# A NOTE ON THE ACTION OF CURARE, ATROPINE, AND NICOTINE ON THE INVERTEBRATE HEART.

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The observations recorded in this note were made in 1903–04 as an item in a more comprehensive work on the physiology of the invertebrate heart. These observations were not published, but some points were elaborated in greater detail on the *Limulus* heart, where unusual anatomical relations permit the analysis of the point of action of drugs, and this latter work on *Limulus* was reported in 1906 (Carlson, 1906–07). Having recently, with Dr. Luckhardt, become interested in the action of alkaloids on the nervous tissues in other automatic organs (lungs, gut, and arteries), the author was led to consult the earlier work on the invertebrate heart, and thus came across this item. Feeling that the observations are of some interest to general physiology they are now recorded as written up in 1904, except for the addition of two references (Carlson, 1906–07, 1909) in the bibliography.

The alkaloids used were of Merck manufacture. The species of invertebrates studied were: molluscs (*Octopus*, *Loligo*, *Ommastrephes*, *Mytilus*, *Mya*, *Tapes*, *Platydon*, *Venus*, *Pecten*, *Cryptochiton*, *Lucapina*, *Haliotis*, *Natica*, *Sycotypus*, *Aplysia*, *Bulla*, *Pleurobranchæa*, *Montereina*, *Triopha*, *Limax*, *Ariolimax*, and *Helix*), and arthropods (*Palinurus*, *Cancer*, and *Limulus*).

## *1. The Action of Curare, Atropine, and Nicotine on Central and Peripheral Ganglia.*

The action of alkaloids in the invertebrates has been the subject of numerous studies. The older researches are cited and reviewed in

*I am indebted to the directors of these laboratories for the facilities so liberally extended.

the works of Plateau (1880) and Krukenberg (1880). According to Bert (1867) and Yung (1879, 1881), the action of curare in the crustaceans and in the cephalopod molluscs is the same as in the vertebrates, only less strong. In the lamellibranch molluscs Yung did not obtain a permanent paralysis by curare. Vulpian (1879) states that curare paralyzes both arthropods and molluscs, but its action is not as strong as in the vertebrates. Krukenberg holds that the action of curare in crustaceans and molluscs is mainly on the central nervous system, but there is also paralysis of motor nerve endings. Biedermann (1890) and Fürst (1890) states that curare will paralyze the motor nerve endings in *Lumbricus* but this is denied by Straub (1900).

A drug may cause paralysis by depressing the motor nerve endings or by paralyzing the central nervous system. My own results go to show that the primary action of curare and nicotine in arthropods and molluscs is on the central nervous system and the peripheral ganglia and not on the motor nerve endings in the muscle. The action on the nerve centers is a primary stimulation followed by temporary or permanent paralysis if the dose is of sufficient strength.

There are great differences in the tolerance or degree of resistance to the action of curare in the different invertebrates. The nudibranchs and the pulmonates (excepting *Ariolimax*) are very sensitive to the drug. *Octopus* is far more resistant than the decapods, and of the latter *Ommastrephes* is more resistant that *Loligo*. Of the arthropods, *Limulus* shows the greatest resistance to the drug.

The stimulating action of curare on the nerve centers appears immediately on the application of the solution. In the squid the stimulation results in spasms and tetanus. The primary stimulation is less in evidence in the crustaceans. In the gasteropods it appears in prolonged and extreme contraction of the body muscles. In all the animals studied stimulation of the motor nerves causes contraction of the skeletal and visceral muscles after a dose of curare that completely paralyzed the central nervous system.

Atropine appears to paralyze motor nerve endings to some smooth muscles (*e.g.* lungs, gastrointestinal tract) in vertebrates. The body muscles of arthropods are of the transversely striated type, but the muscle of molluscs approaches more closely to the smooth variety.

My experiments on several classes of molluscs with the view of paralyzing the motor nerve endings in the muscle by atropine have yielded uniformly negative results. So far, then, we know of no drug that will paralyze the motor nerve endings in invertebrates without materially depressing the muscle itself, after previous paralysis of the central ganglia.

### 2. *The Action of Curare, Atropine, and Nicotine on the Heart Rhythm.*

A review of the literature on the action of the alkaloids on the invertebrate heart discloses considerable disagreement between the results of different observers. Ransom (1884) found that curare in sufficient concentration accelerates the heart rhythm of *Octopus* and *Helix*. According to Yung (1881), curare has sometimes a depressor and sometimes a stimulating action on the heart of lamellibranchs. Plateau (1880) states that curare has a depressor action on the crustacean heart, and according to Dogiel (1877) the drug has no action whatever on the heart of the *Corethra* larva.

Nicotine accelerates the heart rhythm both in crustaceans and in molluscs (Yung, 1881, Plateau, 1880).

Plateau states that atropine has a depressor action on the crustacean heart. Yung and Ransom found that it acts as a stimulant on the molluscan heart. Dogiel states that atropine of sufficient concentration to affect the heart of the *Corethra* larva at all has a depressor action. For an account of the action of these alkaloids on the tunicate heart the reader is referred to the papers by Schultz (1901) and Hunter (1903). My own work does not include the heart of tunicates.

In order to study the action of these drugs on the heart it is, in the first place, necessary to sever the connection of the heart with the central nervous system. All the animals worked on are provided either with inhibitory or accelerator cardiac nerves or both (Carlson, 1909). The action of the alkaloids on the heart when introduced into the intact animal is therefore complicated by their action on the central ganglia or brain and on the peripheral ganglia other than those in the heart. Satisfactory results can for that reason be obtained only on the denervated or excised heart. The solutions of the alkaloids may be applied to the surface of the excised heart and empty heart,

or the heart may be filled with the solution through a cannula. Both these methods were used.

It is furthermore necessary that the solvent is neutral or almost neutral to the heart. Distilled water applied to the molluscan and crustacean heart accelerates the rhythm and produces tonus contractions. It will therefore not do to use distilled water as the solvent. Sea water is almost neutral to the heart of all the marine animals worked on. It has a slight stimulating action but this appears very gradually and only after long immersion of the heart in the sea water. The solution of the alkaloids in sea water will in consequence give fairly accurate results. A still better solvent is the blood plasma of the animals themselves, and this was used in nearly all cases. I was not able to obtain an artificial salt solution that proved to be neutral to the heart of the pulmonates. A solution of the drugs in the blood plasma was the only method available in the work on the snail and the slug heart.

The graphic method was used for recording the change in the heart rhythm.

*Solutions of Curare, Atropine, and Nicotine of Sufficient Concentration to Affect Appreciably the Heart Have a Primary Stimulating Action.*–The acceleration of the rhythm is followed by depression and, if the concentration of the alkaloids is great, by complete cessation of the rhythm, the heart remaining excitable to direct stimulation. Nicotine is in every case the strongest stimulant. There does not seem to be any great difference between the stimulating action of atropine and that of curare.

The hearts of the various invertebrates studied differ greatly in their sensitiveness to the action of the drugs. In the weakest concentrations of curare the stimulating action appears in augmentation of the rate and strength of the beats, and by gradually increasing the concentration this augmentation passes into a condition of incomplete tetanus. This tetanus may be maintained for 5 to 8 minutes, especially in the heart of lamellibranchs and gasteropods and in the gill ventricles of the squid. The relaxation is gradual and may be accompanied by a feeble rhythm. After the rhythm has been abolished by the action of a strong (1 per cent) solution of curare it can usually be restored by bathing the hearts in plasma. This is not

true for the heart of the crab. A solution of curare strong enough to produce incomplete tetanus of the heart in this species abolishes the rhythm permanently.

The latent period of the stimulating action of curare varies with the sensitiveness of the particular animal to the drug as well as with the mode of application of the solution to the heart. In the molluscs the alkaloid acts more quickly when poured into the cavity of the heart than when applied to the surface of the heart. This is probably due to a difference in permeability. When the solution is applied to the surface of the heart it has to penetrate the epicardium in order to act on the nervous and the muscular tissues, while there is no endothelium lining the heart cavity. In the crustaceans the drug acts equally quickly whether applied to the surface of the heart or introduced into the heart cavity. When the curare solution is applied to the nerve cord on the dorsal side of the heart of *Limulus* the action is practically instantaneous. The crustacean heart is more sensitive to curare than the molluscan heart (with the exception of the heart of *Loligo*). Between closely related molluscs there may be a great difference in sensitiveness. Thus the ventricle of *Ariolimax* continues to beat with an accelerated rhythm for 25 to 30 minutes in a 0.5 per cent solution of curare, a concentration producing cardiac tetanus within a minute in the ventricles of *Helix* or *Limax*.

The primary stimulating action of atropine and nicotine on the heart is to all appearances very similar to that of curare. Nicotine has to be used in dilutions of 1:1,000 to 1:10,000 to exhibit the true stimulating action. In greater concentrations it usually stops the arthropod heart at once without any primary stimulation. A strong solution of curare has sometimes the same effect on the feebly pulsating crab heart. This difference is probably due to differences in permeability.

The strong stimulating action of curare in the invertebrate heart (without exception) is in contrast to the relatively slight action of this drug on the heart of vertebrates. Atropine and nicotine, on the other hand, are strong stimulants also to the vertebrate heart. The stimulating action of the curare solution may in part be due to the action of potassium salts which are present as impurities in commercial curare. I have found that a slight concentration of the potassium chloride in

the blood of *Limulus* has a powerful stimulating action on the ganglion cells in the heart. The solution of these drugs in the isotonic solvents changes the osmotic pressure of these liquids and a slight change in the osmotic pressure of the blood has itself an effect on the heart.

The point of the action of these alkaloids in the heart is not yet known. On the myogenic theory of the heart beat their stimulating effects may be due to action on the accelerator nervous mechanism or to a direct action on the heart muscle. On the neurogenic theory the augmentation of the rate of the beats can hardly be accounted for except by direct action on the local ganglia, while the increased amplitude of the contractions may be due to action directly on the muscle. To answer the question whether these and other alkaloids act on the nervous or on the muscular tissue in the heart or on both, several investigators have studied their action on the embryonic heart on the theory that the heart on the embryo begins to beat before any nervous elements are present. Pickering (1893, 1894–95) found that atropine and nicotine accelerate the embryonic (chick) heart, while strong solutions of atropine depress the rhythm without any primary stimulation. Cyrillo (1901) states that atropine depresses the embryonic heart. This investigator finds, moreover, that the action of the principal alkaloids on the embryonic heart is the same as on the heart of adults, from which he concludes that these drugs act primarily on the heart muscle, their action on the nervous tissue in the heart being entirely of a secondary character.

I have come to the very opposite conclusion, or that the primary action of the alkaloids is on the ganglion cells in the heart and not on the muscle. This conclusion is based on the results on the heart of *Limulus* (Carlson, 1906–07). For this line of study the *Limulus* heart is prepared in the following manner:

The nerve cord on the dorsal side of the heart is extirpated in the first three segments, which leaves this part of the heart free from ganglion cells, the rhythm being maintained by the impulses reaching the muscle from the nerve cord of the middle third of the heart along the lateral nerves. It is desirable to isolate further the ganglionated and the ganglion-free ends of the heart by excision of the heart muscle for a distance of 1 cm. in the third segment. This dissection can be

done without the least injury to the lateral nerves. This makes an ideal preparation. It is a simple matter to apply a solution to the first two segments and absolutely prevent it from getting to the hind portion of the heart and, conversely, to bathe the nerve cord on the posterior end of the heart in a solution without the solution reaching to the first two segments. Any change in the rhythm of the first two segments on application of a drug to the nerve cord of the posterior end of this heart preparation can be due only to a change in the activity of the ganglion cells which maintain the rhythm. And again, the change in the rhythm of the first two segments produced by a solution applied to these segments alone is evidently not an action on ganglion cells but must be an action on the nerves and nerve endings in the muscle or on the muscle itself if we assume that local reflexes play no rôle. For accurate determinations of the changes in the rhythm in the two anterior segments in this preparation, the graphic method was always used.

On this preparation an extended series of tests of the principal alkaloids were made, the results yielding the conclusion expressed above. All the alkaloids tested act on the nerve cord. Some of them, especially veratrin, digitalin, and nicotine act also on the muscle, and it is probable that all of them act on the muscle if in very strong solution; but their action on the nerve cord or ganglion cells is much more rapid and intense, and all the alkaloids, moreover, act on the nerve cord in a dilution which has no or at least a very slight and gradual action on the muscle. Thus a solution of 1 per cent curare or atropine in plasma or sea water stimulates the nerve cord powerfully at the very instant of application, while no change in the rhythm follows its application to the muscle. Further work in this line will probably show that difference in the action of the alkaloids on the nervous and on the muscular tissues in the heart is only one of degree, the ganglion cells being more permeable to the drugs and the muscle cells having lower excitability.

The action of these alkaloids (curare, atropine, and nicotine) on the whole heart of crustaceans and the molluscs is in all essentials the same as their action on the nerve cord or ganglion cells in the *Limulus* heart. The conclusion seems obvious that their action on the crustacean and molluscan heart is also primarily on the ganglion cells.

### 3. *The Effects of Curare, Atropine, and Nicotine on the Cardioregulative Nervous Mechanism.*

Curare, atropine,and nicotine do not paralyze the cardioaccelerator nerves in the gasteropod molluscs or the intrinsic motor nerves in the heart of *Limulus*. The following experiment on *Ariolimax* may be quoted as typical.

Apr. 4, 1904. Heart of *Ariolimax*.

9.35 a.m. Heart exposed; stimulation of visceral nerve effective. Heart bathed in a 0.5 per cent curare solution in plasma; acceleration.

9.45 a.m. Stimulation of visceral nerve effective. Curare continued.

10 a.m. Stimulation of visceral nerve effective. Curare continued.

10.25 a.m. Stimulation of visceral nerve effective. Curare continued.

10.45 a.m. Accelerator action of nerve partly impaired. Curare solution replaced by blood.

11.10 a.m. Stimulation of visceral nerve accelerates heart.

11.30 a.m. Stimulation of visceral nerve accelerates heart. Blood replaced by 0.5 per cent curare solution. Acceleration of rhythm.

11.50 a.m. Stimulation of visceral nerve has only a very slight action on heart. Rhythm very feeble and slow.

12.15 p.m. Stimulation of visceral nerve still accelerates heart. Curare solution replaced by blood.

1.30 p.m. Heart quiescent. Stimulation of visceral nerve (interrupted current) produced a series of beats in ventricle.

The diminution of the influence of the motor nerves on the heart after prolonged action of these drugs is evidently due to diminished excitability of the heart. The final failure of the accelerator nerves appears only after such a prolonged action of the alkaloids on the heart that the rhythm is almost, if not entirely, abolished. In *Ariolimax* it is possible to produce a series of contractions in the ventricle brought to a standstill by curare, atropine, or nicotine on stimulation of the visceral nerve with the tetanizing current.

I am not in a position to say whether these alkaloids paralyze the accelerator nerves that connect the heart of *Limulus* with the central nervous system, but neither curare, atropine, nor nicotine abolishes the action of the nerve fibers that pass from the nerve cord on the dorsal side of the heart to the heart muscle.

*Continued Action of Solutions of Curare, Atropine, or Nicotine on the Heart Abolishes the Influence of the Inhibitory Nerves on the Heart*

*both in the Molluscs and the Arthropods.*—The paralysis of the inhibitory nerves may be only temporary, their function being restored by bathing the heart in blood. The paralysis is not produced except by a concentration of the drugs that causes marked primary acceleration of the heart rhythm. I did not find any essential difference in the paralyzing action of the three alkaloids.

In the lamellibranchs (*Tapes, Venus*) the curare solution may act for 20 to 30 minutes before the action of the inhibitory nerves on the heart is abolished. In *Helix* and *Limax* the action is much quicker, or in 4 to 8 minutes. The action is quicker the stronger the solutions. In *Loligo* the injection of a few drops of 0.5 per cent curare or atropine, or 0.1 per cent nicotine in the cephalic vena cava paralyzes the cardioinhibitory nerves in 4 to 5 minutes. I have not succeeded in restoring the function of the nerves in *Loligo* after this paralysis. In *Limulus* the concentration of the drugs must be sufficient to cause great augmentation of the rhythm in order to abolish the influence of the inhibitory nerves on the heart, in which case the paralysis is brought about in a few minutes. In weaker solutions it requires much longer time and may in fact not occur at all.

At what point do these alkaloids act to produce the paralysis of the cardioinhibitory nerves? Atropine paralyzes the vagus fibers in the vertebrate heart, and it is generally held that this action is on the inhibitory nerve endings in the muscle. Nicotine, on the other hand, is supposed to paralyze the synapses of the vagus fibers with the inhibitory ganglion cells in the heart. Turning now to the heart of *Limulus*, it is certain that atropine as well as curare and nicotine act on the ganglion in order to paralyze the inhibitory nerves. If the action of the drugs is confined to the heart muscle and the nerves and the nerve endings in the muscle no paralyzing effects are produced. It would therefore seem that the action of these alkaloids, in abolishing the action of the inhibitory nerves on the heart, is on the ganglion cells in the heart or on the endings of the inhibitory nerves in connection with these cells.

While it is certain that curare, atropine, and nicotine in sufficient concentrations paralyze the cardioinhibitory nervous mechanism in molluscs and arthropods, this action requires, on the whole, a greater concentration of the drugs than that sufficient for the paralysis of the cardiac vagi in the vertebrates.

SUMMARY.

1. The alkaloids (curare, atropine, and nicotine) in molluscs and arthropods stimulate and paralyze the central nervous system and peripheral (visceral) ganglia, but do not paralyze the motor nerve endings to skeletal or visceral muscle.

2. They stimulate and paralyze the denervated heart.

3. They paralyze or block the cardioinhibitory nerves, but not the cardioaccelerator nerves.

4. In the *Limulus* heart these drugs act primarily on the heart ganglion, not on the heart muscle or the intrinsic motor nerve fibers.

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