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10. Sponsored by Commission on Viral Infections, Armed Forces Epidemiological Board, and supported by the U.S. Army Medical Research and Development Command, Department of the Army, under contract No. DA-49-193-MD-2138. We thank Dr. S. K. Taylor of this department and F. G. Anders of University of Houston, for netting bats, L. A. Leonard for assistance with the HI tests, and R. Christian for technical assistance.

2 February 1966

d-Tubocurarine Chloride: Effect on Insects

Abstract. *Injection of* d-*tubocurarine chloride into certain insects produces complete flaccid paralysis. The site of injection is closely related to the region of primary paralysis. The effect depends on concentration, with distinct differences in the optimum concentrations for various species so far tested. A dose-response curve has been prepared for* Calliphora erythrocephala.

While recordings were being made from the chemosensory hairs on the labellum of *Sarcophaga bullata*, undetermined amounts of *d*-tubocurarine chloride were injected into the living adult fly in an attempt to reduce or eliminate random muscular movement that was making it difficult to obtain normal records. The curare was injected by one of us who was not familiar with the copious literature (for example, *1*, *2*) reporting that curare has no effect on insects. We were surprised to find a complete, vertebrate-type, curarine response in the injected fly.

Additional studies have shown that *d*-tubocurarine chloride does indeed affect various different species of insects; injection produces a recognizable syndrome and the affected insect is completely immobilized. In some instances the proboscis is extended. After a specific time period, which varies with the species, spasmodic twitching of the legs begins and there is some fluttering of the wings. Sometime thereafter the insect is able to stand when prodded but moves very little; still later it begins to walk slowly, but the hind legs remain completely paralyzed for extended periods. The insect begins normal walking movements but remains somewhat sluggish; finally comes complete recovery.

The site of injection is important and seems to be very closely related to the ensuing response. If the curare is injected into the anterior end of the thorax or pronotum, the front legs are the first part of the body to show the symptoms and to become paralyzed. If the injection is into the midthorax or mesonotum, the middle pair of legs shows paralysis first; if into the posterior part of the thorax or metanotum, the hind legs are first. Injection into the abdomen affects all legs at about the same time, but the time between injection and response is much longer than when the curare is injected into the thorax. After the insect has shown response to curare (complete body paralysis), the hind legs are always the last part to recover.

The effects described depend on concentration, with distinct differences in optimum concentrations for the species tested. Table 1 lists dosages required to effect complete flaccid paralysis in various insects; in most instances these figures probably represent an overdose.

Most of the species tested were represented by at least two wild-caught specimens. Each insect was fastened to the end of a syringe and injected with curare until there was a noticeable effect; it was then removed and allowed to recover. Insects that were dosed so heavily that they did not fully recover within 3 to 4 hours invariably died. Along with each injection, a control of the same species was injected with a comparable volume of saline with never any observable effect. All injections were made with a microliter syringe (C. H. Stoelting Co.) calibrated at 0.43 μl per turn. The curare used was isotonic *d*-tubocurarine chloride (USP)

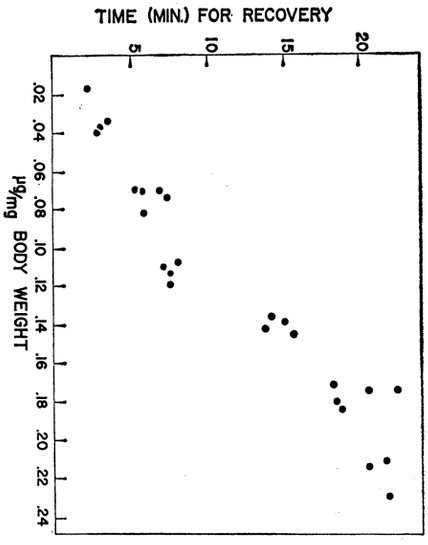

Fig. 1. Dose-response curve for injection of *d*-tubocurarine chloride into *Calliphora erythrocephala.* The amount of curare is plotted against time required for full recovery from the paralysis.

supplied in concentrations of 3 and 15 mg/ml and in pure crystalline form (Abbott Drug Co., No. SM 69997).

We attempted to establish a dose-response curve. Figure 1 is a plot of the amount injected versus recovery time for *Calliphora erythrocephala*; each point represents one fly. Injected with 0.02 to 0.12 μg/mg (here and throughout this report, dosage is per milligram of total body weight), the flies were only partially paralyzed and the recovery period was relatively short; with 0.12 to 0.24 μg the flies were com-

Table 1. Effects on various insects of injection with *d*-tubocurarine chloride. PI, partial to intermediate; CP, complete paralysis.

Genus	Body weight (mg)	Effect of dosage (μg/mg)	
		PI	CP
	Orthoptera		
Nemobius	46	0.702	
Acheta	234	.279	
Tryxalis	247	.261	
Tryxalis	100		1.161
Periplaneta	270	1.110	
	Hemiptera		
Phymata	23.1		1.12
Oncopeltus	36.1		0.895
Metapodius	82.0		1.02
	Coleoptera		
Tetraopes	84		0.461
Diabrotica	6.2		1.04
Epicauta	283.1		0.069
Tenebrio	100.2	0.320	
	Hymenoptera		
Camponotus	11.5		1.12
Bombus	184		0.595
	Diptera		
Calliphora	37.6		.137
Sarcophaga	94.2		.129

pletely immobilized and the recovery time was relatively longer. If the dosage was increased to 0.3 μg, the flies were completely paralyzed and there was never any recovery. Similar curves for *Sarcophaga bullata* and *Phormia regina* closely resemble this curve.

Preliminary experiments with the cockroach *Periplaneta americana* give an indication of its dose response. Injection of 0.1 to 0.4 μg brings no response; 0.5 to 0.9 μg has a partial effect whereby one pair of legs is paralyzed, depending upon the site of injection; there is some drooping of the head and sluggishness in movement. Injection of 1.0 to 1.4 μg has an intermediate effect, with general partial paralysis and very sluggish response to stimuli; paralysis of the limbs at the site of injection is complete, but the animal never completely loses the ability to move. Doses between 1.6 and 1.8 μg effect complete flaccid paralysis. From three to seven insects were tested in each dosage range and all completely recovered. A curve of dose response relative to recovery time has not yet been worked out for cockroaches as they are extremely slow to recover; there is also a much longer time lag between injection and the first appearance of effects. There is, however, a definite relation between dose and severity of the paralysis.

According to Goodman and Gilman (*3*), the mode of action of *d*-tubocurarine chloride is one of competition with acetylcholine for acceptance by the cholinergic receptors of the motor end plate, with no depolarization of the cell membrane and consequently no contraction of the muscle fiber. Workers indicate that curare has no effect on insect neuromuscular junctions or neuromuscular transmission (*1–3*).

The obvious difference between our study and earlier work is that Roeder (*1*) and others used a dosage, ranging from 10^{-3} to $10^{-7}M$, that works very well in vertebrates but produces no detectable syndrome in insects. Higher doses of curare do produce a typical effect in the insects so far studied.

Quite obviously there is no direct correlation between body weight of the insects and the dosage of curare required to produce paralysis (Table 1): the Coleoptera, *Epicauta*, weighing 283.1 mg, requires only 0.069 μg/mg for complete paralysis, while a cockroach nymph (*Periplaneta americana*), weighing 270 mg, requires 1.11 μg/mg to show only partial paralysis. And *Acheta*, weighing approximately the same as the cockroach nymph, showed only a partial effect after a dose of 0.279 μg/mg.

The extremely high dosage required to paralyze insects may reflect a number of factors, all of which remain conjecture. Quite obviously curare works as a paralyzing agent in insects, and it will be of significant interest to determine the site of action and type of chemical response at the cellular level.

JOSEPH R. LARSEN
DONALD M. MILLER*
TOSHIO YAMAMOTO
Department of Entomology and Physiology, University of Illinois, Urbana

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* Present address: Department of Zoology, University of California, Los Angeles.

18 February 1966

Seasonal Variation in Mating Behavior in Cats after Desensitization of Glans Penis

Abstract. *The glans penis in 14 sexually experienced cats was desensitized by section of the nerves dorsalis penis. These males mounted the estrous female readily but they were so disoriented that they could not achieve intromission. Reduced sensory feedback resulting from the operation and from lack of intromissions caused a decided drop in sexual activity in the fall with recovery in early winter. A latent sexual cycle in male cats is revealed, which corresponds in time to the established female cycle.*

In the comprehensive theory of the regulation of mammalian sexual behavior developed by Frank Beach (*1*), the sensory input into the system is considered nonspecific and additive. This conclusion, which was derived from his own research on rodents and from a survey of the literature (*2*), stems from a variety of observations and experiments that show that sensory deprivations (visual, auditory, tactile from snout and genitalia), regardless of modality or area, cause a decline in sexual activity, but do not cause qualitative changes in sexual behavior. Conversely, increasing the stimuli derived from the sexual partner or from the environment increases sexual activity, while the gonadal hormones and neural activity, particularly of the neocortex, adjust the threshold for appearance of the various behavioral acts.

In 1962 we reported (*3*) preliminary observations on a sensory deprivation in male cats, observations that seemed, at first thought, to be at variance with this part of Beach's theory. By surgical procedure the nerves dorsalis penis of several males were severed bilaterally. This operation desensitizes the glans penis but does not interfere with erection. These males showed no observable decrease in sexual activity. They mounted the female as readily as before operation but were so disoriented that they were unable to insert the penis into the vagina. Thus, by a small circumscribed sensory deprivation we produced major qualitative changes in behavior with no immediate loss in sexual arousal. As testing continued, however, decrements in sexual behavior appeared; not as a continuous decline, which would be predicted from Beach's theory, but as a pronounced seasonal decline in the fall with a return to higher levels of sexual activity in the winter.

We are now presenting an interim report of this experiment based on the behavior of 14 male cats that have been observed for 2 to 26 months after operation (average 18 months). Seven of these males are still being observed. The subjects were domestic short-hair males of unknown ancestry. Seven were obtained as adults and were presumed to have had sexual experience. The other seven were obtained as kittens and raised in laboratory cages, and all of the mating activities of these animals were controlled and observed. Since our analysis is not concerned with the effects of experience, and since there were no apparent differences in sexual behavior between the two groups, the data of the two groups were pooled.

Sex tests and methods of observation were similar to those used by Rosenblatt and Aronson (*4*). The major items of the normal mating pattern observed are (i) the male grips the back of the female's neck with teeth; (ii) mounts the back of the female; (iii) makes stepping movements with hind limbs; (iv) exhibits pelvic thrusting which is followed by (v) a single brief intromission with ejaculation after which (vi) the male dis-