to endotoxin. Findings of the present study support the view that the intestine of the dog does not play a major role in promoting development of the irreversible phase of endotoxin shock.

Summary. Previous reports have suggested that the gastrointestinal tract plays a major role in development of irreversible endotoxin shock in the dog. However, results presented here do not support this view but show a statistically significant stepwise decrease in survival time as an increasing amount of visceral tissue is removed from the dog. These data indicate that the gut of the dog is not the "shock organ" but rather serves a protective role in endotoxin shock. A possible mechanism for development of the irreversible phase of endotoxin shock in the cat, rabbit, dog and monkey is suggested.

Appreciation is expressed to Mr. Chin Hyong Kim for technical assistance.

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Received October 18, 1961. P.S.E.B.M., 1962, v109.

Tremorine Tremor in Chronic Spinal Rats. (27152)

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Everett and his co-workers, who discovered the characteristic properties of Tremorine (1,4-dipyrrolidino-2-butyne), reported that 5 to 20 mg/kg by any route of administration produced a marked, sustained tremor in all animals tested(1,2,3). The tremor was seen particularly in the head and limbs. They initially noted that acute spinal rats exhibited Tremorine tremors above the level of section, but none caudal to it. Nash and Emerson later reported that Tremorine (35 mg/kg, i.v.) given to 2-week chronic spinal cats would induce tremor and spasticity at all levels above and below the section(4). Kaelber and Hamel recently reported their inability to reproduce this "spinal" tremor in 2-week chronic spinal cats given Tremorine, 35 mg/kg, i.v.(5). They suggested that the phenomenon reported by Nash and Emerson may really have been the twitching of clonus associated with various mass reflexes commonly seen in chronic spinal animals.

The use of Tremorine as a tool for production of "spinal" tremor led us to plan a detailed study of the phenomenon, using more than 75 chronic spinal rats maintained up to one month post-operatively.

Methods. All operations were performed aseptically under ether anesthesia upon female Wistar albino rats weighing 200 g. Spinal cordectomy was accomplished by laminectomy and removal of a 2-3 mm segment of cord between the sixth and ninth thoracic vertebrae. Subsequent dorsal root sections in chronic spinal rats were performed either unilaterally or bilaterally by laminectomy and division of the dorsal root fibers central to the spinal ganglia of the fifth, sixth and

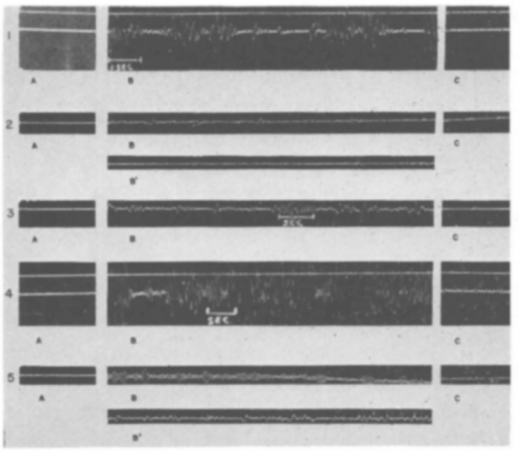
* This investigation was carried out during the tenure of a USPHS Predoctoral Fellowship.

seventh lumbar nerves. Limb denervation was effected by section of these same spinal nerves peripheral to the bifurcation of the dorsal and ventral roots. All sections were confirmed at termination of the experiments by examination of formalin-fixed spinal cord and roots.

The animals were tested daily following cord section for the return of spinal reflexes in response to toe pinch, and for the ability of Tremorine to elicit tremors in the hind legs. All injections were administered intraperitoneally. After trying transducers and electromyography, the following method was used for obtaining visual recordings of tremor activity. The rat was placed prone in a rat holder with its hind limbs hanging free. A small magnet (obtained from a magnetic automatic pencil) was taped to the bottom of the foot and a coil of copper wire placed close to the magnet. Movements of the magnet induced minute currents in the coil which were amplified by a Grass P-5 preamplifier and displayed on a Tektronix 502 Dual-beam oscilliscope. Grass C-4 Kymograph camera recordings were taken of these patterns.

Results. Tremorine. Immediately after spinal section, rats responded to hindlimb toe pinch by feeble ipsilateral flexion only. Tremorine in doses up to 20 mg/kg produced a pronounced tremor above the level of section, but none in the rear extremities. By the second day this ipsilateral flexion response was pronounced; in addition, the contralateral limb responded to the toe pinch by a feeble adduction of the limb up toward the body midline. By the third to fifth day this contralateral response developed into a crossed extension in which the rat extension kicked the contralateral limb toward the pinched foot. Return of these contralateral responses was generally accompanied by the ability of Tremorine to induce active tremors in the hind limbs. While the hind limbs of rats showing contralateral adduction did not always respond to Tremorine, invariably with the return of the crossed extension pattern, spinal tremors could be elicited with 5 to 10 mg/kg of Tremorine.

Fig. 1 demonstrates typical magnet recordings of Tremorine tremors taken from the



"Magnet" recordings from hind limbs of chronic spinal rats. (A) control; (B) after tremor drug; (C) after procyclidine, 15 mg/kg.

FIG. 1. Tremorine, 10 mg/kg, both hind limbs intact.

FIG. 2. Tremorine, 10 mg/kg; (B) left hind limb intact, (B') right hind limb dorsal roots sectioned.

FIG. 3. Arecoline HBr, 10 mg/kg, both hind limbs intact.

FIG. 4. Physostigmine salicylate, 1 mg/kg, both hind limbs intact.

FIG. 5. Physostigmine salicylate, 1 mg/kg; (B) left hind limb intact, (B') right hind limb dorsal roots sectioned.

right hind foot of a spinal rat 2 weeks postoperatively. Prior to Tremorine the hind limbs were quiet. Fifteen minutes after Tremorine, 10 mg/kg, typical 1 to 2 second bursts of tremor at a rate of 10/sec. were seen in the right hind foot. These bursts represented alternation of tremor back and forth between the right and left hind limbs. This alternate tremor was periodically interrupted by unilateral or bilateral limb flexion accompanied in many cases by hip abduction at the height of flexion. The pattern shown in Fig. 1 consists of pure tremor with no flexion.

Above the level of section the Tremorine response was a nearly continuous moderate tremor of the head and forelimbs. This forelimb tremor could not be satisfactorily recorded for comparison with the hind limb events since the front limbs could not be kept in a stationary position for magnet recordings. The synthetic anti-Parkinson drug, procyclidine (Kemadrin®), 15 mg/kg, quickly blocked the tremor of the hindlimbs (as

well as that above the level of section).

Fig. 2-B′ shows the abolition of Tremorine tremors in a limb following section of its dorsal roots. Note also the reduction of tremor in the left, intact limb of this animal (Fig. 2-B).

Cholinergic drugs. As a number of findings suggest that Tremorine acts by a central cholinergic mechanism, we studied the cholinergic drugs, arecoline, physostigmine, and prostigmine in chronic spinal rats.

The type of response obtained with arecoline HBr, 10 mg/kg, is shown in Fig. 3. Pfeifer has reported a Parkinson-like tremor with this drug in normal rats(6). In chronic spinal rats a tremor of about 10/sec. was seen to alternate in intensity from right limb to left. The duration of this tremor was only about 10 minutes as compared to 1 hour with Tremorine. Procyclidine also blocked the spinal tremors of arecoline. Unilateral dorsal root section blocked arecoline tremors in the sectioned limb.

Fig. 4 demonstrates the ability of physostigmine salicylate, a tertiary amine which inhibits cholinesterase, to produce tremors in the chronic spinal rat. These tremors, although slightly greater in amplitude and rate than those produced by Tremorine, also alternated in intensity from limb to limb. Procyclidine effectively blocked the spinal tremors.

Unilateral dorsal root section blocked the physostigmine tremors in the sectioned limb (Fig. 5-B′), but also markedly diminished the tremor amplitude in the intact limb (Fig. 5-B). The slow activity during drug action in the limb with root section, and in the intact limb after procyclidine (Fig. 5-C), represents a peripheral component of the action of physostigmine in the form of a slow, visible, muscle fasciculation. Dorsal root section or acute limb denervation did not abolish this action; the magnitude of this fasciculation artifact, however, did not contribute significantly to the apparent tremor response.

In contrast to physostigmine, the quaternary amine inhibitor of cholinesterase, prostigmine methylsulfate in doses of 0.1 to 1 mg/kg, produced no typical spinal tremors in chronic spinal rats, but only the slow, visible, muscle fasciculation of the hind limbs. This fasciculation was not blocked by acute limb denervation nor procyclidine, and it even persisted 4 to 5 minutes after death.

Discussion. Hind limb tremors were consistently observed in chronic spinal rats given doses of Tremorine which were also in the minimal range for eliciting upper body tremors. Our study of this phenomenon indicates the possibility that the qualitatively similar tremors produced in the hind limbs of chronic spinal rats by Tremorine, arecoline, and physostigmine are centrally mediated, but subject to feedback control. These drugs possess tertiary amine structures and thus pass readily into the spinal fluid to exert their action. In contrast, the quaternary amine compound, prostigmine, evidently does not penetrate into the spinal fluid to exert a direct effect. The apparent reduction of Tremorine and physostigmine tremors in the intact limb of a chronic spinal rat where the opposite leg has undergone a sectioning of its dorsal roots, combined with the finding that tremor induction paralleled the return of the crossed extensor reflex, suggests further that adequate input across the cord is necessary for reinforcement of these tremors.

The ability of the clinically effective anticholinergic, anti-Parkinson agent, procyclidine, to block the tremors of Tremorine, arecoline, and physostigmine, but not the fasciculations of physostigmine and prostigmine, implicates central cholinergic sites for tremorogenic activity. Thus far, cholinergic transmission in the central nervous system has been definitely established only for the motor-axon collaterals to the Renshaw cells(7). Renshaw cell stimulation by cholinergic agents depresses motoneuron activity. Feldberg *et al.* present evidence which supports the existence of cholinergic synapses at interneurons in the polysynaptic reflex pathways from dorsal to ventral root(8). The action of Tremorine on these systems is now being studied in chronic spinal dogs.

Summary. Tremorine and 2 other centrally-acting cholinergic compounds, arecoline and physostigmine, have been shown to induce tremors of the hind extremities in

chronic spinal rats. These tremors have been blocked by the anticholinergic, anti-Parkinson agent, procyclidine, as well as by dorsal root section. In contrast, prostigmine methylsulfate produced only muscle fasciculations which were not blocked by procyclidine or acute limb denervation. The implications of these findings are briefly discussed.

The authors gratefully acknowledged Dr. G. M. Everett, Abbott Laboratories, for supplying Tremorine.

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Received October 20, 1961. P.S.E.B.M., 1962, v109.

Trachoma Viruses Isolated in the United States. V. Growth Rates and Drug Inhibition Patterns in Embryonated Eggs.* (27153)

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It has been known for some years that clinical trachoma and inclusion conjunctivitis respond to several antimicrobial drugs. In fact it is likely that trachoma could be controlled throughout the world, and perhaps eliminated altogether, if infected persons would undergo adequate treatment with tetracyclines or other effective drugs. When trachoma viruses became available for laboratory study it was found, as expected, that they were strongly inhibited by tetracyclines, less markedly by penicillin or chloramphenicol and virtually unaffected by streptomycin (1,2,3). After isolation of the first trachoma viruses from the United States in our laboratory(4) pronounced strain-specific behavior was encountered with tetracycline and penicillin. Each strain tended to be inhibited in its lethality for eggs by a constant and distinct quantity of a given antimicrobial drug. These initial results suggested the possibility that the reaction patterns to antimicrobial drugs might be genetically stable markers of trachoma strains(3). Consequently it was desirable to submit a larger number of isolates of trachoma and inclusion conjunctivitis (IC) viruses to tests with antimicrobial drugs. This report summarizes quantitative experiments on the action of penicillin, tetracycline and tylosin tartrate on 5 strains of trachoma and 2 strains of IC viruses. The action of antimicrobial drugs on viruses on the psittacosis-LGV-trachoma group is remarkably similar to their action on bacteria. The latter is greatly influenced by metabolic activity and growth rate of microorganisms. Therefore it was of interest to compare the growth rates of different trachoma and IC viruses in embryonated eggs and attempt to correlate them with susceptibility to drugs. Results of such studies are presented here.

Materials and methods. Viruses. Isolates from the United States were employed at 2 or more passage levels with the following egg LD_{50} titers (ELD_{50}/ml): Trachoma strains BOUR YS 9-11 ($10^{4.7}$ to $10^{7.0}$), ASGH YS 7-9($10^{5.1}$ to $10^{7.4}$), APACHE #1 YS 7-9 ($10^{6.5}$ to $10^{6.8}$), APACHE #2 YS 8-10 ($10^{5.6}$ to $10^{5.9}$); and inclusion conjunctivitis strains IC Cal 1 YS 6-9($10^{5.0}$ to $10^{6.8}$) and IC Cal 3 YS 7-8 ($10^{6.3}$ to $10^{6.8}$). In addition one of

* Supported by grants from USPHS and Burroughs Wellcome Fund.

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