THE OBSERVABLE EFFECTS OF ACETYLCHOLINE, SUCCINYLCHOLINE, D-TUBOCURARINE AND QUININE AND THEIR BINARY COMBINATIONS ON TARDIGRADES

An abstract of a Thesis by
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The problem. This study seeks to determine the observable effects of acetylcholine, succinylcholine, d-tubocurarine, and quinine on tardigrades and to compare these effects to known mammalian reactions. Evidence of synergism or antagonism is sought by combining each drug compound with the other three individually.

Procedure. Three species of tardigrades (Milnesium tardigradum, Hypsibius oberhauseri, and Macrobiotus richtersi) were subjected to serial dilutions from 4.0% to 0.0% (weight/volume) of each of the four compounds and their binary combinations. Observations were made through a 25X binocular dissecting microscope at the time periods: 15 minutes, 1 hour, and 5 hours. Controls were kept in distilled water and in spring water. All test animals were kept in a moist-chamber to avoid evaporation and subsequent concentration of the test solutions. Anesthesia was tested by returning affected animals to spring water and watching for movement, indicating recovery.

Findings. Contraction of the tardigrades' bodies and legs was brought about within an hour by a minimum of 0.4% acetylcholine or 1.6% succinylcholine. In that same time period the tardigrades were relaxed, with the bodies and legs fully extended, by 1.2% d-tubocurarine; but no change was seen with up to 2.0% quinine. By 5 hours, 0.8% quinine had caused a relaxation of the animals. Acetylcholine and succinylcholine were highly synergistic. Both quinine and d-tubocurarine sensitized the tardigrades to acetylcholine. D-tubocurarine delayed the action of succinylcholine. Small amounts of d-tubocurarine negated effects of 2.0% or less succinylcholine, causing no observable effects. Quinine caused a delayed sensitization of the tardigrades to succinylcholine. D-tubocurarine and quinine delayed and reduced the effectiveness of each other. The effect of these test solutions was anesthesia, not narcosis. This was demonstrated by the recovery of the affected tardigrades when they were returned to spring water.

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INTRODUCTION

Tardigrades comprise a small group of animals generally considered to be a separate phylum. Although small and inconspicuous animals, they have been known for a long time. Goeze, in 1773, described "Kleiner Wasserbar." In 1776, Spallanzani called them "Il Tardigrado" (Riggen, 1962). During the past two centuries since Goeze coined the term water-bear, sporadic observations have been made in many parts of the world, including North America. But most of the serious work on them has been carried out in Europe; it has centered on taxonomy and morphology. Little is known of tardigrade physiology or embryology.

The basic morphology of both the nervous and muscular systems of the tardigrades has been described. The tardigrade nervous system exhibits the pattern typical of the annelid-arthropod line (one of the reasons tardigrades are usually considered arthropods or close relatives of the arthropods). The bi-lobed tardigrade brain is dorsally placed; it is connected to a subesophagael ganglion and to four ventral trunk ganglia by a pair of ventral nerves (Riggen, 1962).

Tardigrade muscles are no more than delicate bands attached to the cuticle, resembling those of some microcrustacea. These narrow muscle bands, clearly visible as distinct elements, pass through the body at sometimes

unlikely angles, establishing the leverage needed for movement. Five or six strands run longitudinally, arching the body when contracted. Each leg has a set of five or six angled muscles running into it, thus allowing for lateral movements and retraction of each leg independently. However, there are no muscles to extend the legs; only hydrostatic pressure caused by the fluid of the haemocoel can cause leg extension (Meglitsch, 1967). No serious work on the physiological characteristics of either muscles or nerves has been undertaken with tardigrades.

Because of their arrangement, movement is limited; and the tardigrades are clumsy and slow. Each leg is operated independently, moved mostly by rotating it or by contracting it and waiting for it to be slowly re-extended by hydrostatic pressure.

This study seeks to assess the effects of acetylcholine, succinylcholine, d-tubocurarine, and quinine on tardigrade movements. The literature contains no information concerning the effects of these compounds on tardigrades and little information about their effects on invertebrates. Their effects on vertebrates, particularly mammals, are much better known. Observed effects on tardigrades will be compared to known effects on mammals.

Effects of the four compounds used in this study are authoritatively summarized by Goodman and Gilman (1965).

All are obtained in the chloride salt form, but are active in the dissociated ionic form. Thus, references are made to the active ion, not to the chloride salt.

Acetylcholine is released from cholinergic neurons as a natural neural transmitter. It acts upon receptors in skeletal end-plates, smooth muscles, and ganglion cells of the autonomic nervous system. Skeletal muscle contains specialized cholinoceptive structures (end-plates that contain acetylcholine receptors), where nerve fibers terminate. Acetylcholine depolarizes the receptor by changing the permeability to ions of the muscle membrane. Structurally, the compound has a quaternary nitrogen atom, necessary for activity. At the receptors in the end-plate an excess of acetylcholine will cause a neuromuscular blockade. Ganglionic blockage between pre- and post-ganglionic autonomic fibers may also occur with excessive acetylcholine, since it prevents repolarization.

D-tubocurarine is competitive with acetylcholine. It is still being used by South American Indians as a paralytic agent on arrows or darts for hunting wild animals. This alkaloid is also a quaternary ammonium base, and acts as a competitive neuromuscular blocking agent in skeletal muscle, i.e., it combines with the cholinoceptive sites at the postjunctional membrane and thus blocks the transmitter action of acetylcholine. To block ganglionic

transmission, about three times the concentration required to block the postjunctional membrane is needed.

Succinylcholine is another depolarizing agent. Initially a depolarization of the end-plate is caused. In mammals there is normally a short duration of depolarization due to the rapid degradation of the succinylcholine by the cholinesterase of the plasma and liver. (This mechanism could exist in tardigrades, if cholinesterase were present in the haemocoel fluid.) Then, despite repolarization, the mammalian end plate becomes desensitized to the transmitter.

Walts and Dillon (1969) studied the interaction of succinylcholine and d-tubocurarine. In man, a dose of succinylcholine given after a small dose of d-tubocurarine causes a decrease in both the duration and the intensity of the muscle blockade caused by the d-tubocurarine. When succinylcholine is given after a prolonged block with d-tubocurarine, the block is reversed and the succinylcholine has a diminished action.

Quinine, as summarized in Drill's Pharmacology in Medicine (1965), is a competitive inhibitor of cholinesterase, but it also has a distinct curare-like action. This non-quarternized ammonium compound decreases the response of the motor end-plate to acetylcholine in skeletal muscle. Its actions in smooth muscle are inconsequential.

The likelihood of differences between vertebrate and

invertebrate physiological systems is very high. Since there is a large amount of information concerning the effects of chemical compounds on mammalian systems, the best hypothesis is to assume that the tardigrade neuromuscular system is similar to that of the mammal. Both similarities and differences of reactions to the compounds would be informative, since little is known of the physiology of the tardigrade. The compounds chosen in this study are acetylcholine, the most widespread neural transmitter in vertebrates, and three compounds that are either antagonistic or synergistic to acetylcholine. It will be necessary to describe the effects of each compound, as well as the effects of binary combinations of the compounds. These combinations may show synergistic or antagonistic interactions. A systematic study would provide important clues about invertebrate physiology (particularly that of the tardigrade), and add to information describing the effects of the tested compounds on all living systems.

MATERIALS AND METHODS

The animals used in this study were identified as Milnesium tardigradum, Hypsibius oberhauseri, and Macrobiotus richtersi (Riggen, 1962). They were obtained from tree-borne lichens collected in wooded areas near Des Moines, Iowa, between April, 1970 and July, 1971. The

bark samples were floated lichen-side down in clear plastic dishes containing spring water. The tardigrades dropped to the bottom of the container after soaking six to eighteen hours. The animals were either active or relaxed and asphyctic when found. They were transferred to spot plate depressions with a Pasteur disposable pipette, with the aid of a 25X binocular dissecting microscope. A moist-chamber was used to prevent dessication while the animals recovered from asphyxia and transfer shock. Specimens could be kept as long as three weeks in a moist-chamber.

The animals were exposed to four compounds, quinine dihydrochloride (Penik Corporation), d-tubocurarine chloride (Abbott Laboratories), succinylcholine chloride (National Biochemical Corporation), and acetylcholine chloride (National Biochemical Corporation). A 2% (weight/volume) stock solution was prepared of each of the compounds except succinylcholine chloride, for which a 4% stock solution was used.

Each observation consisted of records on the physiological state of animals in paired control-experimental groups. Control groups consisted of ten active tardigrades concentrated in a single depression containing spring water, and ten in a depression containing distilled water. Experimental groups consisted of ten active tardigrades concentrated in a single depression

containing a definite concentration of the experimental compounds. Observations were made with a 25X binocular dissecting microscope. Records were taken of individual reactions after the animals had been under experimental conditions for 15 minutes, 1 hour, and 5 hours.

At each recording time, the number of tardigrades falling into "normal," "relaxed," and "contracted" categories was noted. They were scored as normal when crawling or other such normal activity was noted. "Relaxed" indicated full extension of the body and the legs without movement, as is seen in the asphyctic state. In a "contracted" organism, the body was curled into a tight arch with the legs contracted on the inside of the arch.

After the end of the observation period, some of the animals, randomly picked, were placed in spring water and observed for evidences of recovery. For the purpose of this study, any relaxed or contracted animal that was able to move one leg or more was recorded as "recovered." If an animal did not show movement within a period of five hours, it was considered not recovered. Since this part of the study was only to differentiate narcosis from anesthesia, no regular observation periods were used during recovery.

Approximately 80% of the tardigrades recovered from effects of each of the experimental solutions. No difference was noted among the three species used in their ability

to recover and their rate of recovery. The defined states of "normal," "relaxed," and "contracted" were all exhibited by live organisms.

Each compound tested was applied in a series of concentrations. Each depression contained ten drops of fluid, delivered from a Pasteur disposable pipette. Ten drops of the stock solution were put in the first experimental depression, nine drops of stock and one drop of distilled water in the second depression, etc. Thus, the compounds prepared as a 2% stock solution (quinine dihydrochloride, acetylcholine chloride, and d-tubocurarine chloride) were dispensed in concentrations ranging from 2% to 0%, while succinylcholine chloride, prepared from a 4% stock solution, gave concentrations that ranged from 4% to 0%.

The effects observed in dilutions of single compounds were compared with those seen in combinations of compounds to seek evidence of synergistic or antagonistic actions. Depressions containing combinations of compounds were prepared in the following manner. Ten drops of one of the stock solutions were in the first experimental depression, nine drops of the same stock and one drop of a second stock solution in the second depression, and so forth until ten drops of the second stock were in the last depression.

Each set of dilutions was tested at least three times.

The intensity of the effects of any one compound was reflected by the speed with which behavior modifications developed, and by the concentrations required to elicit responses. Thus, evidences of synergistic or antagonistic effects required comparison of the times when behavior modifications occurred, as well as the concentrations at which behavior occurred for any given period.

RESULTS

The reactions of the tardigrades elicited by the four compounds and their binary combinations are summarized in a series of tables. The tables show the two variables: time and concentration. All the tables express the concentration as a percent (weight/volume) and the time by three observation periods (15 minutes, 1 hour, and 5 hours). The number of tardigrades affected is expressed as a percent of individuals, e.g., "50% are contracted" means 50% were contracted and 50% normal.

Anesthesia was tested by randomly choosing affected tardigrades from each of the twelve test solutions and transferring these animals to spring water. "No recovery" would have indicated narcosis; however, approximately 80% of the tardigrades recovered from their affected state, indicating a form of anesthesia. No specific observation periods or numbers of animals were used in testing for

recovery, as these factors are not an integral part of the study.

As seen in Tables 1 and 2, both acetylcholine and succinylcholine caused a definite contracting reaction. Acetylcholine proved to be a much more effective compound in the sense that very small concentrations caused a reaction within a short time period. Contraction of 50% of the tardigrades within 15 minutes was caused by 0.8% acetylcholine, where it took 1.6% succinylcholine to cause 60% contraction within 15 minutes. Within an hour, the smallest concentration of acetylcholine tested, 0.4%, caused 50% of the animals to contract. All of the tardigrades in 1.6% succinylcholine were contracted within an hour; none were affected in any less concentrated solution of succinylcholine.

D-tubocurarine and quinine (Tables 3 and 4) caused a relaxation of both bodies and legs. Quinine was less effective than d-tubocurarine, in that it took a greater concentration and more time for quinine to bring about relaxation. In an hour all tardigrades were relaxed by a 1.2% solution of d-tubocurarine; none had responded to the 2.0% quinine, the strongest quinine solution tested. By the 5 hour reading, all had responded to the 0.6% d-tubocurarine and to the 0.8% quinine.

Paired combinations of the compounds were used to seek evidence of synergistic and antagonistic actions.

Table 1. Percent of Tardigrades Contracted When Exposed to Various Concentrations of Acetylcholine

Acetylcholine	Time of Exposure		
%	15 min.	1 hr.	5 hr.
0.0	0	0	0
0.4	0	50	50
0.8	50	100	100
1.2	100	100	100
1.6	100	100	100
2.0	100	100	100
2.4	100	100	100
2.8	100	100	100
3.2	100	100	100
3.6	100	100	100
4.0	100	100	100

Table 2. Percent of Tardigrades Contracted When Exposed to Various Concentrations of Succinylcholine

Succinylcholine	Time of Exposure		
%	15 min.	1 hr.	5 hr.
0.0	0	0	0
0.4	0	0	0
0.8	0	0	0
1.2	0	0	0
1.6	60	100	100
2.0	95	100	100
2.4	100	100	100
2.8	100	100	100
3.2	100	100	100
3.6	100	100	100
4.0	100	100	100

Table 3. Percent of Tardigrades Relaxed When Exposed to Various Concentrations of D-tubocurarine

D-tubocurarine	Time of Exposure		
%	15 min.	1 hr.	5 hr.
0.0	0	0	0
0.2	0	0	0
0.4	0	0	0
0.6	0	0	100
0.8	0	0	100
1.0	0	0	100
1.2	0	100	100
1.4	0	100	100
1.6	0	100	100
1.8	0	100	100
2.0	0	100	100

Table 4. Percent of Tardigrade Relaxed When Exposed to Various Concentrations of Quinine

Quinine	Time of Exposure		
%	15 min.	1 hr.	5 hr.
0.0	0	0	0
0.2	0	0	0
0.4	0	0	0
0.6	0	0	0
0.8	0	0	100
1.0	0	0	100
1.2	0	0	100
1.4	0	0	100
1.6	0	0	100
1.8	0	0	100
2.0	0	0	100

Acetylcholine paired with succinylcholine caused a highly synergistic action. In Table 5, 100% of all the animals were shown to have contracted within the first fifteen minutes. This would be expected, since in all instances one or the other of the compounds was present in sufficient concentration to bring about contraction. Table 6 provides clear evidence of synergism. Succinylcholine present as a 1.8% solution is less than the 2.0% used alone, which did not cause 100% contraction in 15 minutes. The presence of very little acetylcholine, one fourth the amount required to get 50% contraction even after 5 hours, produced 100% contraction within 15 minutes. The combination of 0.4% acetylcholine with 1.2% succinylcholine, not enough of either alone to cause 100% contraction even after 5 hours, causes 100% contraction within 15 minutes. But, when 0.9% acetylcholine and 0.2% succinylcholine are together an anomaly occurs. With 0.8% acetylcholine alone, 50% contraction occurs in 15 minutes and 100% in 1 hour. The presence of just a little succinylcholine delays acetylcholine contraction.

On the contrary, acetylcholine is highly antagonistic to d-tubocurarine. Table 7 shows that d-tubocurarine relaxation was prevented by even small amounts (0.1%) of acetylcholine. In 5 hours, 0.4% acetylcholine alone gives 50% contraction; even 0.1% acetylcholine resulted in 100% contraction in 1 hour in the presence of d-tubocurarine.

Table 5. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of Succinylcholine and Acetylcholine

Succinylcholine	Acetylcholine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
3.6	0.4	100	100	100
3.2	0.8	100	100	100
2.8	1.2	100	100	100
2.4	1.6	100	100	100
2.0	2.0	100	100	100
1.6	2.4	100	100	100
1.2	2.8	100	100	100
0.8	3.2	100	100	100
0.4	3.6	100	100	100

Table 6. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of Succinylcholine and Acetylcholine

Succinylcholine	Acetylcholine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
1.8	0.1	100	100	100
1.6	0.2	100	100	100
1.4	0.3	100	100	100
1.2	0.4	100	100	100
1.0	0.5	100	100	100
0.8	0.6	100	100	100
0.6	0.7	100	100	100
0.4	0.8	100	100	100
0.2	0.9	0	80	100

Table 7. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of D-tubocurarine and Acetylcholine

D-tubocurarine	Acetylcholine	Time of Exposure		
%	%	15 min.	1 hr.	5 hrs
1.8	0.1	0	100	100
1.6	0.2	100	100	100
1.4	0.3	100	100	100
1.2	0.4	100	100	100
1.0	0.5	100	100	100
0.8	0.6	100	100	100
0.6	0.7	100	100	100
0.4	0.8	100	100	100
0.2	0.9	100	100	100

Here, the presence of d-tubocurarine makes the tardigrades more sensitive to acetylcholine.

Likewise, acetylcholine greatly antagonizes quinine. In the presence of even 0.1% acetylcholine, the quinine relaxation is prevented. Quinine, as does d-tubocurarine, sensitizes the tardigrades to acetylcholine (Table 8).

In Table 9 one sees evidence that although contraction caused by succinylcholine overrides relaxation caused by d-tubocurarine, some antagonism appears evident. Contraction is delayed in 2.0% succinylcholine by the presence of 1.0% d-tubocurarine, e.g., and also 1.6% succinylcholine worked less quickly in the presence of d-tubocurarine. However, 1.2% succinylcholine (causing no contraction alone) induces 40% contraction in the presence of 1.4% d-tubocurarine. As shown in Table 10 less d-tubocurarine in the presence of 1.2% succinylcholine leads to normality.

Succinylcholine with quinine provides a different set of actions, as can be seen in Table 11. The presence of very small quantities of quinine (0.2%) makes 1.8% succinylcholine more effective than it is by itself. Yet, 0.4% quinine delays contraction by 1.6% succinylcholine. Even by the 5 hour observation period 1.2% and 1.0% succinylcholine would not be expected to cause contraction of tardigrades, but does when accompanied by quinine. As happens with acetylcholine, quinine tends to sensitize,

Table 8. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of Acetylcholine and Quinine

Acetylcholine	Quinine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
0.9	0.2	100	100	100
0.8	0.4	100	100	100
0.7	0.6	100	100	100
0.6	0.8	100	100	100
0.5	1.0	100	100	100
0.4	1.2	100	100	100
0.3	1.4	100	100	100
0.2	1.6	100	100	100
0.1	1.8	100	100	100

Table 9. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of Succinylcholine and D-tubocurarine

Succinylcholine	D-Tubocurarine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
3.6	0.2	100	100	100
3.2	0.4	100	100	100
2.8	0.6	100	100	100
2.4	0.8	100	100	100
2.0	1.0	0	100	100
1.6	1.2	0	100	100
1.2	1.4	0	40	40
0.8	1.6	0	0	0
0.4	1.8	0	0	0

Table 10. Percent of Tardigrades Affected When Exposed to Various Concentrations of the Combined Solution of Succinylcholine and D-tubocurarine

Succinylcholine	D-tubocurarine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
1.8	0.2	0	0	0
1.6	0.4	0	0	0
1.4	0.6	0	0	0
1.2	0.8	0	0	0
1.0	1.0	0	0	0
0.8	1.2	0	0	0
0.6	1.4	0	0	0
0.4	1.6	0	0	0
0.2	1.8	0	0	0

Table 11. Percent of Tardigrades Contracted When Exposed to Various Concentrations of the Combined Solution of Succinylcholine and Quinine

Succinylcholine	Quinine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
1.8	0.2	100	100	100
1.6	0.4	0	100	100
1.4	0.6	0	100	100
1.2	0.8	0	0	100
1.0	1.0	0	0	100
0.8	1.2	0	0	0
0.6	1.4	0	0	0
0.4	1.6	0	0	0
0.2	1.8	0	0	0

Table 12. Percent of Tardigrades Relaxed When Exposed to Various Concentrations of the Combined Solution of D-tubocurarine and Quinine

D-tubocurarine	Quinine	Time of Exposure		
%	%	15 min.	1 hr.	5 hr.
1.8	0.2	0	0	100
1.6	0.4	0	0	100
1.4	0.6	0	0	100
1.2	0.8	0	0	100
1.0	1.0	0	0	0
0.8	1.2	0	0	0
0.6	1.4	0	0	0
0.4	1.6	0	0	0
0.2	1.8	0	0	0

although belatedly, the animal to succinylcholine.

The combination of d-tubocurarine and quinine (as shown in Table 12), caused a relaxation of the tardigrades' bodies, yet it took larger concentrations of each to accomplish this. A solution of 1.2% d-tubocurarine and 0.8% quinine relaxed the tardigrades by the 5 hour observation period. By itself, 1.2% d-tubocurarine can cause a relaxation in 1 hour and 0.6% in 5 hours. Also 0.8% or stronger quinine solutions cause a relaxation within 5 hours. Although both are relaxing agents, they are not synergistic, but actually delay and reduce the effectiveness of each other to the tardigrades.

DISCUSSION

The observable effects of the four compounds are not totally unexpected. When there is an increase of acetylcholine, the chemical neural transmitter in mammals, a depolarization of the receptor results. Acetylcholine causes a contraction of the tardigrade body and leg muscles, suggesting a depolarizing system similar to the mammalian system. Succinylcholine enhances the depolarization of the receptor in mammals. The contraction of the tardigrade caused by succinylcholine is what might be expected, if its action is similar to that in mammals. The two chemicals are highly synergistic in their actions, for less of either

compound was required to produce a reaction when combined, than when used individually. There is one evidence of delay when a very subminimal amount of succinylcholine (0.2%) is present with 0.9% acetylcholine, a sufficient quantity to cause contraction by itself. Rather than the expected immediate contraction, no reactions were observable at the 15 minute observation period and only 80% of the tardigrades were contracted within an hour.

D-tubocurarine and quinine both block the transmission of motor nerve impulses in mammalian systems. These two chemicals cause reactions opposite those of acetylcholine and succinylcholine. The combination of d-tubocurarine and quinine, however, did not act synergistically in tardigrades. Since the two compounds act by different modes and at different levels, this is believable. By delaying and reducing each other's effectiveness, these two chemicals actually show evidence of an unexpected antagonistic effect. The mechanism for this is presently unexplained.

The sensitivity of the tardigrades to acetylcholine in the presence of d-tubocurarine is unlike reactions described for the mammalian system. [Goodman and Gilman (1965) describe the chronically denervated mammalian muscle as being more sensitive to acetylcholine than normal muscle.] In both denervated and normal muscle d-tubocurarine raises higher threshold to acetylcholine. But in tardigrades, in

the presence of 1.6% d-tubocurarine, as little as 0.2% acetylcholine caused a contraction within 15 minutes. This faster action of the acetylcholine at an even lesser concentration than when used alone is a reversal of the actions described in the mammalian system.

Some future studies should deal with introducing the chemicals in sequence rather than at the same time. For instance, one might first relax the tardigrade with d-tubocurarine, then put the relaxed animal in acetylcholine. Since the action of the d-tubocurarine alone is slower than the acetylcholine's, different reactions may occur when the d-tubocurarine is allowed to exert its actions first.

Likewise, with the combination of acetylcholine and quinine, the quinine is much slower in its actions. The acetylcholine's action may take effect before the quinine is able to. The quinine appears to enhance the action of acetylcholine, for even 0.1% acetylcholine causes a contraction of 100% of the animals within 15 minutes. If the tardigrade has a cholinesterase, then the enhancement of the acetylcholine effect by the presence of quinine could be explained. Quinine can act as an inhibitor of cholinesterase, as well as a desensitizer of the end-plate. Action as a cholinesterase inhibitor could account for the increased effectiveness of acetylcholine.

The combination of succinylcholine and d-tubocurarine

provides a unique set of results. Alone, a solution of 1.6% succinylcholine will cause 60% of the tardigrades to contract within 15 minutes and 100% within an hour. But the same concentration in the presence of 1.2% d-tubocurarine causes no reaction within 15 minutes and 100% contraction within an hour.

The same concentration of succinylcholine combined with a lesser amount of d-tubocurarine, 0.4%, causes no change in the animals even after 5 hours. Since there is a delayed contraction and even a stoppage of the contraction, there is a definite antagonism occurring between the two compounds. However, there also is a synergism occurring between succinylcholine and d-tubocurarine. When 1.2% succinylcholine is in the presence of 1.4% d-tubocurarine, 40% of the tardigrades were contracted. The same concentration of succinylcholine in the presence of 0.8% d-tubocurarine causes no apparent action on the tardigrades. Since 1.2% succinylcholine is ineffective by itself, the excess of d-tubocurarine may have sensitized the tardigrade to the succinylcholine. These are not like the results reported by Walts and Dillon in 1969. Their study concerned the interaction between succinylcholine and d-tubocurarine in man. They reported that when succinylcholine was administered after a prolonged block by d-tubocurarine, succinylcholine reversed the block, but its actions were

diminished. If the succinylcholine followed a small dose of d-tubocurarine, the succinylcholine block was significantly reduced in duration and in intensity.

Since their study concerned the compounds administered in sequence rather than simultaneously, no direct comparison can be made. This may account for the differences between the data of the two studies. Future studies with the tardigrades should include sequential administration of chemicals to the animal. Comparison of that data with known facts about mammalian systems might indicate similarities and dissimilarities in mechanisms.

The combination of succinylcholine and quinine is very similar to the combination of succinylcholine and d-tubocurarine in that there is an enhanced delayed reaction. A concentration as low as 1.0% succinylcholine in the presence of 1.0% quinine will cause contraction of the tardigrades within 5 hours. There was no contraction of the animals by the 1.6% succinylcholine in the presence of 0.5% until the 1 hour reading.

Since the tardigrade and man are vastly different in structure and probably in chemical composition, it would be expected of the two types to react differently. At present, many of the reactions of the tardigrade are unexplainable. A series of observations concerning their reactions can lead to information about the mechanisms involved. In 1969,

Kaplan published a survey of anesthetizing techniques for many phyla with pertinent information and references on their respective anesthetizing agents. Tardigrades were not included in this survey, as no literature is available concerning this phylum. The mode of actions are known for only a few of the techniques. Further studies would be useful to learn the mechanisms in all of these invertebrates, as well as in tardigrades.

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