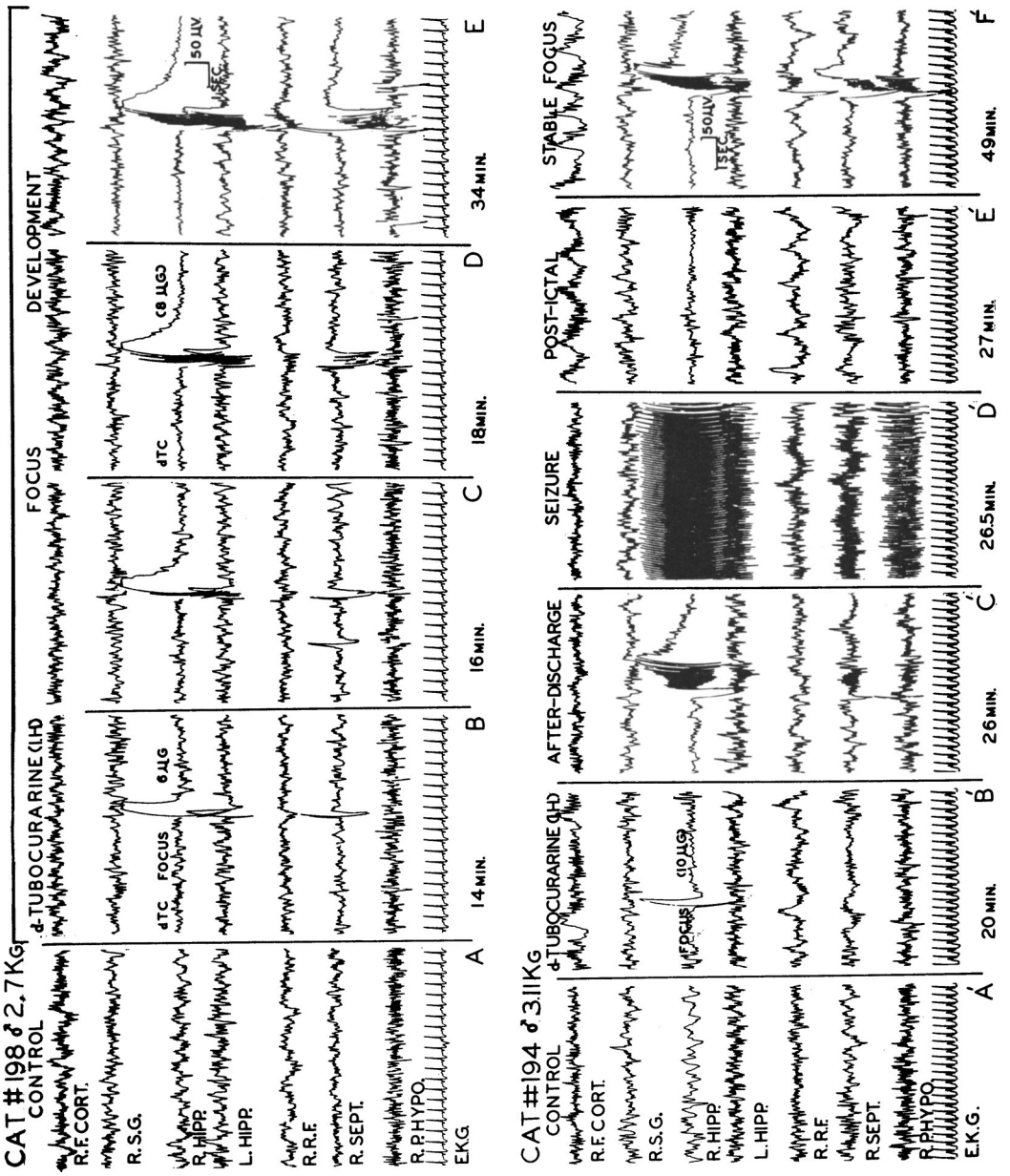

FIG. 1. Hippocampal foci established by intrahippocampal (I.H.) microinjection of d-tubocurarine (dTC). *Upper tracings:* Stages in development of focus and their relationship to cumulative doses (6-8 µg) of dTC. *Lower tracings:* Additional changes in focus produced by an initially larger microinjected dose (10 µg) of dTC. Focus in both experiments established in right hippocampus (R. Hipp.). Indicated time sequence is continuous from the control.

TABLE I. Hippocampal Electrographic Responses to Microinjected Neuromuscular Blockers.

Neuromuscular blockers	Dosage range (μg)	Simple focus	After-discharge	Seizure	Rapid sustained discharge	Additive dTC focus
Stabilizers:						
d-tubocurarine	2- 20	+	+	+	—	.....
Flaxedil	75-370	—	—	+	+	.....
Dihydro-β-erythroidine	160-325	—	—	—	—	+
Depolarizers:						
Decamethonium	60-400	—	—	—	—	+
Succinylcholine	50-350	—	—	—	—	+

responses of the agents are summarized in Table I. As indicated, a typical dTC focal discharge could not be elicited with microinjected gallamine triethiodide (Flaxedil). Yet, both of these drugs are related in chemical structure and act similarly (competitively) on the receptors at the neuromuscular junction. Even at substantially elevated doses (200 μg), Flaxedil failed to induce a discrete focus. Instead, intermittently it produced brief bouts of seizure discharges (20-30/sec) which were followed by periods of post-ictal depression. At still higher cumulative doses (>250 μg) this response was converted into a pattern of sustained spiking (~200/min). This activity, however, unlike that of the seizures, was not interrupted by intervals of post-ictal depression, but rather fired continuously for hours in what might be likened to the output of a pulse generator.

The third member of the series, dihydro-β-erythroidine, also has a "curare-like" action at the neuromuscular junction, but unlike dTC and Flaxedil, contains a tertiary nitrogen base. At doses exceeding 300 μg, it was not possible to establish either a focus or a seizure discharge. It is highly significant that by subsequently microinjecting small amounts of dTC (8 μg) into the same unresponsive site in the hippocampus, a typical dTC focus could readily be established.

The last two members of the series, decamethonium and succinylcholine, differ pharmacologically from the previous 3 agents in that they block transmission by sustained depolarization at the neuromuscular junction. Here again, a focus could not be produced with either microinjected decamethonium or succinylcholine; yet, supplemental microinjections of dTC elicited an active focus in each case.

To characterize further the focus in relation to cholinergic mechanisms, other types of cholinergic blocking agents (scopolamine, tetraethylammonium) and various cholinomimetics (methacholine, carbachol, physostigmine) were microinjected into the hippocampus and their effects on previously established dTC foci examined. The effects of the cholinergic blockers were negligible; whereas, the cholinomimetics markedly enhanced the excitability of the dTC focus, driving it repeatedly into seizures.

Discussion. Of the series of neuromuscular blocking agents microinjected into the hippocampus, dTC was highly selective in establishing discrete foci. For only at substantially higher doses did Flaxedil produce qualitatively different local discharges; whereas, the other 3 neuromuscular blocking agents failed to produce any changes in local electrographic activity. It also becomes quite evident that although these agents act alike pharmacologically on cholinergic receptors at the neuromuscular junction, they differ markedly in their potential for modifying local hippocampal electrographic activity. This lack of parallelism between the mechanisms involved at the two sites gains additional support in that pretreatment with decamethonium or succinylcholine failed to interfere with the subsequent establishing of a dTC focus in the hippocampus. Likewise, it is significant that cholinomimetics that pharmacologically antagonize the actions of dTC at the neuromuscular junction failed to do so against established dTC hippocampal foci. These findings all would tend to

emphasize the differences between the local actions of these drugs on hippocampal and neuromuscular transmission.

The excitatory effects of dTC in several areas of the central nervous system have been attributed primarily to the blocking of inhibitory synapses(11,12). In the cortex the local synchronous discharges produced by topical dTC were likened to strychnine spikes (13) and according to the schema developed by Grundfest(14) dTC would thus act postsynaptically to inactivate inhibitory synapses. On the other hand, in the hippocampus, the characteristics and waveform of the hypersynchronous discharges, as well as the marked potency of dTC, were more analogous to the foci previously described by our group for picrotoxin than for strychnine(8). This would suggest that dTC acts similar to picrotoxin in the hippocampus by interfering with presynaptic inhibitory regulatory mechanisms, thus functioning as a disinhibitor. In fact, it had been proposed that dTC preferentially produces microlesions at presynaptic sites(15). It is reasonable to expect that the pharmacological lesion produced with dTC will offer us another useful tool for analyzing the complex chemical processes that govern local hippocampal excitability.

Summary. Of a series of neuromuscular blocking agents microinjected into the hippocampus, only d-tubocurarine (dTC) was highly selective in establishing a focus at injection sites. These findings coupled with the ineffectiveness of cholinomimetics to antagonize the dTC focus emphasize the differences between the actions of neuromuscular blockers on hippocampal and neuromuscular transmission. Potency of dTC and characteristics of the focus also suggest that dTC acts similar to picrotoxin by interfering with presynaptic inhibition.

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Received October 6, 1966. P.S.E.B.M., 1967, v124.

Production of L Forms of *Neisseria meningitidis* by Antibiotics. (31804)

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Previous reports have demonstrated that L forms of certain bacteria are produced by antibiotics that inhibit bacterial cell wall synthesis(1-3). Four such antibiotics that have been employed for L form production are penicillin, methicillin, cycloserine and cephalothin. More recently bacitracin has been reported to produce L forms of group

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