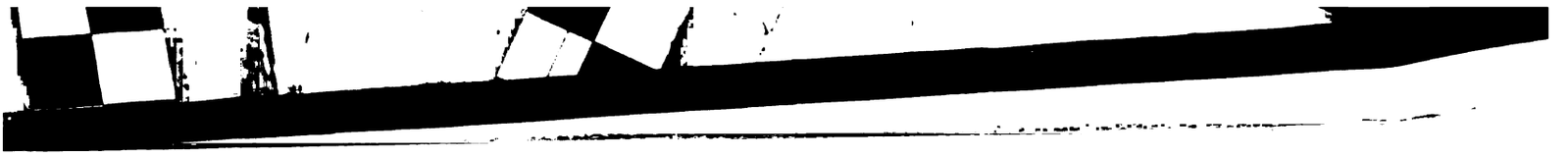


ABSTRACT

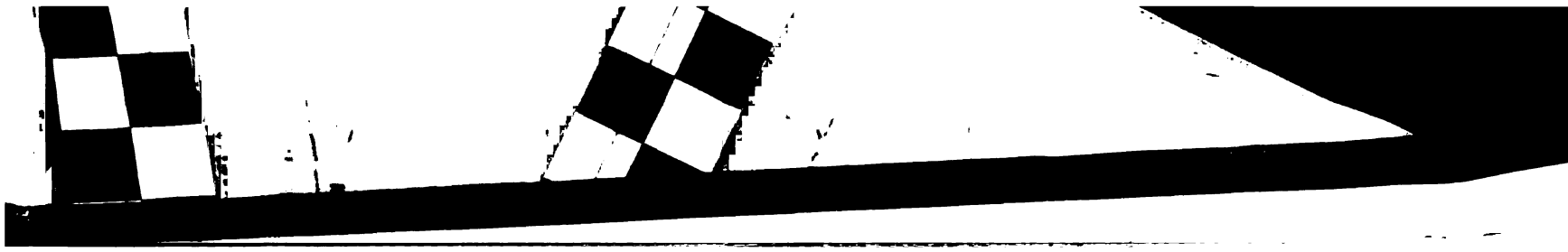
ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES
OF THE HEART OF THE TARANTULA
EURYPELMA MARXI SIMON

By

Robert George Sherman

Little is known about the cardiac physiology of spiders. This study was undertaken to determine the function of the cardiac ganglion in the spider heartbeat and to determine the effects of certain neurologically active drugs on the heart of the spider Eurypelma marxi.

The function of the cardiac ganglion was investigated by means of conventional electrophysiological recording techniques used on isolated heart preparations. The results of the electrophysiological studies show that the endogenous rhythm of the heart resides in the cardiac ganglion and that the ganglion functions to produce unit contractions of the heart. This is seen from the following lines of evidence. Recordings of temporal relationships between cardiac ganglion electrical activity, electrical responses of the cardiac muscle cells and contractions of the myocardium show that the activity of the cardiac ganglion precedes all other activity in the heartbeat. Removal of the ganglion results



in an immediate cessation of the heartbeat. Spontaneous bursts of impulses can be recorded from cardiac ganglia that have been completely isolated from the myocardium. Transection of the ganglion results in two parts of the heart which continue to contract, but out of phase with each other. Unit contractions still occur after transection of the myocardium as long as the ganglion is left intact.

The effects of certain neuropharmacological agents on the E. marxi heart were determined by applying various concentrations of these drugs to isolated heart preparations and monitoring the effects of the drugs by means of a mechanotransducer. The site of drug action on the heart was determined by monitoring the effect of drug treatments on the cardiac ganglion electrical activity and on the myocardial tonus of hearts from which the ganglion had been removed.

The E. marxi heart responds to the cholinergic compounds, acetylcholine (Ach), nicotine and methacholine with a marked increase in heart rate and beat amplitude. The excitatory effects of Ach are potentiated by eserine and partially blocked by atropine, hexamethonium and d-tubocurare. The main effect of methacholine is to produce an increase in the beat amplitude, while the principal effect of nicotine is an increased heart rate. The effects of methacholine are blocked by atropine, but not hexamethonium; the effects of nicotine are greatly reduced by hexamethonium, but not

atropine. The actions of all of these cholinergic drugs occur on the nerve cells of the cardiac ganglion.

Other compounds also produce changes in the E. marxi heartbeat due to their actions on the cardiac ganglion. Epinephrine and norepinephrine produce a marked increase in the rate and amplitude of the heart contractions. Gamma aminobutyric acid (Gaba) and 5-hydroxytryptamine (5-Ht) have an inhibitory effect on the heart, but the heart is much more sensitive to Gaba than to 5-Ht. Picrotoxin does not block the effects of Gaba. d-Glutamic acid, at high concentrations, produces an increase in the heart rate and a decrease in the amplitude of the contractions. 1-Glutamic acid has little effect on the ganglion, but produces sustained contractions of deganglionated hearts. Glycine and d and 1-aspartic acid have little effect on the heart.

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES OF THE HEART OF THE TARANTULA *EURYPELMA MARXI* SIMON

By

Robert George Sherman

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology

1969

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Ralph A. Pax whose advice, criticism and assistance was invaluable throughout the course of this study. Grateful acknowledgment is made of Drs. Paul O. Fromm, Gerard L. Gebber and T. Wayne Porter who served as members of my guidance committee.

Acknowledgment is also made of Dr. Leslie C. Drew who identified the animal used in this study and of Mrs. B. Henderson for her assistance in obtaining materials.

I wish to express my heartfelt thanks to my parents, Mr. and Mrs. George R. Sherman, for their steadfast encouragement and support which has made the completion of this investigation possible. Finally, I thank my wife Barbara and daughter Lisa for making the completion of this study worthwhile.

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INTRODUCTION

Investigation into the cardiac physiology of arthropods have been performed in some detail in three classes; the Crustacea, the Insecta and the Merostomata. Cardiac function in the class Arachnida has been little studied. This is somewhat surprising, for among the arthropod classes arachnids are second only to the insects in numbers of species (Russell-Hunter, 1969).

Among the most important of the arachnids in terms of numbers, size and distribution are the spiders. From a comparative standpoint it would be of interest to compare heart function in spiders to other arthropods for which information is available, since basic functional differences in the cardiac physiology of the various arthropod groups do occur. An example of this is apparent in the genesis of the heartbeat. Heartbeat in Limulus polyphemus (Merostomata) and the decapod and stomatopod Crustacea is neurogenic, i.e., nerve cells located on the heart cause the heart to contract (Prosser and Brown, 1961). On the other hand, heartbeat in certain insects (McCann, 1965; Miller, 1968a) and lower crustaceans (Prosser and Brown, 1961) is myogenic, i.e., the muscle cells of the heart themselves are responsible for initiating contractions of the heart.

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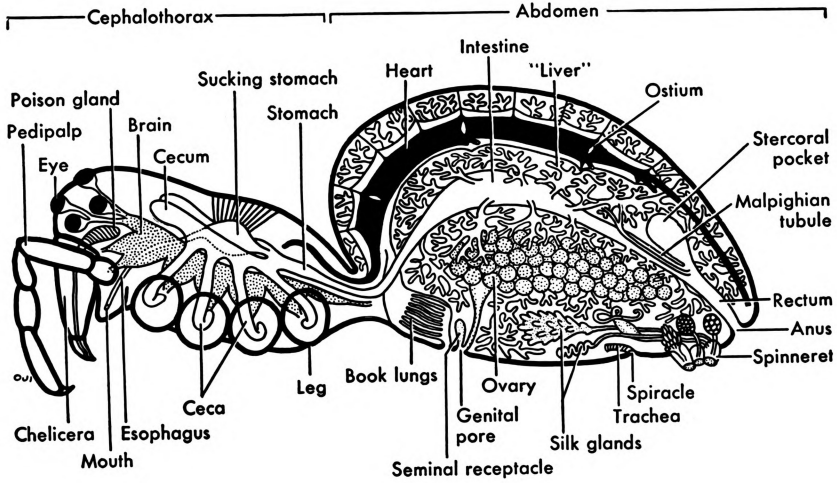
To obtain information about heart function in arachnids, and about spiders in particular, both electrophysiological and pharmacological studies were conducted on the tarantula Eurypelma marxi Simon. This spider was used because of its relatively large size and commercial availability. The results of these studies are reported here with the aim of providing a fuller understanding of the cardiac physiology of spiders and of arthropods in general.

Structural Features of the Heart and Circulatory System

The structure of the hearts of several spiders has been described in a general manner utilizing only the light microscope. Much of what is known about spider heart morphology is due to the work of Petrunkevitch (1910, 1922, 1933) and Millot (1932, 1949), and the description given here is based on these works.

The morphology of the heart is basically the same for all of the spiders that have been investigated. The heart is tubular in shape and lies just below the abdominal exoskeleton in a mid-dorsal position (Figure 1). It extends from the anterior of the abdomen posteriorly for about two-thirds the total length of the abdomen. The heart is held in position by a series of suspensory ligaments attached to the exoskeleton. A thin-walled pericardium surrounds the heart forming a pericardial cavity.

Figure 1. General internal anatomy of a spider (Taken from Hegner and Stiles, 1959).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Intestine
Heart
"Liver"
Sucking stomach
Ostium
Poison gland
Stomach
Pedipalp
Brain
Stercoral pocket
Eye
Cecum
Malpighian tubule
Rectum
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The heart wall is composed of three layers: an outer layer of connective tissue, a thin middle layer of longitudinal muscles and a thick inner layer of circular muscles. The muscles are transversely striated. There is no obvious inner lining of the heart.

Blood from the pericardial cavity enters the heart through paired ostia. A spider may have from two to five ostial pairs depending on the family of spider. In the family of tarantulas (Theraphosidae) there are four pairs. From the anterior of the heart the main efferent vessel carries blood to the prosoma, while the abdomen is supplied by a number of smaller vessels leaving the heart ventrally. Blood is returned to the pericardial cavity by an afferent channel from the large collecting sinus located ventrally in the anterior of the abdomen.

Depending on the family of spider, the respiratory structures consist of either two pairs of book-lungs and no trachea, one pair of book-lungs and tracheae, or only tracheae. In tarantulas there are two pairs of book-lungs and no trachea.

Nerve Innervation of the Heart

In view of the extensive morphological work done on spider hearts by Petrunkevitch (1910, 1922, 1933), Millot (1932, 1949) and others, it is surprising that it was only recently that a cardiac ganglion was reported present in spiders. Sherman and Pax (1967, 1968), Wilson (1967) and Legendre (1968) each working with a different species from a different family of spiders reported a cardiac ganglion

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present on the heart. This ganglion appears as a thread-like structure extending along the length of the heart in a mid-dorsal position. Recently, Sherman et al. (1969) have extended the number of spiders known to possess a cardiac ganglion to 31 species representing 14 different families.

The cardiac ganglion consists of a number of nerve cell bodies and nerve processes which are found throughout the length of the ganglion. The ganglion is enclosed by a connective tissue sheath which is separate from that which surrounds the heart. The nerve cell bodies in the ganglion number about 20 in Scodra calceata (Legendre, 1968), about 50 in Geolycosa missouriensis (Sherman and Pax, 1968) and approximately 85 in Eurypelma marxi (Bursey and Sherman, 1969). In G. missouriensis, nerve fibers from the ganglion innervate the myocardium at about 12 different locations along the length of the heart.

The presence of nerves which enter the heart from the central nervous system have been reported by Wilson (1967) and Legendre (1968). According to these workers the ventral ganglionic mass located in the prosoma gives rise to a nerve tract which runs through the pedicel to the abdomen. There it branches many times. One of these branches divides just posterior to the pedicel and sends two ramifications dorsally to the heart. Although Wilson was unable to locate the point where these nerves join the heart in Heteropoda venatoria, Legendre found that they innervate the cardiac

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ganglion at a point just posterior to the first pair of ostia in Scodra calceata.

Events in the Cardiac Cycle

Wilson (1967), utilizing cine-photography and direct observation, described the sequence of events that occur during each complete cycle of the spider heartbeat. At the beginning of the systolic phase the heart is full of blood, the ostia are open and the suspensory ligaments are shortened. The circular muscles contract, exerting a force on the blood within the heart and at the same time the ostial valves close and the heart extends lengthwise. Blood flows through the arteries as the arterial resistance is overcome and the heart contracts evenly until it reaches its minimum volume. At this point the ligaments are fully extended. The emptied heart now returns to its maximum volume due to the relaxation of the circular muscles and the tension exerted by the shortening ligaments. The heart resumes its original length presumably due to the action of the longitudinal muscles, and the ostia open allowing the heart to fill with blood from the pericardial cavity.

Heart Rate Studies

With the exception of the work of Rijlant (1933) all of the physiological studies on spider hearts before 1965 consisted of determining the spider heart rate. Table 1 gives the rates reported for a number of different spiders.

Table 1. Spider heart rates

Species	Rate (Beats/Min)	Source
Liphistius desultor	26	Bristowe, 1932
Eurypelma marxi	37	Wenk, 1969 (unpublished)
Geolycosa missouriensis	48	Sherman and Pax, 1968
Micrommata virescens	54	Bristowe, 1932
Tegenaria atrica	59	Mikulska and Kokocinski, 1965
Heteropoda venatoria	70	Wilson, 1967
Pholcis phalangioides	134	Willem, 1917
Epeira diadematа	139	Willem, 1917

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From these values it appears that there is no characteristic heart rate for spiders in general, the rate varying markedly from species to species. However, the way in which the rates were determined was not the same in each of the studies. The first four values probably represent true resting rates in undisturbed spiders, but the last four rates were obtained from spiders which had been restrained in order to facilitate recording of heart rates. Sherman and Pax (1968) found that restraining the spider G. missouriensis resulted in heart rates nearly three times those of the same spider measured under unrestrained conditions. From this, it is likely that most of the variability in reported spider heart rates is due to the manner in which they were recorded rather than due to species differences.

A variety of extrinsic factors aside from restraint are also capable of producing elevated heart rates. Forced exercise typically results in a transient three to seven-fold increase in heart rate (Bristowe, 1932; Sherman and Pax, 1968). Changes in temperature (Mikulska, 1961) and bright light shone in the eyes of spiders (Mikulska and Kokociński, 1965) are also capable of producing elevated heart rates.

Electrical Activity of the Heart

Rijlant (1933) was the first to record electrocardiograms of spiders. He made recordings from three genera of spiders (Empeira sp., Tarentula sp. and Mygale sp.) and found that with each contraction of the heart there occurred

a burst of electrical activity consisting of from 10 to 30 electrical oscillations and lasting from 200 to 500 msec. The frequency of the oscillations ranged from 40 to 100 per second.

Sherman and Pax (1968) recorded the electrocardiogram of G. missouriensis and the extracellular electrical activity of the cardiac ganglion. In both instances the pattern of electrical activity recorded consists of a large number of oscillatory potentials which closely resemble the records obtained by Rijlant (1933). Sherman and Pax (1969) also recorded the electrical activity from within single cardiac muscle cells of G. missouriensis using microelectrode techniques. They find that the electrical responses of the cardiac muscle cells consist of a large initial depolarization which is followed by a lesser maintained depolarization which lasts up to 650 msec. Superimposed on the maintained depolarization are a number of smaller potentials. Because of the characteristics of the maintained depolarization phase and because a number of discrete steps are seen in the initial depolarization, Sherman and Pax suggest that the active response of the G. missouriensis heart muscle cells represents a summation of a number of postsynaptic junctional potentials.

Pharmacology of the Spider Heart

The only pharmacological study on spider hearts has been that of Kadziela and Kokociński (1965). They injected

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epinephrine and acetylcholine into the abdomen of Tegenaria atrica and measured the change in heart rate which resulted. Epinephrine in a dose of 5.2×10^{-6} mg/mg of preparation produced a rapid decrease in the amplitude of the heartbeat and an increase in the heart rate. The return of the rate to normal took about 90 seconds. An acetylcholine injection of 2.6×10^{-6} mg/mg of preparation was followed by a slowing of the heart rate and a decrease in the amplitude of the beat. The return to normal was quicker than for epinephrine, taking about 60 seconds.

Origin of the Spider Heartbeat

In the animal kingdom heart contractions are initiated by the endogenous activity of either muscle or nerve cells. If the initiation of the heartbeat is due to nerve cells, the heart is said to be neurogenic. On the other hand, if the heartbeat originates in the heart muscle, the heart is said to be myogenic. Several indirect criteria have been used to distinguish between the myogenic and neurogenic heartbeat (Prosser and Brown, 1961). The more important ones are:

(1) The presence or absence of nerve cells on or in the heart. If present the heartbeat may be neurogenic; if absent, the heart is presumably myogenic.

(2) The pattern of the electrocardiogram recorded. The pattern consists of numerous high frequency potentials in neurogenic hearts and a few slow waves in myogenic hearts.

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(3) Effects of certain drugs, particularly ether and acetylcholine, on heart activity. Ether inhibits neurogenic hearts and has little effect on myogenic ones, while acetylcholine slows myogenic hearts and may produce rate increases in neurogenic hearts.

Studies into the genesis of the spider heartbeat have provided conflicting information as judged from the above criteria. The cardiac ganglion present on the heart of a variety of spiders and the pattern of the electrical activity recorded from the heart are characteristics of neurogenic hearts. However, the decrease in heart rate produced by acetylcholine is a response characteristic of myogenic hearts.

Objectives of this Study

Due to the small number of physiological studies on spider hearts there are many specific areas within spider cardiac physiology that deserve study. In view of the apparent contradiction in current information concerning the genesis of the spider heartbeat, the most pertinent investigation at this point would be one which attempted to resolve this conflict. For this reason electrophysiological studies were undertaken on the tarantula Eurypelma marxi with the aim of providing conclusive information as to the origin of the spider heartbeat. In addition to these studies, pharmacological investigations involving acetylcholine and epinephrine were conducted in an attempt to re-evaluate the results reported by Kadziela and Koiociński (1965).

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Besides acetylcholine and epinephrine, other chemicals which have been reported to be active on other arthropod hearts were also studied as to their effects on the E. marxi heart, with the hope of providing a fuller understanding of cardiac pharmacology in arthropods.

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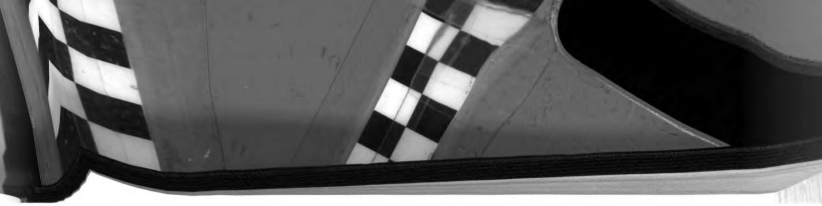
MATERIALS AND METHODS

Source and Maintenance of Animals

Specimens of the tarantula Eurypelma marxi Simon were purchased from Southern Biological Supply Co., McKenzie, Tennessee. The spiders were shipped air express from the company's collector in Lyford, Texas. Both male and female spiders ranging from 44 to 55 mm in length were used and no differences in results obtained were noted due to size or sex.

Spiders were kept individually in 3.8 liter glass jars. The bottom of each jar was covered with about four centimeters of sand over which was spread a thin layer of commercial aquarium stones. The tops of the containers were covered with cheesecloth which was held in place with rubber bands. Water was provided by soaking small pieces of sponge and placing one in each container. Ordinarily the spiders were used within two weeks of their arrival, but if they were to be kept for longer periods of time they were fed a cockroach nymph (Blaberus discoidalis) each week.



Physiological Spider Saline

The saline used is that described by Rathmayer (1965) for a leg nerve-muscle preparation of the spider Eurypelma hentzi Girard. The composition of the saline is as follows: 217 mM NaCl, 5.0 mM KCl, 4.0 mM $CaCl_2$, 3.0 mM $NaHCO_3$ and 1.1 mM $MgCl_2$. The pH of the saline ranged from 7.1 to 7.3. The saline was kept at room temperature which ranged from 23 to 28°C. However, for any one experiment the room temperature remained quite constant.

Isolation of the Heart

Spiders were immobilized by placing them in a refrigerator at 5°C for 15 to 20 minutes. When the spider appeared to be acting "sluggish" its legs were removed by cutting them off close to their origins. The abdomen was then separated from the prosoma by cutting through the pedicel. The abdomen was then placed ventral-side-up in a 10 cm finger bowl which had been filled three fourths with paraffin. A cut was made along both lateral margins of the abdomen beginning just dorsal to the spinnerets and ending at a point just short of the pedicel and just ventral to it. A large insect pin was placed at the posterior of the abdomen to hold it to the paraffin. At this point sufficient saline to cover the abdomen was added to the dissecting dish and the saline was periodically changed during the remainder of the isolation procedure.

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The ventral exoskeleton was removed by drawing it up and forward to a point in front of the rest of the abdomen and then freed by completing the cut made previously. The viscera were then removed en masse by lifting them at their posterior and pulling them up and forward while at the same time teasing them away from around the heart. This procedure exposes the heart which at this point is in its mid-dorsal position still attached to the dorsal exoskeleton.

To completely isolate the heart, the suspensory ligaments were severed and the pericardium teased away from the dorsal exoskeleton. The heart was then lifted free by use of forceps placed at its posterior end. Care was taken to avoid stretching the heart, and to avoid damaging the cardiac ganglion, forceps were never placed on the mid-dorsal surface of the heart. After the heart was freed it was placed in the recording dish which consisted of a Syracuse glass dish filled three fourths with paraffin. The heart was covered with saline and the saline changed approximately every three minutes thereafter. The isolated heart along with the cardiac ganglion and some of the ostia is shown in Figure 2.

Preparation of Hearts for Recording

Four different isolated heart preparations were used, the one used at any time depending on the nature of the experiment. For nearly all experiments the preparation consisted of an isolated heart pinned dorsal-side-up in the

Figure 2. Dorsal view of the isolated heart of *E. marxi*. Anterior is to the bottom. The cardiac ganglion can be seen as a light line along the mid-dorsal heart surface. The transverse slits toward the posterior of the heart are ostia. (20x)

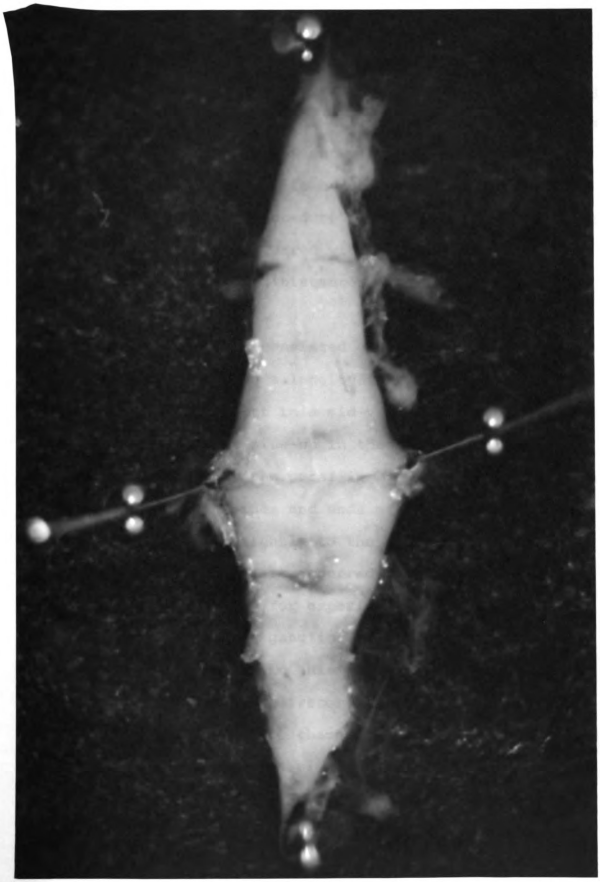
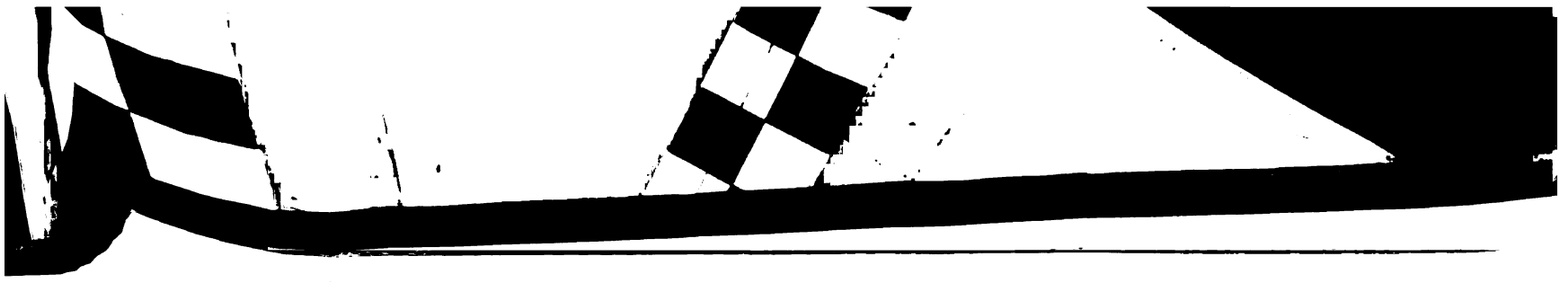


Figure 2

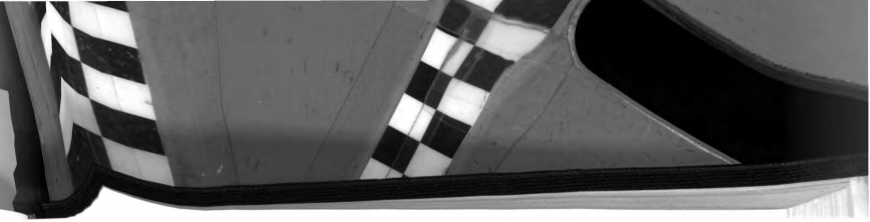


recording dish by means of fine insect pins (size 000).

In certain instances it was advantageous to slightly modify the heart preparation described above. When sampling of the intracellular electrical activity of the muscle cells was to be done, the heart was not pinned to the paraffin, but instead was allowed to lie on one side completely free of any restraint. This procedure greatly reduced the amount of heart movement that occurred with each contraction and thereby facilitated the maintenance of a microelectrode in a contracting muscle cell.

A third preparation consisted of a heart which was split ventrally by making a longitudinal incision along the entire length of the heart in a mid-ventral position. This heart was placed dorsal-side-up in the recording dish and pinned out flat against the paraffin by means of insect pins positioned along both sides and ends of the heart. In this way the heart was held tightly to the paraffin and very little movement of the heart occurred with each contraction. This preparation was used for experiments in which the effects of drugs on cardiac ganglion electrical activity were studied. Hearts prepared in this manner had the advantage of not pulling away from the recording electrode when solutions bathing the heart were changed.

The fourth heart preparation consisted of a heart from which the cardiac ganglion had been removed, but which was otherwise left intact. The ganglion was easily dissected



by simply teasing it free from the external heart surface with fine forceps. Deganglionated hearts were used to study the effects of certain drugs on the myocardium, free from any influence of the cardiac ganglion.

Recording Apparatus and Procedures

Cardiac Ganglion Electrical Activity

A glass suction electrode was used to record the electrical activity of the cardiac ganglion. A 15 cm piece of Kimax glass tubing having an outside diameter of four millimeters was drawn-out at one end over a flame to a tip diameter of 200 micra. A 10 cm length of Tygon tubing with an outside diameter of five millimeters was pulled over the other end of the glass tubing. The other end of the Tygon tubing was connected to a plastic Y having an outside diameter of five millimeters. One arm of the Y was joined to another length of Tygon tubing (30 cm) to which was attached a 12 ml disposable plastic syringe. An insulated length of copper wire to which was soldered a four centimeter piece of 34 gauge silver wire was inserted into the other arm of the plastic Y and directed into the glass tubing, silver wire first. The wire was inserted until it reached a point about three millimeters from the tip of the drawn-out end of the glass.

The glass suction electrode was positioned onto the heart with a Narishige micromanipulator. The electrode was

lowered onto the cardiac ganglion and the plunger of the syringe withdrawn until a small length of the ganglion was pulled just into the tip. Enough saline to bridge the distance between the electrode tip and the silver wire was also drawn into the electrode along with the cardiac ganglion.

The wire lead from the electrode was connected to a Grass P5 preamplifier. Another piece of silver wire was placed in the recording dish as a ground. The signal from the preamplifier was displayed on a Tektronix 502A oscilloscope and filmed with a Polaroid C-27 oscilloscope camera.

Heart Muscle Electrical Activity

Intracellular recording of electrical activity necessitates the use of microelectrode recording techniques. Glass microelectrodes were prepared by drawing out Kimax glass capillary tubing (outside diameter of 800 micra) with a Narishige microelectrode puller. Approximately 20 such micropipettes were placed on a glass slide and held secure with a rubber band. The slide was then placed in a Coplin jar with the tips of the micropipettes directed upward. Methanol was added to the Coplin jar until the pipette tips were covered by about three centimeters of methanol and the jar was placed in a vacuum desiccator. A vacuum was pulled until the air in the micropipettes was displaced by the methanol. Then the methanol was decanted and the Coplin jar filled with distilled water. After 15 minutes the

distilled water was replaced with three molar KCl. The electrodes were left in three molar KCl for at least 48 hours before use. Only electrodes with low tip potentials and with resistances from 10 to 40 megohms were used. It was observed that electrodes left in KCl for up to 14 days were often still suitable for use.

A Narishige micromanipulator was used to position microelectrodes into the heart. The recording leads consisted of silver wire coated with silver chloride. The leads were connected to a W-P Instruments Model Four Electrometer and the signal displayed and filmed, using the same apparatus as described for the cardiac ganglion electrical activity.

Mechanical Recording of the Heart Contractions

A displacement mechanotransducer was used to monitor the contractions of the heart. The transducer was connected to the heart in the following way. A small piece of insect pin was bent into a hook and the hook attached to a short length of fine thread by means of a drop of melted paraffin. The other end of the thread was connected to the movable arm of the transducer. The insect pin hook was then inserted into the lateral margin of the heart at a point near the second pair of ostia. This was the region of the heart showing the greatest amount of movement with each contraction. Additional insect pins were placed at both ends of the heart and along the side opposite to that where the

transducer was attached. This served to anchor the heart in place.

The mechanotransducer used was the E and M Instrument Co. Model F-50 Microdisplacement Transducer. This transducer has a maximum sensitivity of 100 mg/cm of pen displacement. The transducer output was monitored on an E and M Instrument Co. Model Four Physiograph.

Pharmacology Study Procedures

Source and Preparation of Drugs

The drugs used and the source from which they were obtained are as follows:

(1) Cholinergic Compounds

Acetylcholine chloride--Nutrit. Biochem. Corp.

Nicotine--Nutrit. Biochem. Corp.

Methacholine(acetyl-beta-methylcholine)chloride--Mann Research Labs.

Eserine(physostigmine)sulfate--Sigma Chem. Co.

Atropine sulfate--Sigma Chem. Co.

d-Tubocurarine chloride--Sigma Chem. Co.

Hexamethonium chloride--Mann Research Labs.

(2) Monoamines

l-Epinephrine hydrochloride--Sigma Chem. Co.

l-Norepinephrine hydrochloride--Sigma Chem. Co.

5-Hydroxytryptamine creatine sulfate--Sigma Chem. Co.

(3) Other Drugs

Gamma aminobutyric acid--Nutrit. Biochem. Corp.

Picrotoxin--Nutrit. Biochem. Corp.

Glycine--Sigma Chem. Co.

d-Glutamic acid--Sigma Chem. Co.

l-Glutamic acid--Sigma Chem. Co.

d-Aspartic acid--Sigma Chem. Co.

l-Aspartic acid--Sigma Chem. Co.

Stock solutions of each of the drugs to be used for a particular experiment were made as shortly before an experiment as possible, using spider saline as the solvent. All concentrations are expressed as the salt, except for nicotine which is expressed as the base.

Experimental Procedures

Drugs were administered by simply removing the saline bathing the heart with a syringe and replacing the saline with the drug solution. The volume of drug solution applied in each case was four milliliters. This amount was sufficient to completely cover the heart. Usually drugs were left on the heart for three minutes and in a few instances for as long as 30 minutes, the time depending on the drug and the experiment. After each application the rate and amplitude of the heartbeat were allowed to return to the level seen before the treatment. During this time several saline washes were made. In each case at least five minutes were alloted between drug treatments and in general this

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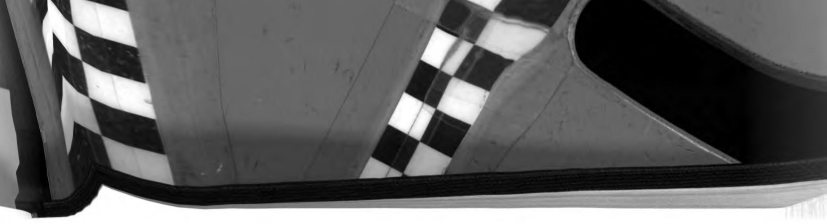

period was 10 to 15 minutes in duration. At least three saline washes and as many as seven were made during the recovery period.

Any one heart was used for only one drug unless the interactions of two or more drugs were to be investigated. The duration of the experiments approximated two and one-half hours and in no case were drugs tested on a heart that had been in isolation for longer than three hours. Hearts used had heart rates between 49 and 22 beats/min during the course of an experiment. If upon isolation the initial heart rate was greater than 49 beats/min, the heart was allowed to beat in saline until the rate dropped to less than 50 beats/min. This was done because a greater amount of variability in rates was observed for hearts beating faster than 50 beats/min than for those with lower rates.

Data Reduction

For all drugs except acetylcholine, heart rates were measured for the minute just preceding each treatment and for each minute thereafter. In the case of acetylcholine, the minute intervals were subdivided into six equal intervals of 10 seconds each. This was done because acetylcholine was observed to have its maximum effect immediately after application and the effect was often only a transient one. Only by measuring the rate over a shorter time interval than one minute was the maximum response to acetylcholine determined. Since all rates are expressed as beats/min, the

precision of the acetylcholine rate data is only plus or minus six beats/min.

The amplitude of the heart contractions was measured in terms of mg of pull on the transducer. The heartbeat amplitude was determined for the 10 beats which occurred just prior to the beginning of a drug treatment and for the 10 beats immediately following the start of a treatment. In addition, 10 consecutive beats were also measured one and two minutes after the drug treatment was begun. The precision of the amplitude measurements is plus or minus five milligrams.

RESULTS

Electrophysiological Studies

Though a number of studies have provided indirect evidence for a nervous origin of the heartbeat in spiders, conclusive evidence for this is lacking. Even though a cardiac ganglion is present and the cardiac electrical activity patterns are those characteristically seen for neurogenic hearts, one cannot conclude solely from this evidence that the cardiac ganglion functions as the heart pacemaker. One must demonstrate that cardiac ganglion activity precedes all other overt activity in the heartbeat and that removal of the ganglion arrests the heartbeat. With this in mind I have undertaken electrophysiological studies on the role of the cardiac ganglion in the heartbeat of the spider Eurypelma marxi.

Cardiac Ganglion Electrical Activity

The spontaneous activity of the cardiac ganglion was recorded by means of a suction electrode placed on the ganglion at a point near either the first or third ostial pairs. The pattern of activity recorded from the E. marxi heart is essentially the same as that recorded for the heart of G. missouriensis and will be only briefly described.

Figure 3A (lower trace) shows the characteristic pattern of electrical activity obtained. The activity consists of a series of sustained bursts of electrical potentials with a period of little or no activity between each burst. The number of potentials in a burst varies from one recording site to another and is difficult to determine precisely since each deflection in the electrical recording may represent the summation of a number of individual potentials and some of the potentials are of such low magnitude that they are difficult to distinguish from background noise. In any case, as many as 50 potentials in a burst were seen.

The duration of the burst varies considerably from animal to animal and also for any one heart. This variation is due in part to the heart rate at the time of the recording. For example, a burst duration of 500 msec was measured for one heart which was beating at a rate of 50 beats/min. When the heart rate had declined to 24 beats/min, the burst duration was about one second. The burst duration also varies from one recording site to another.

Cardiac Muscle Electrical Activity

The electrical responses of individual heart muscle cells were recorded using microelectrode techniques. A total of four hearts were used to sample the electrical activity and the results for the first 25 cell penetrations of each heart were recorded. For each heart 20 of the penetrations

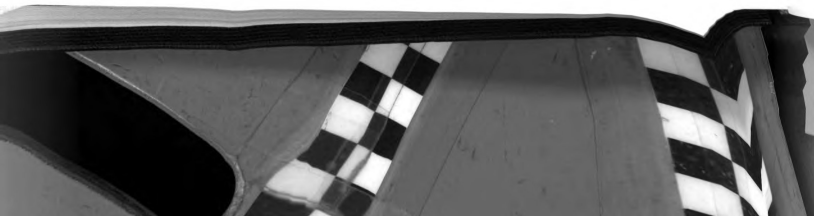

Figure 3. Electrical and mechanical activity of the heart. A and B: extracellular recordings of cardiac ganglion electrical activity (lower traces) and heart mechanical activity (upper traces). Amplitude cal: 16 uV/mm, lower traces; 16 mg/mm, upper traces. Time cal: 80 msec/mm (A); 160 msec/mm (B).

C and D: intracellular recordings of cardiac muscle cell electrical responses (lower traces) and heart mechanical activity (upper traces). Amplitude cal: 2 mV/mm, lower traces; 16 mg/mm, upper traces. Time cal: 80 msec/mm (A); 160 msec/mm (B).

E and F: extracellular recordings of cardiac ganglion electrical activity (lower traces) and intracellular recordings of cardiac muscle cell electrical responses (upper traces). Amplitude cal: 16 uV/mm, lower traces; 2 mV/mm, upper traces. Time cal: 30 msec/mm (A); 80 msec/mm (B).

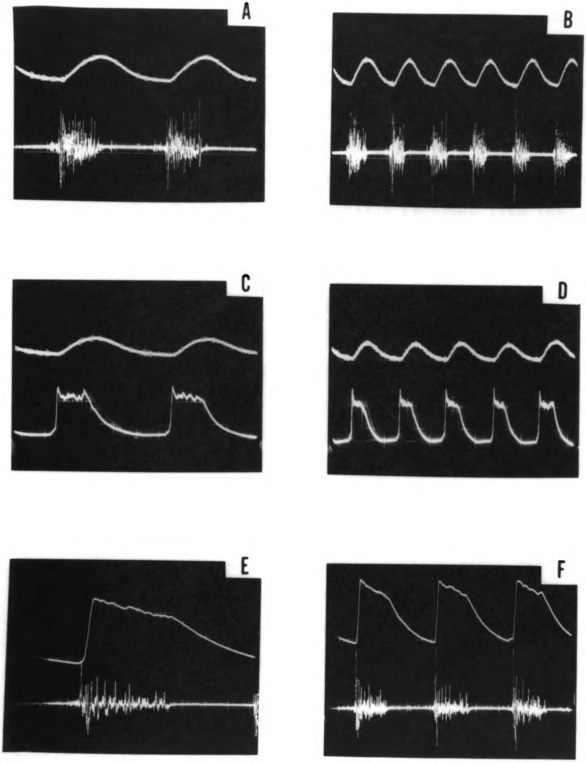

Figure 3

were made in the region of the heart between the first and fourth ostial pairs. This is the area of the heart which characteristically shows strong contractions. The remaining five penetrations of each heart were made either anterior to the first ostial pair or posterior to the last pair of ostia. These end regions of the heart show only weak contractions. All penetrations were made within one hour after isolation of the heart. Resting membrane potentials recorded from the middle regions averaged - 65 mV (SE = 5mV), while those recorded from the ends of the heart averaged - 48 mV (SE = 3mV). No decrease in the resting potentials were apparent with time in isolation.

The pattern of the active electrical responses recorded from the muscle cells of the tarantula heart is essentially the same as that described previously for the G. missouriensis heart. The pattern is basically the same for all cells. Each response consists of a rapid initial depolarization which is followed by a maintained depolarization, forming a plateau (Figure 3C, lower trace). The level of the plateau depolarization is usually 75 to 90% that of the initial depolarization and the plateau lasts for up to one second. Superimposed on the plateau is a series of oscillatory potentials of varying number and amplitude. Up to 15 such small potentials were measured for some responses. The time for the initial depolarization to reach maximum varies considerably from cell to cell and even for consecutive responses of the same cell.

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This time was as brief as 25 msec for recently isolated hearts, but for hearts in isolation for one hour it was as long as 90 msec. Repolarization of the cell membrane is much more gradual than the initial depolarization, often taking as long as one second. The active response recorded from the heart muscle cells appears to represent a summation of a number of excitatory postsynaptic potentials, since a number of discrete steps are often discernible on the rising and falling phases of the response and the peak depolarization never reaches zero potential.

The size of the active response of the heart muscle cells is larger in the middle regions of the heart where the contractions of the muscle cells are strong. For these heart regions the active response averaged 35 mV (SE = 3 mV), while the size of the active response recorded from cells at the ends of the heart averaged only 12 mV (SE = 2 mV). Correspondingly, the contractions seen in these regions are characteristically weaker than those in the middle of the heart.

Mechanical Activity of the Heart

Contractions of the heart were recorded by means of a mechanotransducer to monitor the time course of the heart contractions. The pattern of the myogram is shown in Figure 3A (upper trace). The myogram consists simply of a smooth upstroke and downstroke, signaling the beginning and the end of the effective stroke of the heart.

Temporal Relationship Between Cardiac Ganglion Activity and Heart Contractions

If the cardiac ganglion elicits contractions of the heart, the electrical activity of the ganglion must occur before the heart contractions. To determine the temporal relationship between cardiac ganglion activity and contractions of the heart, simultaneous recordings were made of ganglionic electrical activity and the mechanical activity of the heart.

Figure 3 (A and B) shows such a recording made at two different recording speeds. These records show that the beginning of each ganglionic burst occurs before the start of the upstroke of the heart contractions. The time lapse between the start of the ganglionic activity and the beginning of the contraction is approximately 300 msec. This number is only an estimate since the electrical events of the ganglion were recorded at a point on the ganglion, while the myogram represents the tension exerted by a large region of the heart. The activity of the ganglion continues throughout the rising phase of the myogram, ending about the same time that the upstroke reaches its peak amplitude.

Temporal Relationship Between Heart Muscle Electrical Activity and Contractions of the Heart

To determine the temporal relationship between the depolarizations of the *E. marxi* cardiac muscle cells and contractions of the heart, simultaneous recordings of both

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events were made. Figure 3 (C and D) shows the relationship between the depolarizations of a heart muscle cell and the contractions of the heart. The muscle cell electrical response is seen to begin about 200 msec before the start of the contraction of the heart. Since the myogram represents the contraction of a region of the heart, and the electrical response is that of a single cell, this 200 msec figure is at best only an approximation of the actual delay. The time course of the electrical responses of the muscle cells closely parallels the time course of the myogram. The electrical response reaches its maximum amplitude just prior to the maximum amplitude of the contraction and the electrical response begins to decline just before the downstroke of the myogram.

Temporal Relationship Between Cardiac Ganglion Electrical Activity and Heart Muscle Electrical Events

The electrical response of each muscle cell appears to consist of a series of excitatory postsynaptic potentials which summate to produce a sustained depolarization. This muscle response begins prior to tension development by the heart musculature. A burst of ganglionic activity also begins before each contraction of the heart. Therefore it seems likely that the neurons in the ganglion elicit the summating postsynaptic potentials recorded from individual muscle cells.

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To determine if a burst of ganglionic potentials begins prior to the onset of the muscle cell electrical response, records of both events were made at the same time. Figure 3 (E and F) shows such recordings obtained at two different film speeds. In this figure the onset of a burst of potentials in the ganglion is seen to precede the beginning of the depolarization of the muscle cell membrane. When fast recording speeds are used (Figure 3E) the onset of ganglionic activity occurs approximately 40 msec before the muscle cell begins to depolarize. This number was obtained for muscle cells about two millimeters away from the recording electrode on the cardiac ganglion. It is apparent from these recordings that cardiac ganglion activity precedes all other overt events in the heartbeat of E. marxi.

Effect of Ganglion Removal on the Heartbeat

Since the cardiac ganglion activity occurs first in the heartbeat, a pacemaker function can be ascribed to the ganglion. To further demonstrate the nervous origin of the heartbeat, the cardiac ganglion was carefully lifted free from the heart and the effect on the heart contractions noted. As the ganglion was removed, regions of the heart where the ganglion was still attached continued to beat, while those regions of the heart no longer innervated by the ganglion no longer contracted. When the ganglion had been completely removed, the heart stopped contracting.

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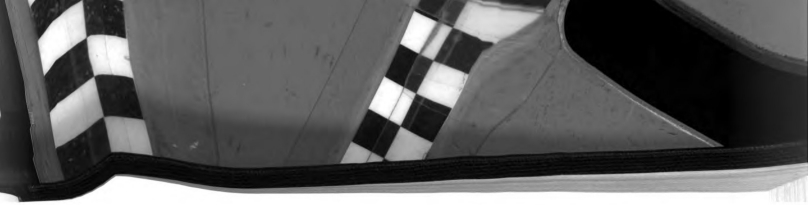
To eliminate the possibility that the heart stopped beating due to damage to the muscle cells during the ganglion removal procedure, a number of muscle cells were penetrated with microelectrodes after deganglionation to see if any decrease in the values of the membrane resting potentials had occurred. When this was done, resting potentials comparable to those of cells of intact hearts were obtained, indicating that the muscle cells had not been disrupted. At the same time no spontaneous active electrical responses were recorded from these muscle cells.

Although the electrical responses of the heart muscle cells were abolished by removal of the cardiac ganglion, spontaneous bursts of electrical activity could still be recorded from completely isolated cardiac ganglia. Thus the cardiac ganglion is the source of the endogenous rhythm of the heart.

Coordination of the Unit Contractions of the Heart

One of the initial observations made upon isolation of a heart is how well the various regions of the heart are coordinated to produce a simultaneous contraction of the entire myocardium. That the heart contracts as a unit is shown in Figure 4. In this figure the electrical responses of two different muscle cells are shown. One of the cells was located near the first ostial pair and the other near the fourth. As shown in A, the electrical responses of



these two cells occurred at the same time even though the cells were located at opposite ends of the heart. In B, recordings of the depolarizations of the muscle cells have been superimposed on one another to further demonstrate that each cell becomes depolarized simultaneously.

Such fine coordination of the muscle cell electrical responses to produce unit contractions of the heart could be achieved by extensive electrical coupling between adjacent muscle cells, as occurs in the mammalian heart (Woodbury et al., 1965) or by polyneuronal and multiterminal innervation of the myocardial cells by the cardiac ganglion, as appears to be the case for the Limulus polyphemus heart (Abbott et al., 1969).

If unit contractions of the heart occur because of electrical coupling of adjacent muscle cells, a transverse cut made through the myocardium should result in two sections of the heart which contract out of phase with each other. When such a cut was made, leaving the cardiac ganglion intact, but completely separating the myocardium into two parts, the two parts continued to beat in phase. This result is illustrated in Figure 4 (C and D). Here, microelectrode recordings from within two cells, one located in each of the two parts of the heart, are shown. Although the responses of one of the cells are considerably smaller than those of the other cell, both muscle cells become depolarized at the same time. This is perhaps more clearly seen in Figure 4D

Figure 4. Intracellular recordings of cardiac muscle cell electrical responses. See text for details.
Amplitude cal:
A and B: 4 mV/mm, both traces of both records
C and D: 2 mV/mm, both traces of both records
E and F: 1 mV/mm, upper trace of both records
4 mV/mm, lower trace of both records
Time cal: 160 msec/mm, all records

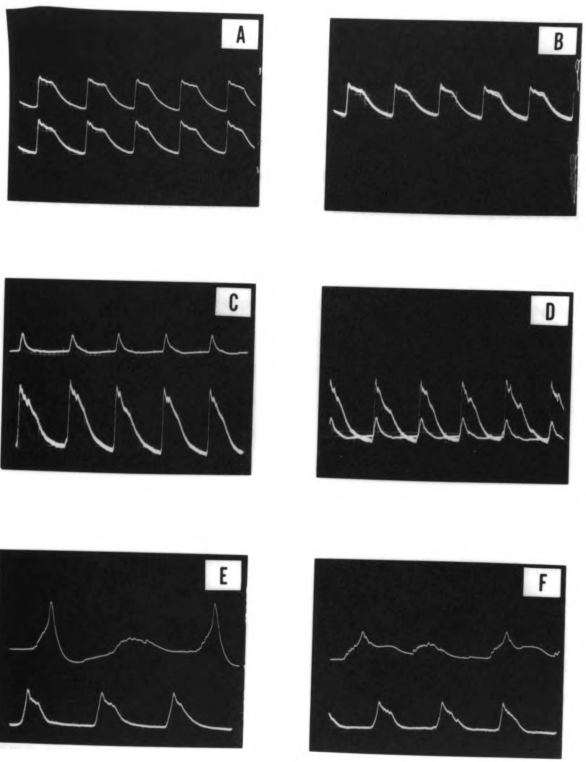


Figure 4

where an attempt was made to superimpose the responses of the two cells on one another. Therefore unit contractions of the heart are not dependent upon an intact myocardium.

In the above experiment the cardiac ganglion was left intact, bridging the gap made by the cut through the myocardium. When the transverse cut was extended through the ganglion, unit contractions of the two completely separated parts of the heart continued to occur, but the contractions of the two parts occurred out of phase with each other. This result is shown by the electrical recordings in Figure 4E and F. Here, the responses recorded from four different muscle cells are shown. The responses shown in the upper traces of these records were recorded from two different cells located in the posterior piece of the heart, while the responses in the lower traces of each record were recorded from two different cells in the anterior piece. After the cardiac ganglion was transected, the depolarizations of the cells in the posterior heart piece were no longer synchronized with the depolarizations of the cells in the anterior piece.

The active electrical responses shown in the upper traces of E and F of Figure 4 were recorded from a piece of heart which was only four millimeters in length (total heart length was 12 mm). The form of these responses is different from that normally recorded from intact hearts. In these responses, the large initial depolarization either does not

occur or else is displaced toward the end of the response. The altered electrical responses of these muscle cells are also characterized by numerous small peaks, each one perhaps representing individual excitatory postsynaptic potentials.

Although the unit contractions of the heart were not disrupted by cutting through the myocardium, the possibility that the myocardium functions in beat coordination, along with the cardiac ganglion, cannot be eliminated by the above experiments. To show that the myocardium alone cannot coordinate unit contractions of the heart, a transverse cut was made through the cardiac ganglion, while leaving the myocardium intact. After the cut, the myocardium on both sides of the cut continued to beat, but again out of phase with each other. Therefore unit contractions of the heart are achieved by the activity of the cardiac ganglion and not by the myocardium. This, however, does not eliminate the possibility of electrotonic connections between adjacent muscle cells of the heart.

Pharmacological Studies

There is very little information about the pharmacology of spider hearts, and about arachnid hearts in general. In spiders, only one pharmacological study has been made and in this study only two drugs were used.

A number of neurologically active drugs are known and some of them have a common physiological role in widely

differing animals. Some of these drugs were investigated here as to their effects of the heart of E. marxi. These studies were conducted with the aim of determining how the neuropharmacology of spider hearts compares to that of other neurogenic arthropod hearts.

Control Hearts

To establish a basis for making a comparison between the results of studies involving changes in heart rates, five hearts were isolated and allowed to beat in isolation for three hours while bathed only in saline. Throughout this three hour period both the rate and amplitude of the heartbeat were monitored by means of a mechanotransducer. Each recording period was begun approximately 15 minutes after the heart isolation procedure was completed.

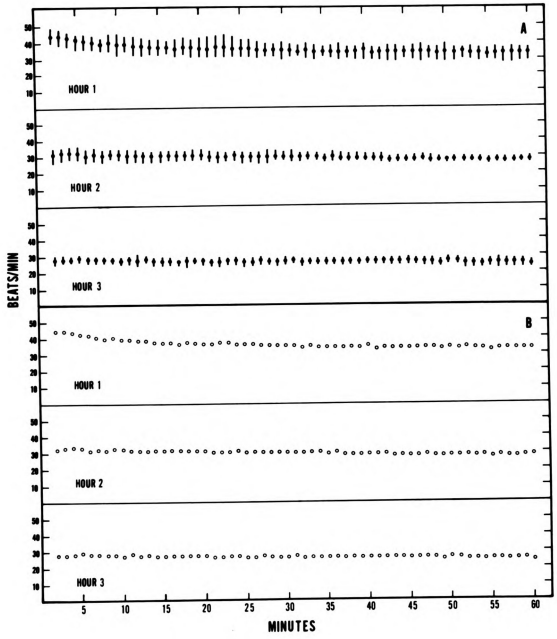
The heart rate for the five hearts was measured at minute intervals for the entire three hour recording period. Figure 5A shows the mean heart rate for the five hearts along with the range in rates seen. The mean heart rate initially seen was 45 beats/min, with a range in rates from 42 to 49 bests/min. The rate gradually declined during the recording period to a mean rate at the end of 24 beats/min.

Although there was some variability in the heart rate between isolated hearts, as evident by the range in rates, there was very little variability in rate in any one heart. The greatest change in heart rate ever seen from one minute to the next for any heart was plus or minus two beats/min.

Figure 5. Heart rate of isolated hearts.

A: mean heart rate for five hearts; vertical lines represent the range.

B: heart rate of one isolated heart.


A
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HOUR 1
HOUR 2
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BEATS/MIN
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35
40
45
50
55
60

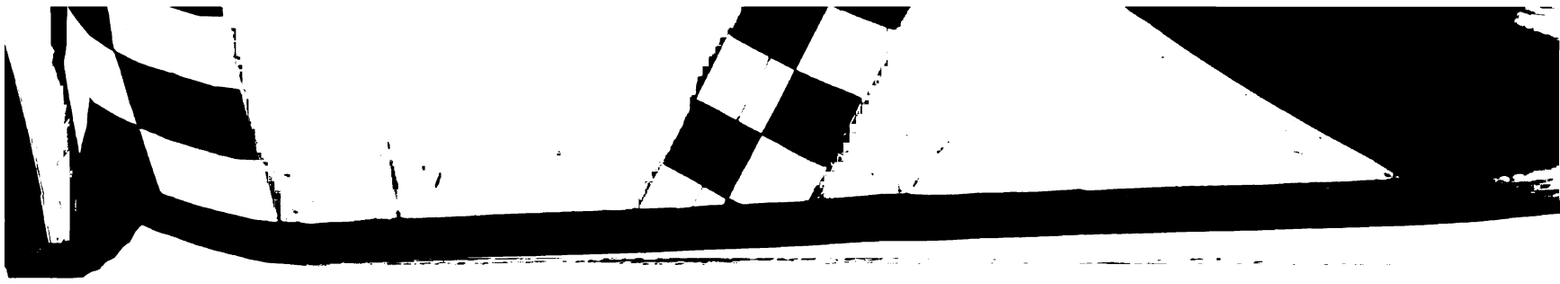
Figure 5

The small intra-animal variability in heart rate can be seen in Figure 5B where the heart rate of one of the control isolated hearts is shown.

Measurements of the amplitude of the heart contractions were also made. The amount of pull on the transducer is a function of the amount of tension placed on the heart. The tension placed on the heart was adjusted so that the amplitude of the heartbeat usually ranged from 40 to 60 mg. No attempt was made to determine the absolute amount of tension developed by the heart with each contraction and thus changes in the amplitude of the heartbeat are only relative ones.

To obtain a measure of the variation in the amplitude of the heartbeat, the amplitude of each of 10 consecutive heartbeats were measured at 10 minute intervals for each of the control hearts throughout the recording period. The tonus of the myocardium did not vary during the recording period but some variation in the amplitude of the heartbeat was seen. In general, the variation within a 10 beat interval was only plus or minus five milligrams, but changes as great as 20 mg were occasionally seen. The variation between the mean of one 10 beat interval and the next for any one heart was considerably less. In this case there usually was no difference in the mean amplitude and the greatest change ever observed was plus or minus 10 mg.

In order to determine what effect, if any, the periodic saline washes had on the contractions of the heart, heart



rates and heartbeat amplitudes were measured before and after each wash. Heart rates were measured for the minute just prior to and immediately after the completion of each wash. The mean change in the rate due to the saline washes for the five hearts was plus or minus one beat/min. The largest changes in rate ever seen due to a wash were minus two and plus three beats/min. Since the action of one drug (acetylcholine) on the heart rate was measured for 10 second intervals instead of whole minute intervals, the effect of the saline washes was also determined for intervals of 10 seconds. This was done by measuring the rate of beating for the 10 seconds just preceding and immediately following each wash. In this case, the mean change in rate was six beats/min, and the largest changes ever seen were minus six and plus twelve beats/min. Because of the short intervals measured, the precision of these measurements is only plus or minus six beats/min.

The effect of the saline washes on heartbeat amplitude was also determined. This was done by measuring the 10 beats that occurred just prior to the beginning of a wash and the 10 beats which occurred just immediately after completion of the wash. There was no change in the mean amplitude of the heartbeat due to the washes for the five hearts. The greatest changes seen due to a wash were minus 15 mg and plus 10 mg.

Cholinergic Compounds

Response of the Heart to Acetylcholine

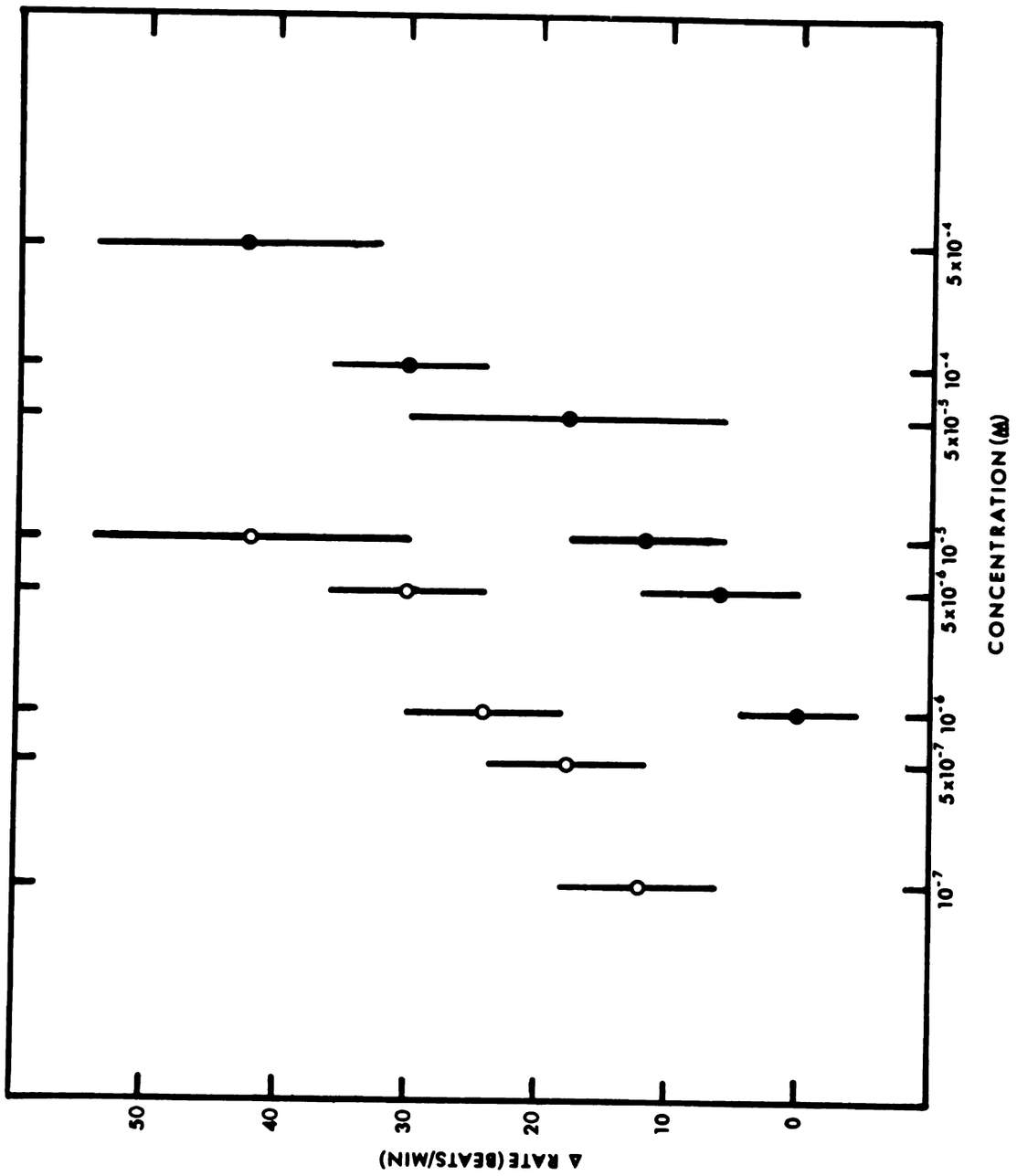
Acetylcholine (Ach) produces an increase in the heart rate of decaped crustaceans (Welsh, 1939a,b) and L. polyphemus (Garrey, 1942), but Ach is reported to be inhibitory on the heart of scorpions (Zwicky, 1968) and the spider Tegenaria atrica (Kadziela and Kokociński, 1965).

Ach, when applied to the heart of E. marxi, produces both an increase in the rate and amplitude of the heart contractions. Figure 6 (closed circles) shows the mean increase in heart rate produced by several different concentrations of Ach. From this figure it appears that concentrations as low as 5×10^{-6} M are effective in increasing heart rate.

The effect of Ach on the amplitude of the heartbeat is given in Table 2. Each value represents at least five replications made on at least five different hearts. At concentrations of 1×10^{-5} M and below, the response, if any, is reflected in an increased amplitude of the heartbeat. At concentrations of 5×10^{-5} M and higher, Ach produces an increase in the amplitude of the beat and an increase in myocardial tonus was well as sustained contractions in some cases.

Figure 7A and B shows the various effects of Ach on the heart. In A, 1×10^{-4} M was used and the immediate effect on the heart was an increased myocardial tonus and

Figure 6. Dose-response curve for the rate effect of Ach on the heart before and after eserine treatment. Each point represents the mean response to at least five replications made on at least five different hearts. Closed circles, before eserine; open circles, after eserine. Vertical lines represent one standard deviation.


Δ RATE (BEATS/MIN)
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30
20
10
0
CONCENTRATION (M)
10^{-7}
5×10^{-7}
10^{-6}
5×10^{-6}
10^{-5}
5×10^{-5}
10^{-4}
5×10^{-4}

Figure 6

Table 2. Effects of acetylcholine and eserine on heartbeat amplitude

Concentration (M)	Mean Heartbeat Amplitude Change In Mg (Range)	Mean Increase in Tonus In Mg (Range)	Number of Tone Increases	Mean Time in Tetany In Sec (Range)	Number Showing Tetany	Total N
			Acetylcholine			
1×10^{-6}	0 (-10 to 10)	0	0	0	0	5
5×10^{-6}	10 (-5 to 15)	0	0	0	0	6
1×10^{-5}	25 (10 to 55)	0	0	0	0	10
5×10^{-5}	40 (15 to 100)	20 (0 to 30)	4	0 (0 to 3)	2	10
1×10^{-4}	45 (20 to 85)	60 (20 to 105)	8	2 (0 to 5)	5	8
5×10^{-4}	70 (35 to 120)	120 (20 to 180)	5	4 (0 to 8)	4	5
			Eserine			
1×10^{-5}	40 (15 to 85)	0	0	0	0	8
			Acetylcholine After Eserine			
1×10^{-7}	15 (0 to 25)	0	0	0	0	7
5×10^{-7}	15 (5 to 25)	0 (0 to 10)	1	0	0	5
1×10^{-6}	25 (-10 to 50)	30 (10 to 70)	5	0	0	5
5×10^{-6}	20 (-15 to 45)	50 (10 to 120)	5	0 (0 to 1)	1	5
1×10^{-5}	35 (-10 to 80)	210 (50 to 340)	6	11 (2 to 30)	6	7

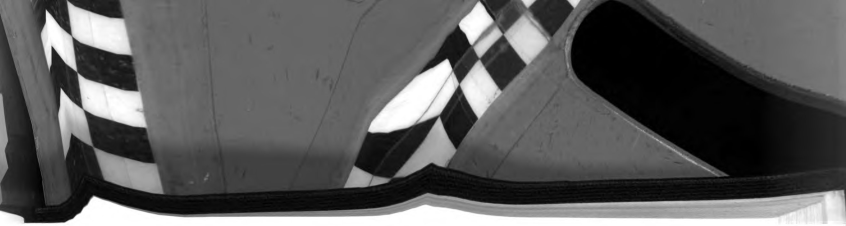


Figure 7. Effects of Ach and eserine on the heart.

A: saline removed at first arrow and 1×10^{-4} M Ach applied at second arrow.

B: same as A, except 1×10^{-5} M Ach applied at second arrow.

C: same as A, except 1×10^{-5} M eserine applied at second arrow.

D: eserine removed at first arrow after a five minute treatment and 1×10^{-5} M Ach applied at second arrow.

Amplitude cal: 10 mg/mm

Time mark: 1/sec.

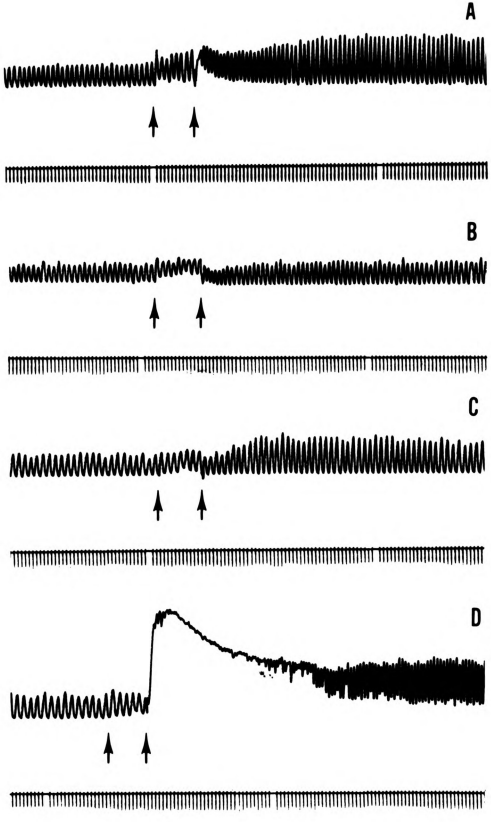


Figure 7

sustained contraction. After a few seconds the increased tonus and sustained contraction subsided, giving way to individual beats which occurred at an increased frequency. As the increased tonus declined, the amplitude of the heartbeat increased and soon after reached its maximum magnitude. The effect of Ach is immediate and reaches its maximum in the first few seconds after application. Figure 7B shows the lesser effects produced by 1×10^{-5} M Ach.

The Ach effect begins to diminish about 30 seconds after application, but an increased rate and beat amplitude are seen throughout the period that the drug is on the heart. In one experiment, 1×10^{-4} M Ach was left on the heart for 10 consecutive minutes. The rate in the first minute that the drug was on, was 60 beats/min. After 10 minutes, the rate had fallen to 48 beats/min, but this rate was still higher than the 33 beats/min seen before drug treatment. The increased amplitude of the beat seen at the beginning of the experiment was only slightly less at the end of the 10 minutes.

After the removal of Ach, the rate and amplitude of the contractions quickly return to control levels. The time for recovery is as brief as two minutes for low concentrations, but after higher doses are applied, this time may be as long as five minutes.

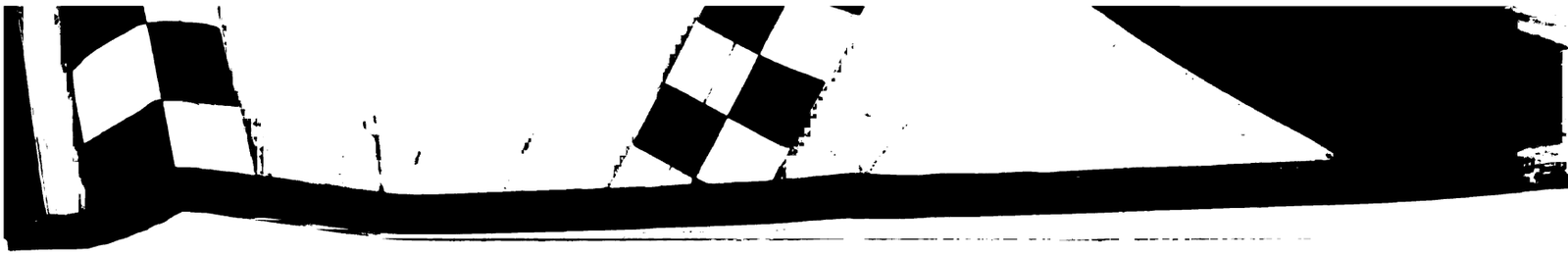
Although the response to Ach declines when a single prolonged treatment is made, there is no apparent change in the

sensitivity of the heart to Ach as a result of repeated treatments, when each exposure is separated by a period of saline washes. In one experiment, 5×10^{-5} M Ach was applied to the heart for three minutes followed by a 10 minute period of saline washes. This procedure was repeated seven times and the response to the drug measured. Of the seven treatments with Ach, there were four cases in which the rate increase was 24 beats/min, one in which the increase was 18 beats/min and two in which the rate increased 30 beats/min. The first and last treatments both gave increases of 24 beats/min and no trend was seen in the occurrence of the other responses.

Eserine and Ach

The presence of an acetylcholinesterase has been demonstrated for a number of systems in which Ach is believed to be a chemical mediator of nervous transmission (Florey, 1962). The physiological role of this enzyme is to terminate the action of Ach. Eserine is known to inhibit cholinesterase activity and thus potentiate the effects of Ach. To determine if such an enzyme might be present in the E. marxi heart, eight hearts were treated with 1×10^{-5} M eserine for five minutes prior to the administration of Ach.

Eserine alone produces an increase in the rate and amplitude of the heartbeat. The increase in heart rate during the first minute of eserine treatment averaged 11 beats/min (SD = four beats/min) for eight replications, one



on each of eight different hearts. The rate was elevated for the entire five minutes of treatment, but after the first minute it gradually declined. Eserine alone also produces an increase in the heartbeat amplitude by an average of 40 mg for the same eight hearts (range: 15 to 85 mg). Sustained contractions were never seen due to eserine alone and only slight changes in tonus were observed. The effect of eserine on one of the hearts is shown in Figure 7C.

The effect of the eserine treatment on the response of the heart to 1 x 10^{-5} M--the same as that shown in Figure 7B--is shown in Figure 7D. The potentiating effects of eserine on the response to Ach is readily apparent. After eserine treatment, the response to Ach only slowly declines upon removal of Ach, with the time required for recovery lasting as long as 15 minutes.

In Figure 6 (open circles), the rate changes produced by Ach treatment after eserine treatment are shown. Ach in concentrations of 5 x 10^{-6} M and 1 x 10^{-5} M gave rate increases three to four times those seen before treatment with eserine, and concentrations which gave no rate increases before eserine, such as 1 x 10^{-6} M, produced increased rates after eserine. Concentrations of Ach greater than 1 x 10^{-5} M were not included in this figure because they generally produced a prolonged tetany of the heart.

Eserine also potentiated the effects of Ach on the amplitude of the heart contractions, the magnitude of the

tonus increase and the length of the sustained contractions. This is shown in Table 2. Again, concentrations of Ach greater than 1×10^{-5} M were not used after eserine treatment because of the prolonged tetany produced by them.

The potentiating effect of eserine was not readily reversible. In every case, application of Ach in any concentration from 1×10^{-7} M to 1×10^{-5} M at any time for up to two hours after a five minute treatment with 1×10^{-5} M eserine resulted in a potentiation of the response to Ach.

Atropine and Ach

Atropine is a specific blocker of Ach activity in many systems and is known to block the effect of Ach on the decapod heart (Davenport, 1941) and on the cardiac neurons of a cockroach (Miller, 1968b). To see if atropine blocks the effect of Ach on the E. marxi heart, five isolated hearts were treated with 1×10^{-4} M atropine for five minutes and then with 1×10^{-4} M atropine plus 1×10^{-4} M Ach.

Atropine alone produced little change in rate during the first minute that it was on the heart, but by the fifth minute a mean decrease in rate of five beats/min was seen. Atropine also produced an increase in the heartbeat amplitude which ranged from 10 to 40 mg with a mean of 20 mg.

When Ach is applied with atropine, after an atropine treatment, the response to Ach is greatly diminished, although not completely abolished. The mean increase in rate to 1×10^{-4} M Ach before atropine was 24 beats/min, while after

atropine it was only six beats/min. Atropine also greatly diminished the effect of Ach on the amplitude of the contractions. Ach after atropine treatment produced no increase in heartbeat amplitude and in only one case was there an increase in myocardial tonus and this was only slight.

One example of the responses seen with atropine and Ach treatment is shown in Figure 8. In A, the heart was treated with 1×10^{-4} M Ach alone; in B, with 1×10^{-4} M atropine; in C, with 1×10^{-4} M Ach together with 1×10^{-4} M atropine.

The atropine effect appeared to be reversible, for after a prolonged saline wash of 30 minutes, responses to Ach approaching those observed before atropine treatment were obtained.

d-Tubocurarine and Ach

d-Tubocurarine (curare) also blocks the action of Ach on the decapod heart (Davenport, 1942), and its ability to alter the effect of Ach on the E. marxi heart was also investigated. For these experiments four isolated hearts were treated with 1×10^{-4} M curare for 30 minutes and then with 1×10^{-4} M Ach plus 1×10^{-4} M curare.

Curare alone produced an initial mean increase in the heart rate of four beats/min, but by the fourth minute the heart rate was slightly less than that seen before the drug was applied. After 15 minutes in curare, a mean decrease in rate of seven beats/min was seen and after 30 minutes, the rate had dropped an average of nine beats/min. Curare

Figure 8. Effects of Ach and atropine on the heart.

A: saline removed at first arrow and 1 x 10^{-4} M Ach applied at second arrow.

B: same as A, except 1 x 10^{-4} M atropine applied at second arrow.

C: atropine removed at first arrow after a five minute treatment and 1 x 10^{-4} M Ach plus 1 x 10^{-4} M atropine applied at second arrow.

Amplitude cal: 10 mg/mm

Time cal: 1/sec.

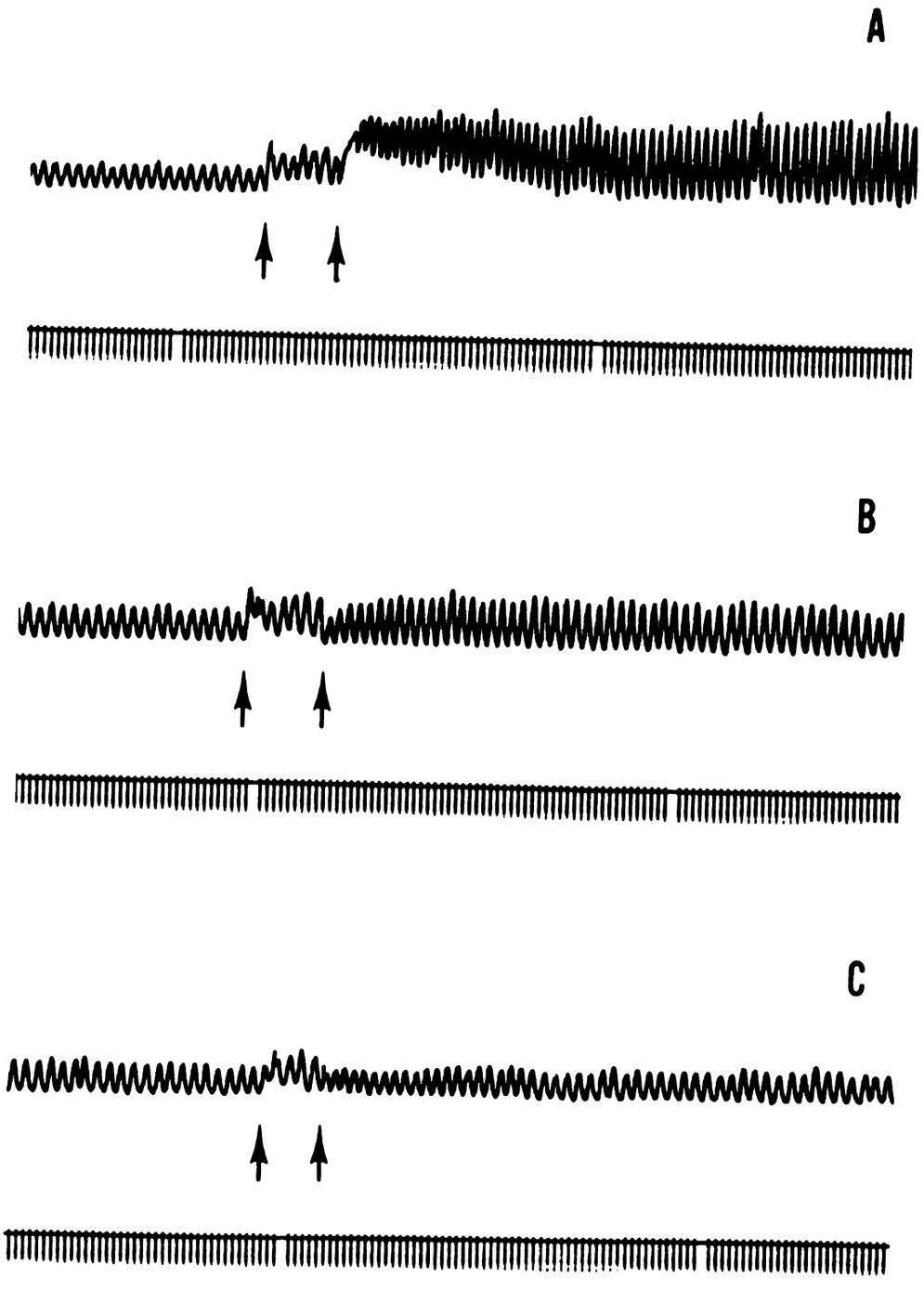


Figure 8

alone had little effect on the amplitude of the contractions; in two hearts a small decrease in beat amplitude occurred.

In two of the four hearts treated with curare the effect of Ach on heart rate was considerably diminished. Before curare, 1×10^{-4} M Ach caused a mean increase of 30 beats/min, while after curare the increase was only 12 beats/min. A third heart also gave a decreased response to Ach after curare; 18 beats/min after curare compared with 30 beats/min before. In the fourth heart, curare had no effect in altering the action of applied Ach. Figure 9 shows one example of the experiments done with curare and Ach.

Curare did produce a decrease in the amplitude response to Ach, but as for the rate response, curare did not abolish the amplitude effect of Ach altogether.

The action of curare on the Ach effect was reversible, for after washing the heart in saline for several minutes, responses to Ach occurred which were comparable to those seen before curare treatment.

Hexamethonium and Ach

Hexamethonium (C6) is another compound which acts to antagonize the effects of Ach. To determine if C6 blocks the action of applied Ach on the heart of E. marxi, three isolated hearts were treated with 5×10^{-4} M C6 for 15 minutes and then with 5×10^{-4} M Ach along with 5×10^{-4} M C6.

Figure 9. Effects of Ach and d-tubocurarine on the heart.

A: saline removed at first arrow and 1 x 10^{-4} M Ach applied at second arrow.

B: same as A, except 1 x 10^{-4} M d-tubocurarine applied at second arrow.

C: d-tubocurarine removed at first arrow after a 30 minute treatment and 1 x 10^{-4} M Ach plus 1 x 10^{-4} M d-tubocurarine applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

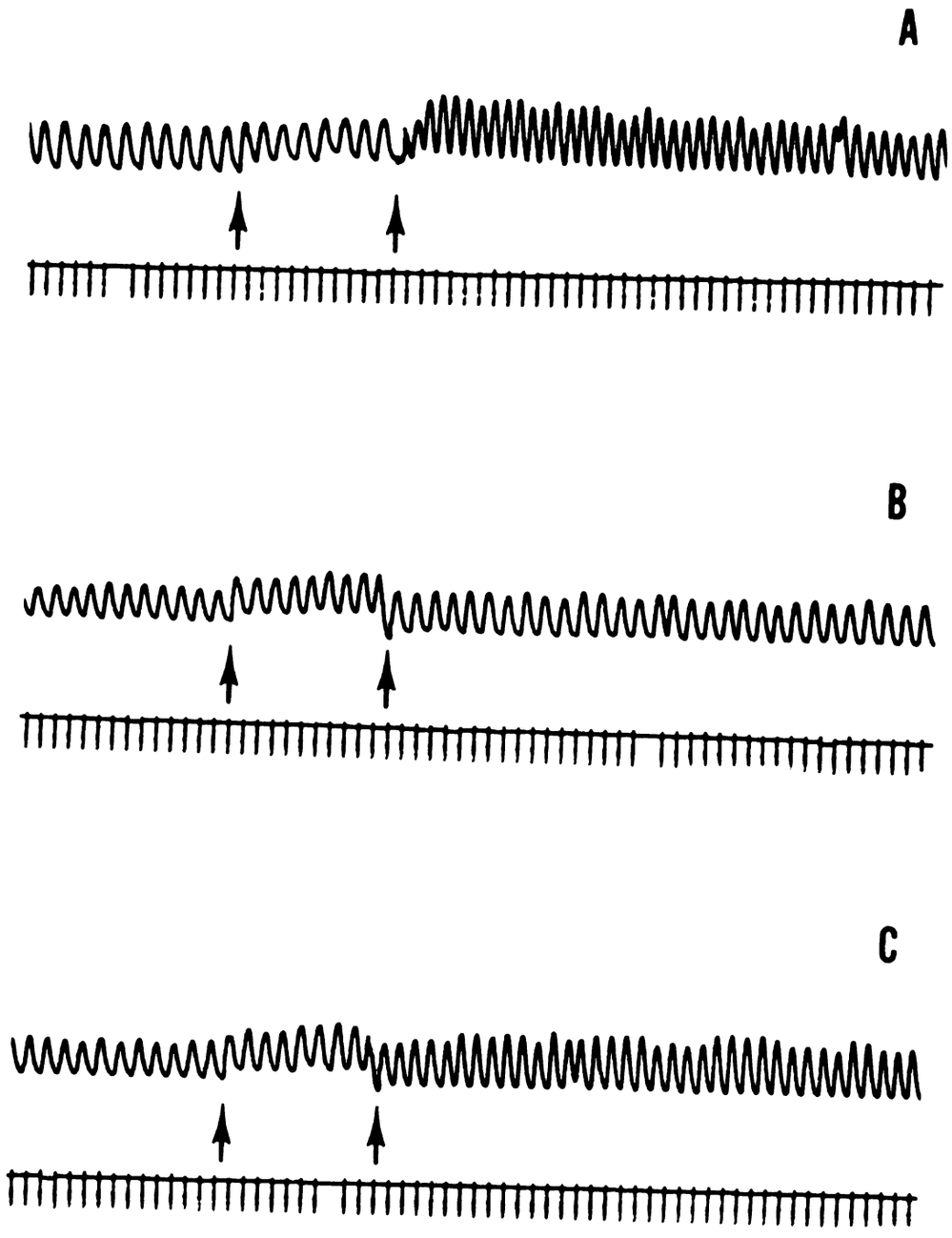


Figure 9

C6 alone produced little immediate effect on the heart, but in the fifteenth minute after its application the rate had decreased an average of eight beats/min. No apparent change in the amplitude of the contractions was seen.

The effectiveness of Ach in changing the heart rate was reduced by an average of 12 beats/min by treatment with C6, but there was no difference in the amplitude response to Ach after C6 treatment. One example of the responses obtained when a heart was treated with C6 and Ach is shown in Figure 10. In this example, a decrease in the rate response to Ach of 12 beats/min as well as a blockade of the initial transient tonus increase are evident due to treatment with C6.

Site of Action of Ach

There are two principle locations on the heart where changes in the rate and amplitude of the heartbeat can be brought about; the cardiac ganglion and the heart musculature. To determine if Ach has an effect on the myocardium, Ach was administered in a number of instances to hearts after their cardiac ganglia had been removed. In no case was a recordable change in myocardial tonus seen upon the application of Ach to deganglionated hearts. Thus the response of the intact heart to Ach appears to be due to the action of Ach on the cardiac ganglion.

To demonstrate more clearly that the site of Ach and eserine action is the cardiac ganglion, the electrical

Figure 10. Effects of Ach and hexamethonium on the heart.

A: saline removed at first arrow and 5×10^{-4} M Ach applied at second arrow.

B: same as A, except 5×10^{-4} M hexamethonium applied at second arrow.

C: hexamethonium removed at first arrow after a 15 minute treatment and 5×10^{-4} M Ach plus 5×10^{-4} M hexamethonium applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

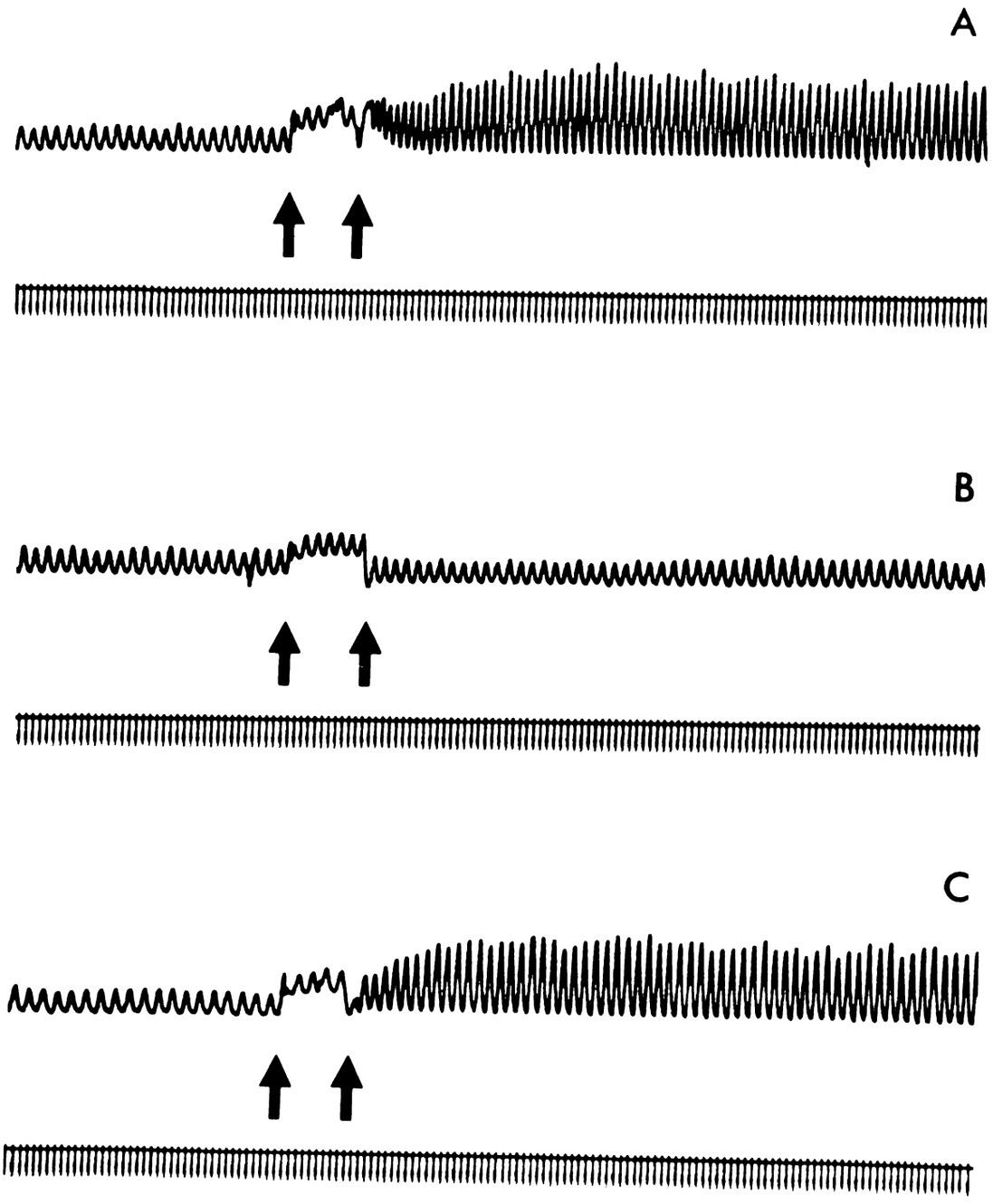


Figure 10

activity of the ganglion was monitored while Ach and eserine were applied to the heart. Figure 11 shows the effects of Ach and eserine treatment on the electrical activity of the cardiac ganglion. The potentiating action of eserine on the effect of Ach on the ganglion is clearly visible in Figure 11E. It is apparently the continuous firing of the ganglion seen here which produces the sustained contractions seen in the mechanical recordings of eserine and Ach treated hearts.

The responses of the cardiac ganglion to Ach and atropine treatment are shown in Figure 12. This figure was obtained from the same heart as was Figure 11, but after the heart had been washed in saline for 15 minutes. Figure 12A shows the normal burst pattern; B, the burst pattern during treatment with 1×10^{-5} M Ach. Comparison of this trace with trace B of Figure 11 clearly demonstrates that the previous eserine treatment was still effective. Figure 12C shows the burst pattern after a saline wash period of 10 minutes; D, the effect of 1×10^{-4} M atropine; and E, the effects of atropine and Ach given together. The ability of atropine to block Ach activity is clearly visible.

Muscarinic and Nicotinic Receptor Sites

Certain cholinergic neurons of the vertebrate nervous system have two types of receptor sites for Ach (Koelle, 1965b). These two types of receptors are distinguished on the basis of their responses to certain cholinomimetic

Figure 11. Effects of Ach and eserine on cardiac ganglion electrical activity.

A: in saline.

B: in 1×10^{-5} M Ach.

C: in saline after removal of Ach.

D: in 1×10^{-5} M eserine.

E: in 1×10^{-5} M Ach after five minute eserine treatment.

F: in saline just after Ach removed.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/min.

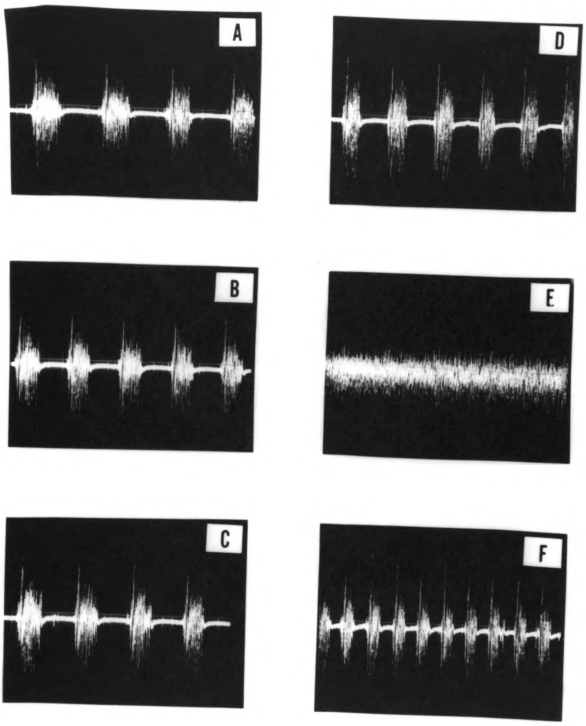


Figure 11

Figure 12. Effects of Ach and atropine on cardiac ganglion electrical activity. Records obtained from same heart as in Figure 11.

A: in saline.

B: in 1 x 10^{-5} M Ach; large response to Ach due to previous eserine treatment (Figure 11D).

C: in saline, 10 minutes after removal of Ach and addition of saline.

D: in 1 x 10^{-4} M atropine.

E: in 1 x 10^{-5} M Ach plus 1 x 10^{-4} M atropine, after five minute atropine treatment.

F: after Ach and atropine removed and saline added.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

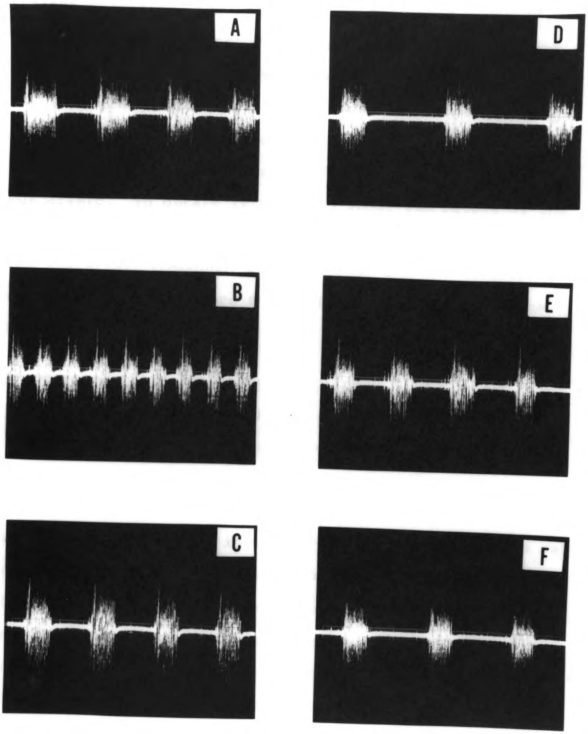


Figure 12

substances. One type of receptor is activated by nicotine, but not muscarine, and is called the nicotinic receptor. The other type of receptor responds to muscarine, but not nicotine, and is called the muscarinic receptor.

Certain cholinomimetic substances may selectively stimulate either one type of receptor or the other and on this basis they have been classified as either nicotinic or muscarinic. To obtain information about the nature of the Ach receptor sites present in the E. marxi cardiac ganglion, the muscarinic compound, methacholine, and the nicotinic compound, nicotine, were tested for their effects on the heart.

Response of the Heart to Methacholine

One example of the effects of two different concentrations of methacholine on an isolated heart is shown in Figure 13 (A and B). Immediately upon application there is an increase in the rate and amplitude of the contractions as well as an increase in tonus. While the increase in rate and beat amplitude occurs throughout the time that the drug is on the heart, the increased tonus is only transient in nature. Upon removal of methacholine, the heart quickly recovers from the drug effects (generally within five minutes). The rate response of the heart to several different concentrations of methacholine is shown in Figure 14. Although concentrations as low as 1×10^{-6} M produce some increase in rate, even at high concentrations rate increases in excess

Figure 13. Effects of methacholine and atropine on the heart.

A: saline removed at first arrow and 1×10^{-4} M methacholine applied at second arrow.

B: same as A, except 5×10^{-5} M methacholine applied at second arrow.

C: same as A, except 1×10^{-4} M atropine applied at second arrow.

D: atropine removed after a five minute treatment at first arrow and 5×10^{-5} M methacholine plus 1×10^{-4} M atropine applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

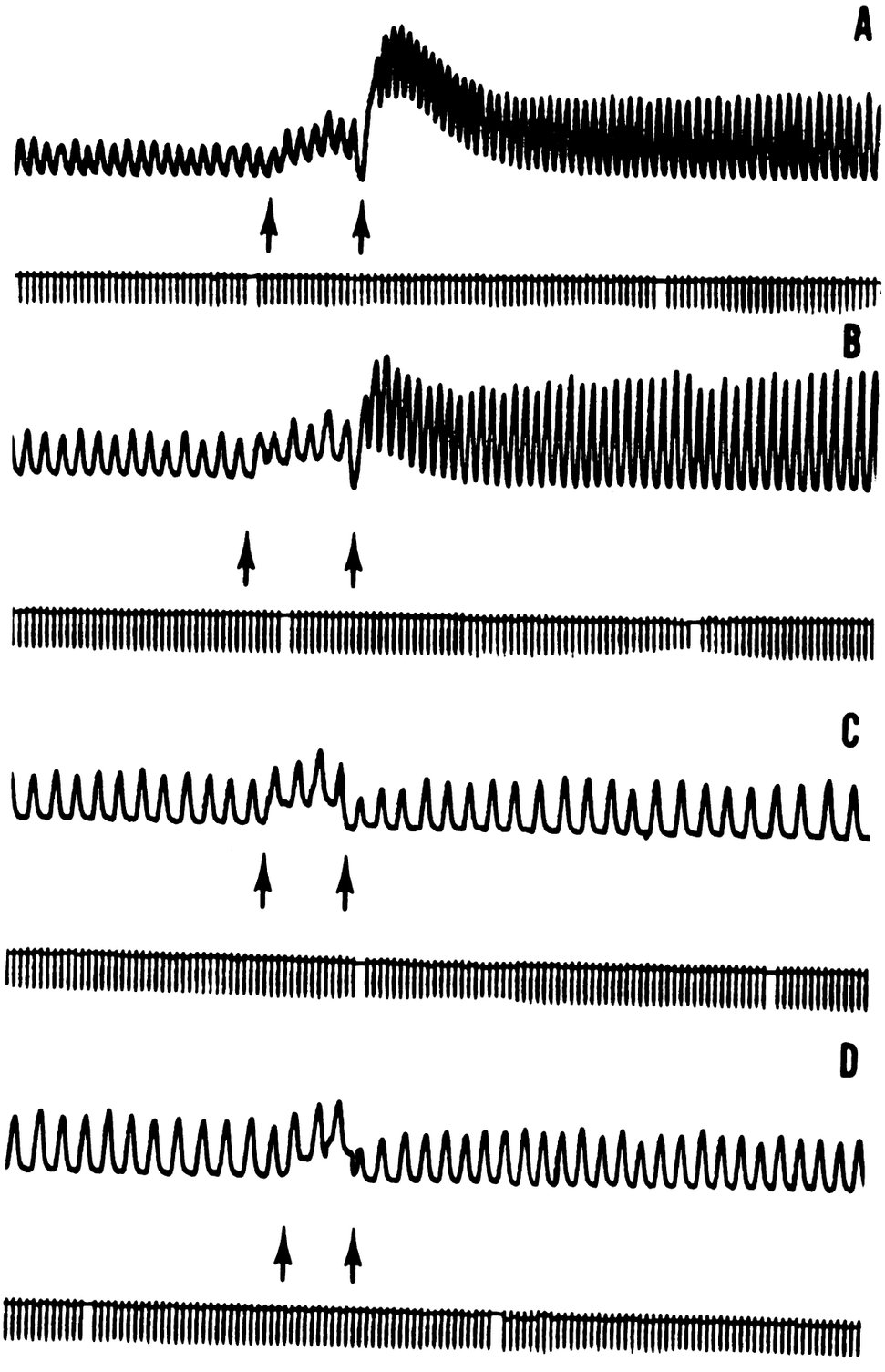


Figure 13

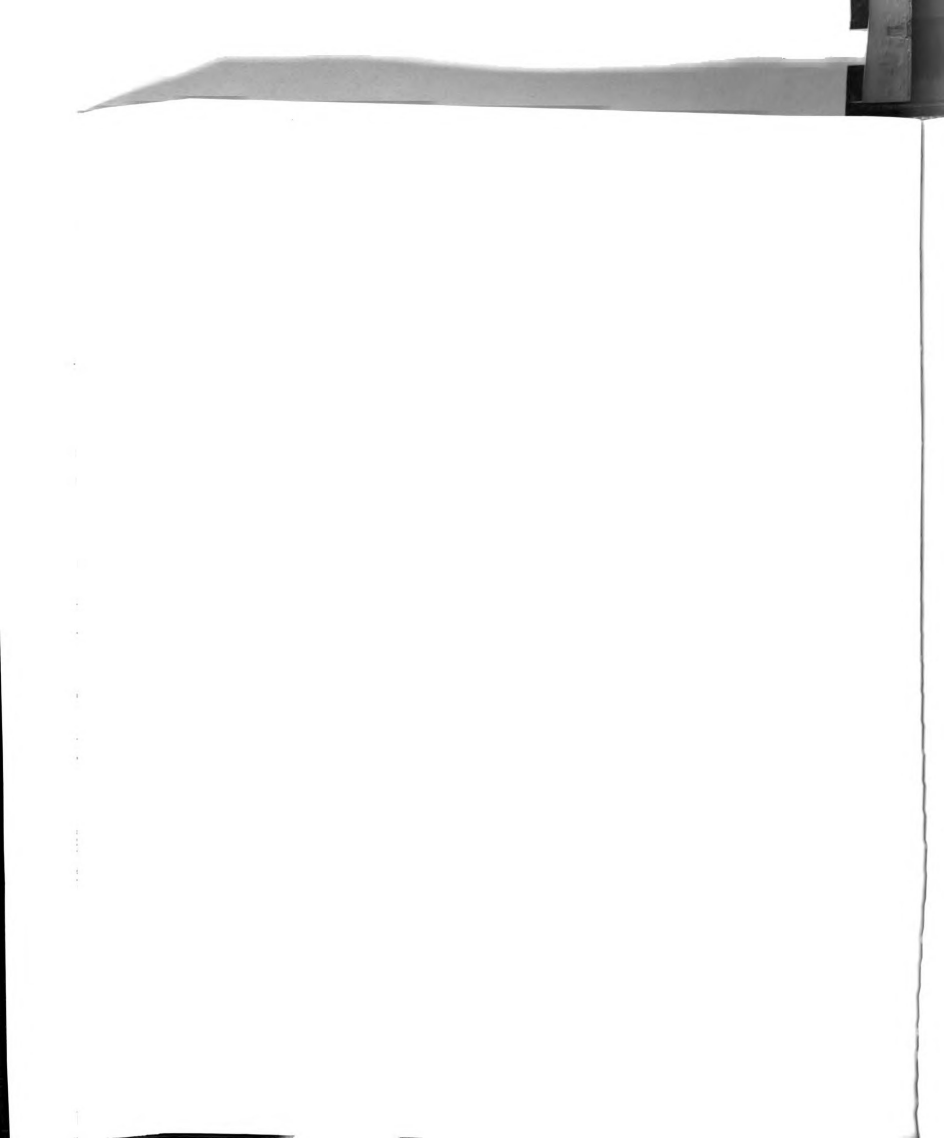


Figure 14. Dose-response curve for the rate effect of methacholine on the heart. Each point represents the mean response to at least six replications made on at least three different hearts. Vertical lines represent one standard deviation.

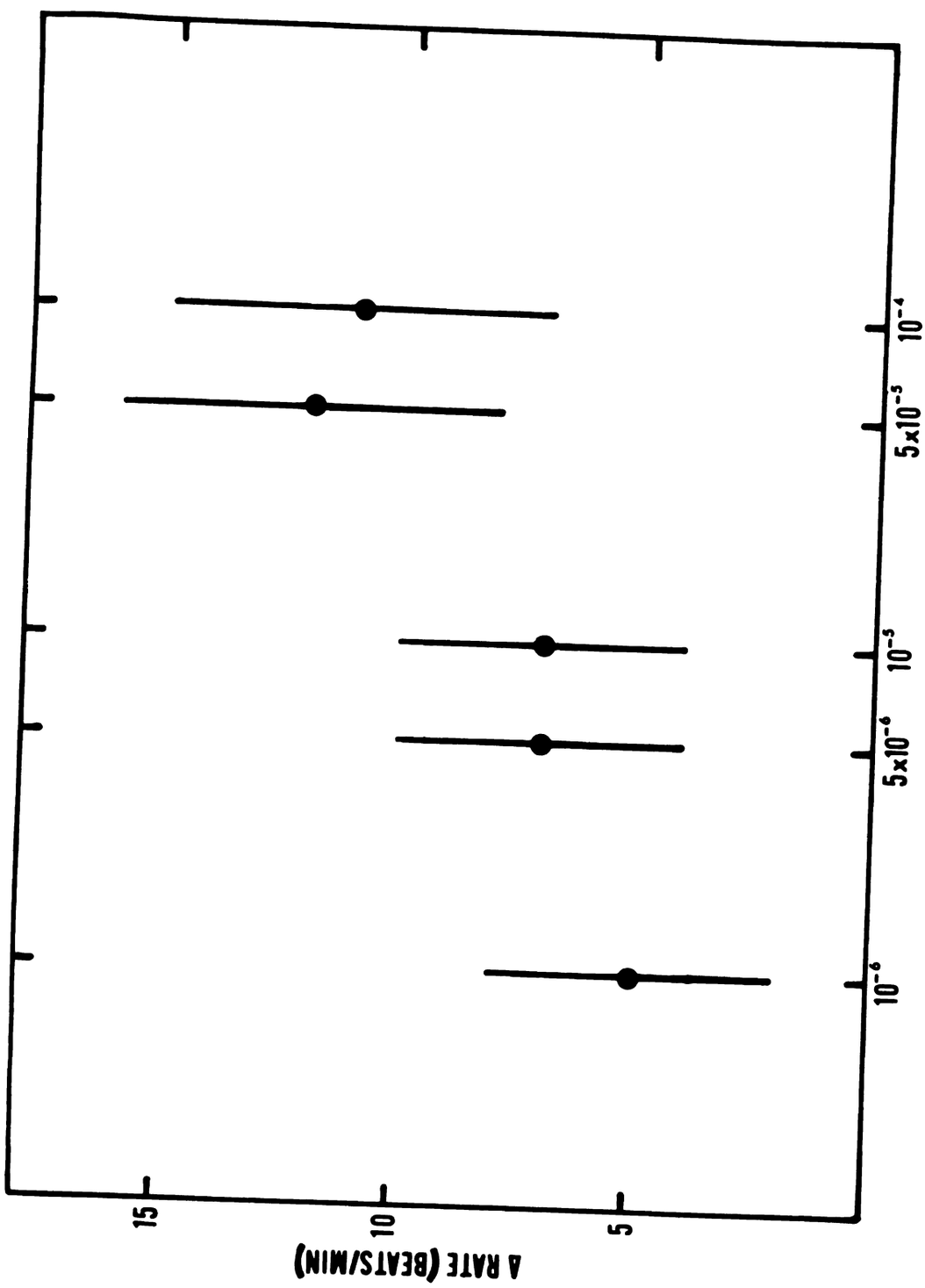
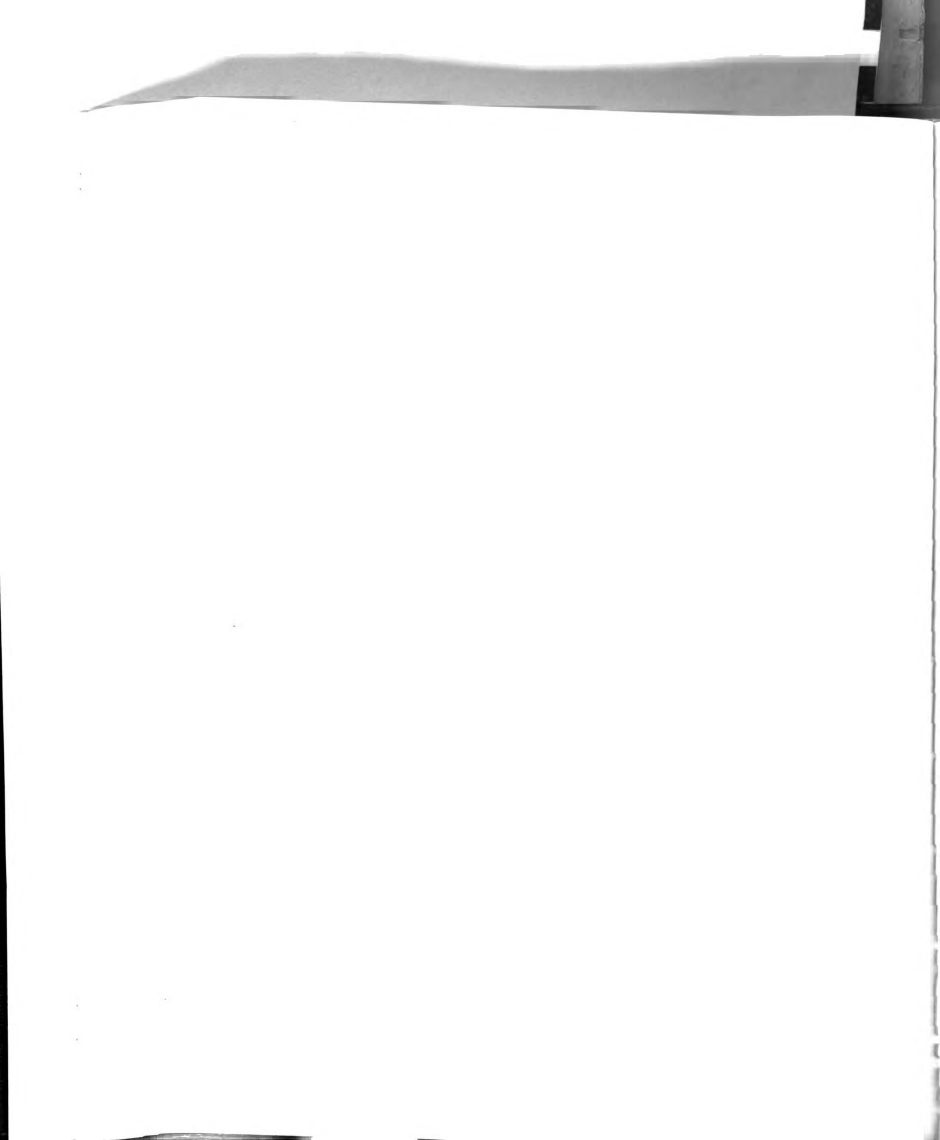

Δ RATE (BEATS/MIN)
15
10
5
CONCENTRATION (M)
10^{-6}
5×10^{-6}
10^{-5}
5×10^{-5}
10^{-4}

Figure 14



of 15 beats/min are seldom seen.

The effect of methacholine on the amplitude of the heart contractions is shown on Table 3. Each value represents at least six replications made on at least three different hearts. At each concentration of methacholine used there was an increase in the heartbeat amplitude as well as an increase in myocardial tonus, but only one sustained contraction was ever seen and it lasted only a few seconds.

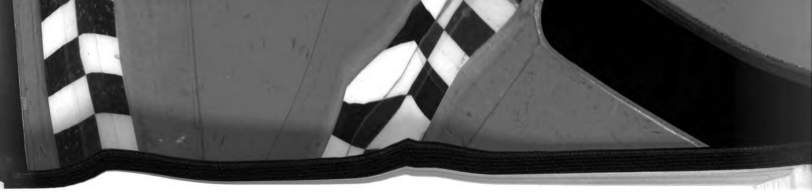
From these results it appears that although methacholine produces an increase in the heart rate, it is much more effective in increasing the amplitude of the heart contractions.

Eserine and Methacholine

Methacholine, like Ach, is hydrolyzed by acetylcholinesterase, but at a much reduced rate (koelle, 1965a). To see if eserine also potentiates the effect of methacholine on the E. marxi heart, one experiment was conducted in which a heart was treated with 1×10^{-5} M eserine for 10 minutes prior to the application of 5×10^{-5} M methacholine. In this experiment there was only a slight increase in response to methacholine due to the eserine treatment. The increase in response to methacholine before eserine treatment was 13 beats/min, while after eserine it was 15 beats/min. No apparent increase in the amplitude response to methacholine was seen after treatment with eserine.

Table 3. Effects of methacholine on heartbeat amplitude

Concentration (M)	Mean Heartbeat Amplitude Change In Mg (Range)	Mean Increase In Tonus In Mg (Range)	Number of Tone Increases	Mean Time In Tetany In Sec (Range)	Number Showing Tetany	Total N
1×10^{-6}	35 (15 to 65)	5 (0 to 10)	4	0	0	6
5×10^{-6}	40 (5 to 105)	10 (0 to 40)	4	0	0	8
1×10^{-5}	40 (15 to 90)	20 (0 to 50)	4	0	0	8
5×10^{-5}	40 (15 to 90)	20 (0 to 60)	7	0	0	8
1×10^{-4}	50 (25 to 75)	45 (10 to 110)	8	1 (0 to 5)	1	8



Atropine and Methacholine

Certain Ach blocking agents are known to selectively block either nicotinic or muscarinic receptor sites. Atropine is a muscarinic blocking agent, since it blocks the action of the muscarinic type of cholinomimetics (Koelle, 1965b).

To determine if atropine blocks the effect of applied methacholine, four isolated hearts were treated with 1×10^{-4} M atropine for five minutes and then with 1×10^{-4} M atropine plus 5×10^{-4} M methacholine. The mean increase in rate produced by methacholine before atropine treatment was 12 beats/min; after atropine the increase was only one beat/min. The increase in heartbeat amplitude produced by methacholine before atropine did not occur after atropine treatment. Thus atropine effectively blocks the action of applied methacholine. These results are shown for one of the hearts in Figure 13C and D.

Hexamethonium and Methacholine

Hexamethonium is a nicotinic blocking agent, since it blocks the action of the nicotinic type of cholinomimetics (Koelle, 1965b). To see if hexamethonium alters the response of the E. marxi heart to methacholine, four isolated hearts were treated with 1×10^{-4} M hexamethonium for 15 minutes and then with 1×10^{-4} M hexamethonium plus 5×10^{-5} M methacholine. Before hexamethonium the mean rate increase due to methacholine was 14 beats/min. After hexamethonium,

it was 16 beats/min. No effect of hexamethonium was seen on the increased amplitude of the contractions evoked by methacholine. Thus hexamethonium does not block the action of methacholine on the heart. An example of the records obtained from experiments of this kind is shown in Figure 15.

Site of Action of Methacholine

Methacholine, like Ach, did not produce any recordable change in myocardial tonus when applied to deganglionated hearts. The effect of methacholine on the electrical activity of the cardiac ganglion is shown in Figure 16. Both an increase in the rate of bursting and an increase in the amount of activity in each burst are seen upon treatment of the heart with methacholine.

Response of the Heart to Nicotine

Nicotine is the classical "type" substance for the nicotinic cholinomimetic compounds and it is known to produce marked increases in the heart rate of decapod crustaceans (Davenport, 1941) and L. polyphemus (Carlson, 1906).

Figure 17A shows the effect of 1×10^{-5} M nicotine on an isolated E. marxi heart. Nicotine causes an immediate increase in the heart rate; in this case, 27 beats/min. Along with the increase in rate, a transient increase in the amplitude of the contractions may occur, but this is not always seen. In fact, a decrease in the amplitude of the heartbeat is sometimes observed. From these observations it

Figure 15. Effects of methacholine and hexamethonium on the heart.

A: saline removed at first arrow and 5×10^{-5} M methacholine applied at the second arrow.

B: same as A, except 1×10^{-4} M hexamethonium applied at second arrow.

C: hexamethonium removed after a 15 minute treatment at first arrow, and 5×10^{-5} M methacholine and 1×10^{-4} M hexamethonium applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

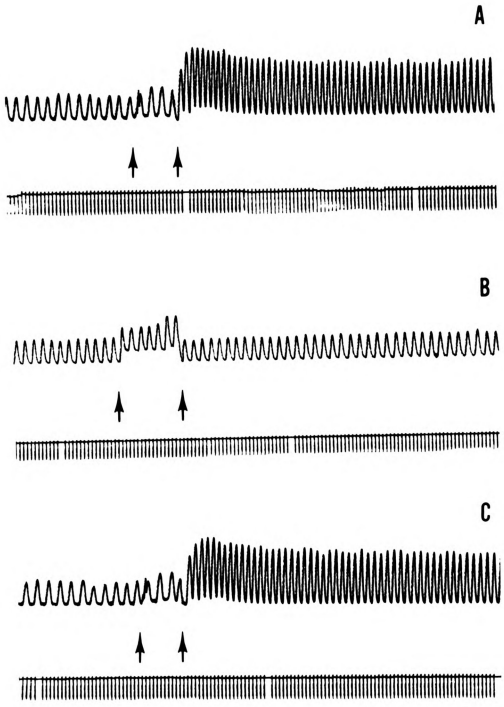


Figure 15

Figure 16. Effect of methacholine on cardiac ganglion electrical activity.

A: in saline.

B: 10 seconds after application of 5×10^{-5} M methacholine.

C: same as B, except one minute later.

D: immediately after methacholine removed and saline added.

E: same as D, except one minute later.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

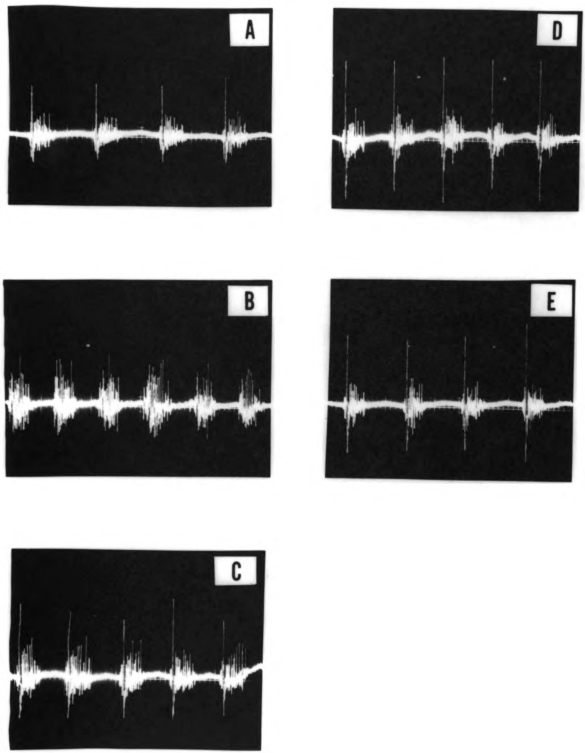


Figure 16

Figure 17. Effects of nicotine and hexamethonium on the heart.

A: saline removed at first arrow and 1 x 10^{-5} M nicotine applied at second arrow.

B: same as A, except 1 x 10^{-4} M hexamethonium applied at second arrow.

C: hexamethonium removed after a 15 minute treatment at first arrow and 1 x 10^{-5} M nicotine plus 1 x 10^{-4} M hexamethonium applied at second arrow.

D: same as A, except 5 x 10^{-5} M nicotine applied at second arrow (different heart).

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

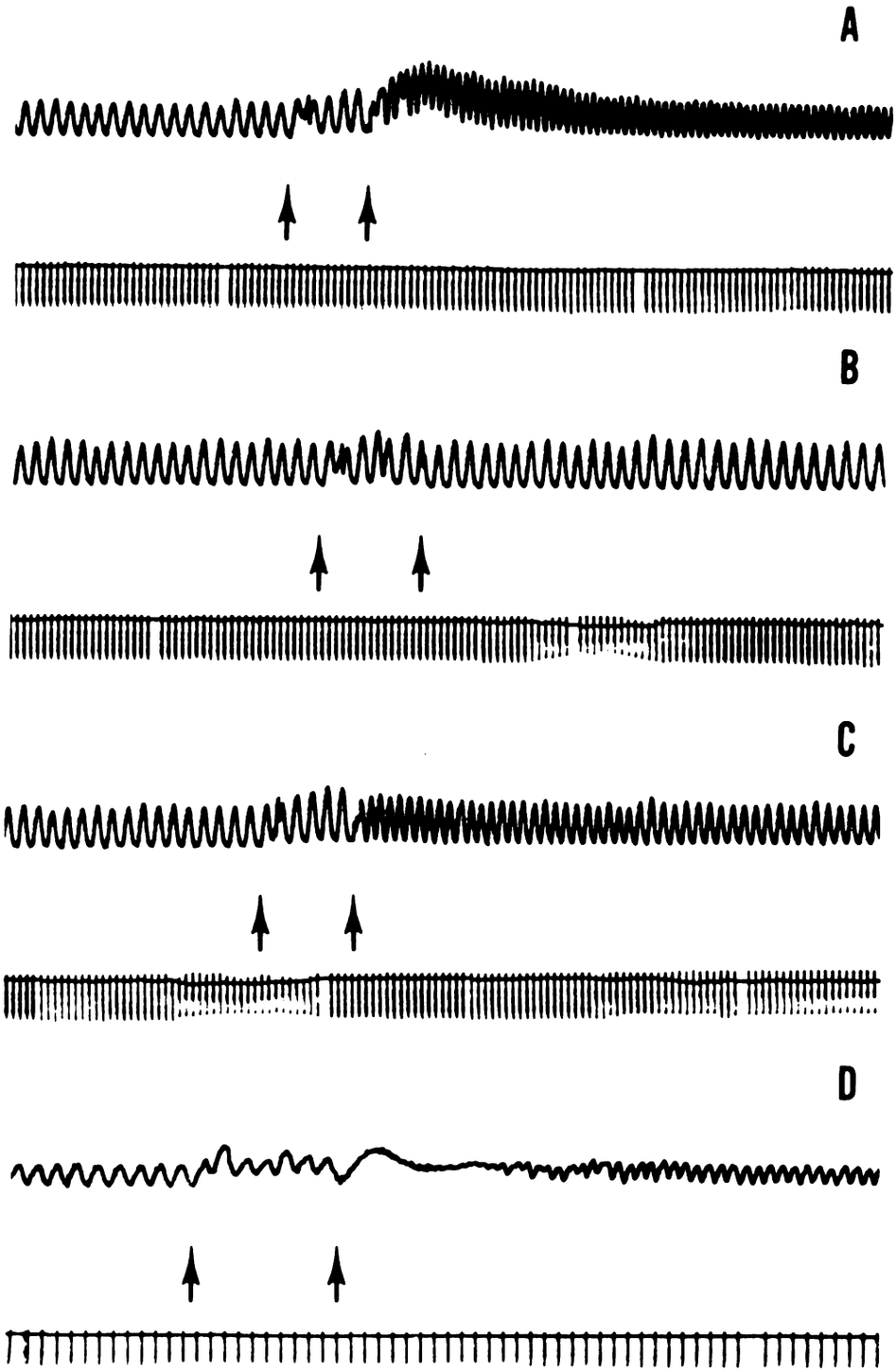


Figure 17

seems that the principal effect of nicotine is an increase in the heart rate.

The increase in rate due to nicotine begins to decline soon after its application to the heart, and when low doses are applied, the rate often returns to control value even though nicotine is still on the heart. These doses range from 5×10^{-7} M to 10^{-6} M. At concentrations higher than 1×10^{-5} M, some hearts respond with sustained contractions which last for up to one minute, followed by very weak beats that are generally irregular in occurrence. The effect of 5×10^{-5} M nicotine on one heart is shown in Figure 17D.

The observation that the response to nicotine begins to decline even though the drug was still on the heart suggests that the heart becomes desensitized to nicotine upon prolonged exposures. Support for this observation was obtained from experiments in which 1×10^{-5} M nicotine was applied to two different hearts, nine different times, for a period of three minutes each. Between the three minute treatments, each heart was washed in several changes of saline over a period of 10 minutes. The initial response to nicotine for these hearts was a mean increase in heart rate of 15 beats/min with little change in the amplitude of the contractions. With each successive application there was a progressive decline in the response to nicotine and when the last nicotine treatment was made, a mean increase of only four beats/min was seen.

Because of the apparent desensitization of the heart to nicotine and because at concentrations higher than 1×10^{-5} M nicotine often produced irregular beating, no attempt was made to obtain a dose-response curve for this chemical.

Hexamethonium and Nicotine

Hexamethonium blocks the action of nicotine on the cardiac neurons of a cockroach (Miller, 1968b). The ability of hexamethonium to alter the effect of nicotine on the E. marxi heart was investigated by treating three isolated hearts with 1×10^{-4} M hexamethonium for 15 minutes and then with 1×10^{-4} M hexamethonium plus 1×10^{-5} M nicotine. The mean rate increase with nicotine treatment before hexamethonium treatment was 19 beats/min, while after hexamethonium the mean rate increase was eight beats/min. The increase in tonus due to nicotine was also reduced by hexamethonium. The partial blocking action of hexamethonium on the nicotine effect is shown in Figure 17B and C. The blocking action of hexamethonium was reversible, since with several minutes of saline washes, responses to nicotine comparable to those originally seen were obtained.

Atropine and Nicotine

Atropine effectively blocks the action of muscarinic, but not nicotinic cholinomimetics, when both nicotinic and muscarinic receptor sites are present (Koelle, 1965b).

To determine if atropine prevents the response of the E. marxi to nicotine, five hearts were treated with 1×10^{-4} M atropine for five minutes and then with 1×10^{-4} M atropine plus either 5×10^{-6} M or 1×10^{-5} M nicotine.

In three of the five hearts there was little difference in the response to nicotine before and after atropine treatment. The results obtained for one of these hearts is shown in Figure 18A and B. Here, the increase to nicotine was nine beats/min before atropine and eight beats/min afterwards, but the increased tonus seen before atropine did not occur after atropine. This could possibly be due to desensitization of the heart to nicotine. In the other two hearts treated with atropine the mean increase in rate to nicotine before atropine was 15 beats/min, while after atropine treatment the mean increase was seven beats/min. The results obtained for one of these hearts is shown in Figure 18C and D. Although there appears to be a partial blocking of the nicotine effects by atropine in these two cases, it is possible that a greater than usual desensitization of the heart to nicotine may have occurred in these two hearts.

Site of Action of Nicotine

Nicotine did not produce any recordable change in myocardial tonus when applied to deganglionated hearts. The effect of nicotine on the electrical activity of the cardiac ganglion is shown in Figure 19. After addition of 5×10^{-5} M

Figure 18. Effects of nicotine and atropine on the heart.

A: saline removed at first arrow and 5×10^{-6} M nicotine applied at second arrow.

B: atropine removed after a five minute treatment at first arrow and 5×10^{-6} M nicotine plus 1×10^{-4} M atropine applied at second arrow.

C and D: same as A and B respectively, except different heart.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

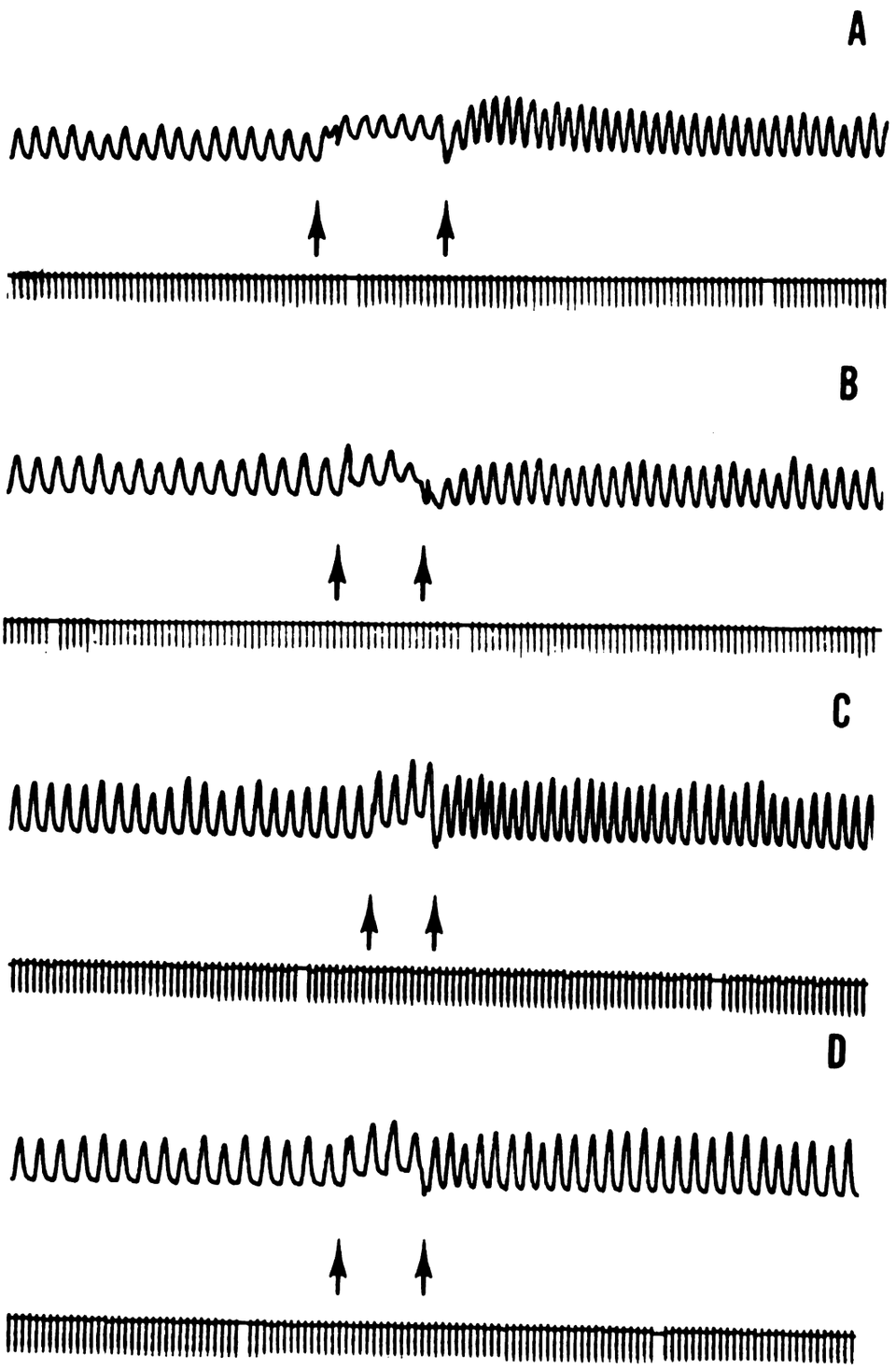


Figure 18

Figure 19. Effect of nicotine on cardiac ganglion electrical activity.

A: in saline.

B: in 5×10^{-5} M nicotine.

C: same as B, except one minute later.

D: in saline just after removal of nicotine.

E: same as D, except one minute later.

F: after fifth treatment with 5×10^{-5} M nicotine, each treatment separated by 10 minute saline wash.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

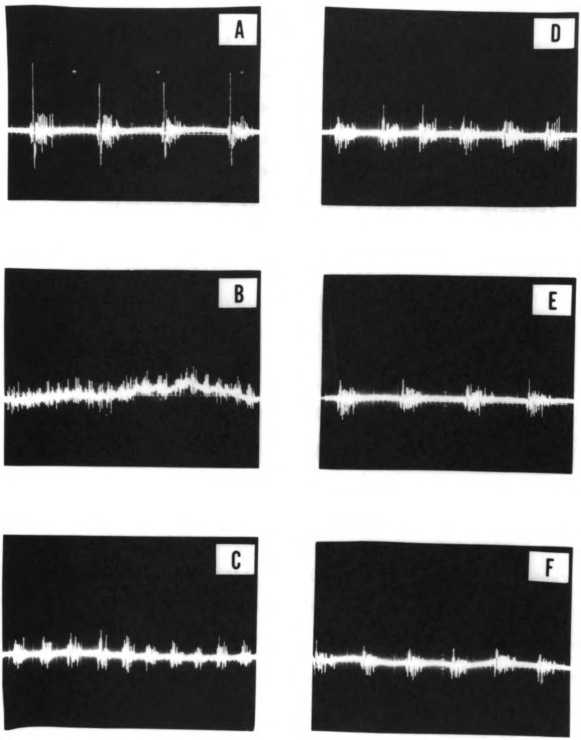


Figure 19

nicotine to the heart, small and irregular bursts of impulses occurred at an increased frequency. This effect is shown in Figure 19B. After 10 minutes of saline washes, the rate of bursting had returned to control levels, but the amplitude of the bursts was still reduced, as shown in E. Record F was obtained after the heart had been treated with four different applications of 5×10^{-5} M nicotine, each one separated by a saline wash period of 10 minutes. Upon the fifth nicotine application, as shown in F, the response of the ganglion was considerably less than that seen initially.

Nicotine and Methacholine

The principal effects of nicotine and methacholine on the E. marxi heart are quite different. The action of nicotine is characterized by a pronounced increase in the heart rate, but not an increase in the amplitude of the contractions. Methacholine characteristically produces a large increase in the amplitude of the contractions, but only a small increase in the heart rate. To determine if the individual effects of these two cholinomimetics sum when both are applied to the heart at the same time, the following experiment was performed. A heart was treated first with nicotine, then methacholine and then both of them simultaneously. Between each of the treatments the heart was washed in saline for 10 minutes. Figure 20 shows the results of this experiment. Nicotine alone in a concentration of 5×10^{-6} M

Figure 20. Effects of nicotine, methacholine and atropine on the heart.

A: saline removed at first arrow and 5×10^{-6} M nicotine applied at second arrow.

B: same as A, except 5×10^{-5} M methacholine applied at second arrow.

C: same as A, except 5×10^{-6} M nicotine plus 5×10^{-5} M methacholine applied at second arrow.

D: atropine removed after a five minute treatment at first arrow and 5×10^{-6} M nicotine, 5×10^{-5} M methacholine and 1×10^{-4} M atropine were applied simultaneously at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

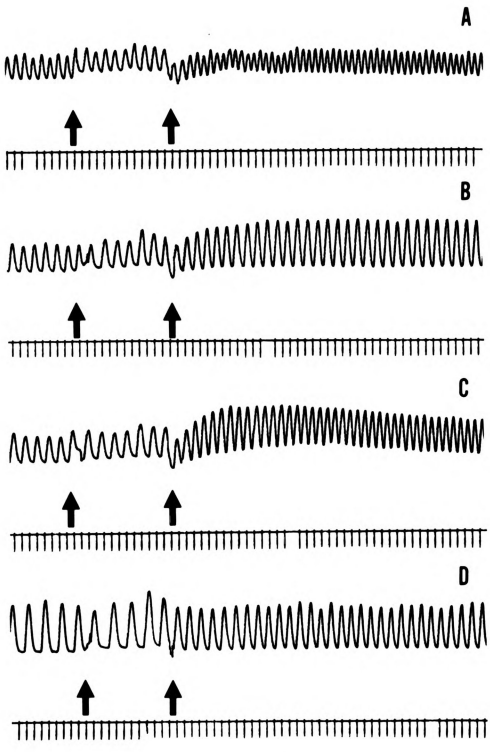
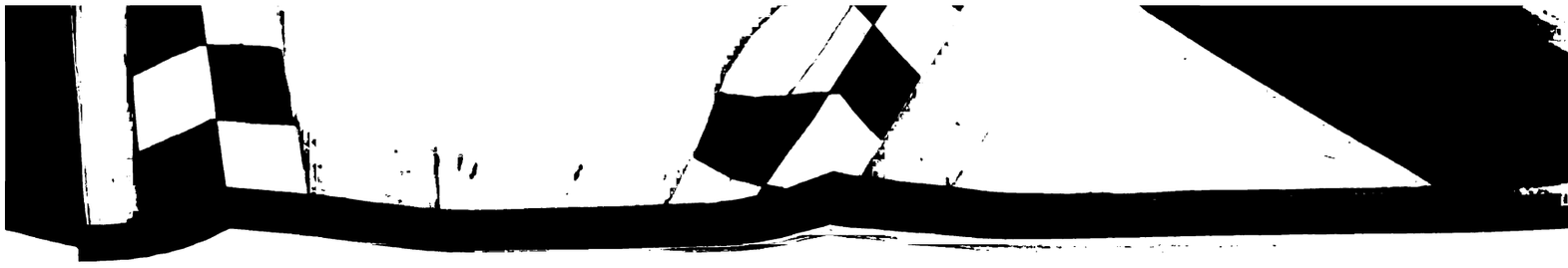


Figure 20

produced an increase in the rate of 20 beats/min. Little change in the amplitude of the beat occurred, but there was a small transient increase in tonus (Figure 20A). Methacholine alone, at 5×10^{-5} M, produced a six beat/min increase in the rate and a pronounced increase in the amplitude of the beat (about 50 mg). A slight increase in tonus was also seen (Figure 20B). When both compounds were applied together, both a large increase in rate (21 beats/min) and in beat amplitude (40 mg) were seen. In addition, an increase in the tonus of the heart over that produced by either drug alone occurred (Figure 20C). Although the absolute rate increase after treatment with both of the drugs together was somewhat less than the sum of each of them alone (21 in comparison to 26 beats/min), the rate increase was greater than three times that seen for methacholine alone. Furthermore, a rate increase less than 26 beats/min might be expected, since as already shown, the response to nicotine becomes progressively smaller with repeated treatments with this drug.

To further investigate the additive effects of methacholine and nicotine, this same heart was then treated with 1×10^{-4} M atropine for five minutes and then the same doses of nicotine and methacholine as used above were applied to the heart together with 1×10^{-4} M atropine. Figure 20D shows the effects of this treatment on the heart. Before atropine treatment, nicotine and methacholine, when applied



together, produced a rate increase of 21 beats/min and an increase in both beat amplitude and myocardial tonus. After atropine, the simultaneous treatment with nicotine and methacholine produced a rate increase of 15 beats/min, with no increase in either the heartbeat amplitude or myocardial tonus. Since methacholine alone initially produced a rate increase of six beats/min and an increase in the amplitude of the heartbeat, it appears that when atropine, nicotine and methacholine were applied simultaneously to the heart, atropine selectively blocked the action of methacholine, while leaving that of nicotine unaffected.

Catecholamines

Response of the Heart to Epinephrine and Norepinephrine

Epinephrine and norepinephrine have a pronounced excitatory action on a number of different neurogenic hearts (Carlson, 1906; Welsh, 1939b; Florey, 1968). To determine if these catecholamines have a similar action of the E. marxi heart, they were applied to 10 different hearts in concentrations ranging from 1×10^{-6} M to 1×10^{-4} M. Figures 21 and 22 show the effect of 5×10^{-5} M epinephrine and norepinephrine on the heart contractions. The records are continuous from upper left to lower right. For both epinephrine and norepinephrine, an increase in the heart rate occurs which reaches its maximum in about 45 seconds.

Figure 21. Effect of epinephrine on the heart. Continuous record from top left to bottom right. Saline removed at first arrow and 5×10^{-5} M epinephrine applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

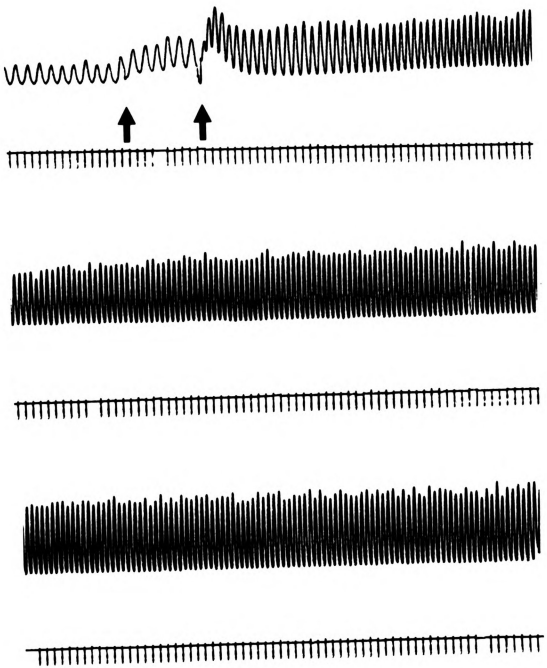


Figure 21

Figure 22. Effect of norepinephrine on the heart. Continuous record from top left to bottom right. Saline removed at first arrow and 5 x 10^{-5} M norepinephrine applied at the second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

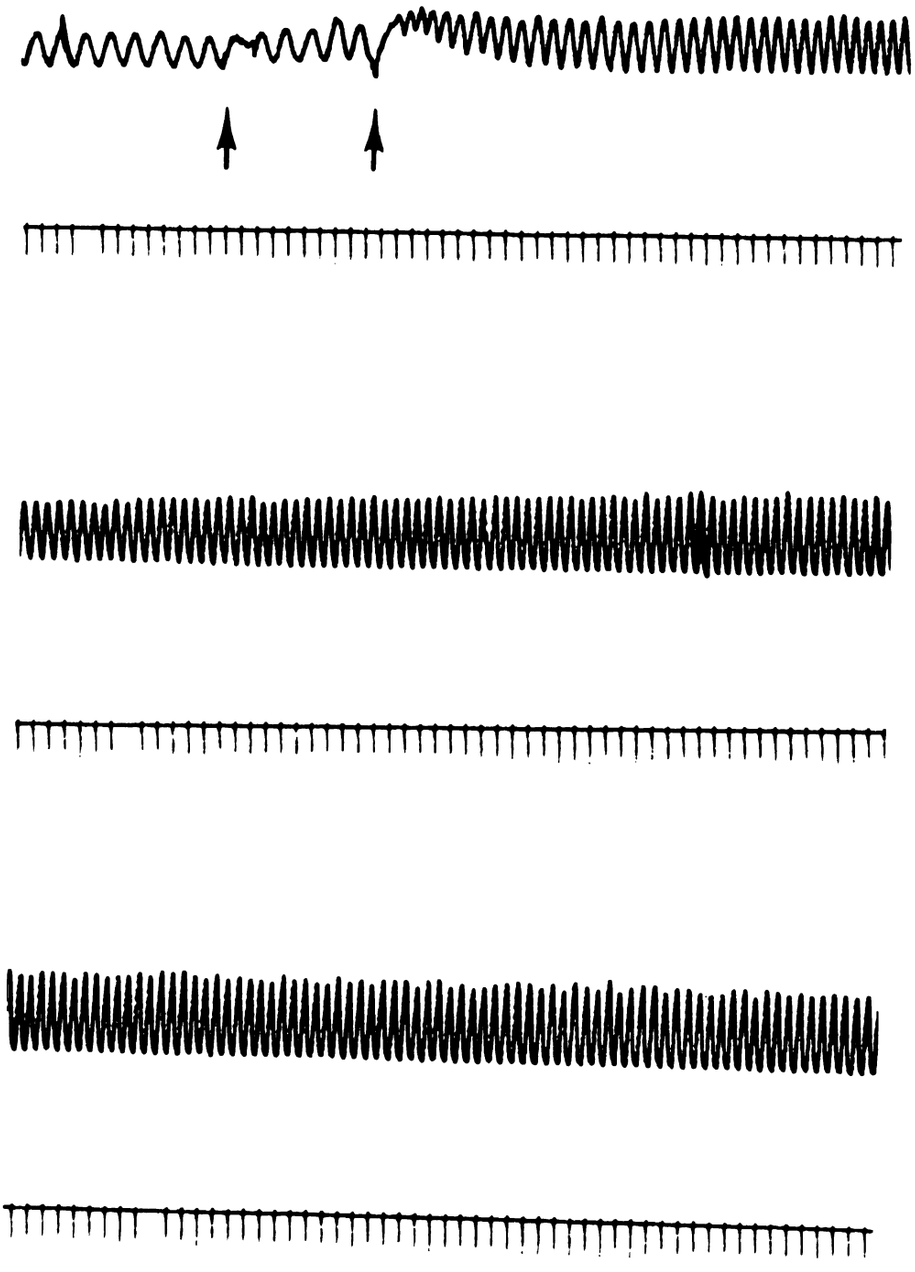


Figure 22

An increase in the amplitude of the heartbeat and with high doses, a transient increase in myocardial tonus also occurs. The increased rate and beat amplitude do not decrease appreciably throughout the time that epinephrine and norepinephrine are on the heart. Upon removal of these drugs, the response only gradually declines and recovery to control levels usually requires 10 to 15 minutes.

No apparent change in the sensitivity of the heart was noted due to repeated applications of epinephrine and norepinephrine. Responses to any particular dose did not change appreciably even after eight different treatments with these drugs.

Figures 23 and 24 are dose-response curves for the rate effects of epinephrine and norepinephrine on the heart. Threshold concentration for the rate response for both is about 1×10^{-6} M.

Table 4 gives the effects of epinephrine and norepinephrine on the amplitude of the heart contractions. Each value represents seven replications made on at least four different hearts. As seen in the table, the amplitude of the heartbeat increases as the concentration used increases, as does the myocardial tonus. In general, norepinephrine does not produce as great an effect on the amplitude of the contractions as does epinephrine.

Figure 23. Dose-response curve for the rate effect of epinephrine on the heart. Each point represents the mean response to seven replications made on at least four different hearts. Vertical lines represent one standard deviation.

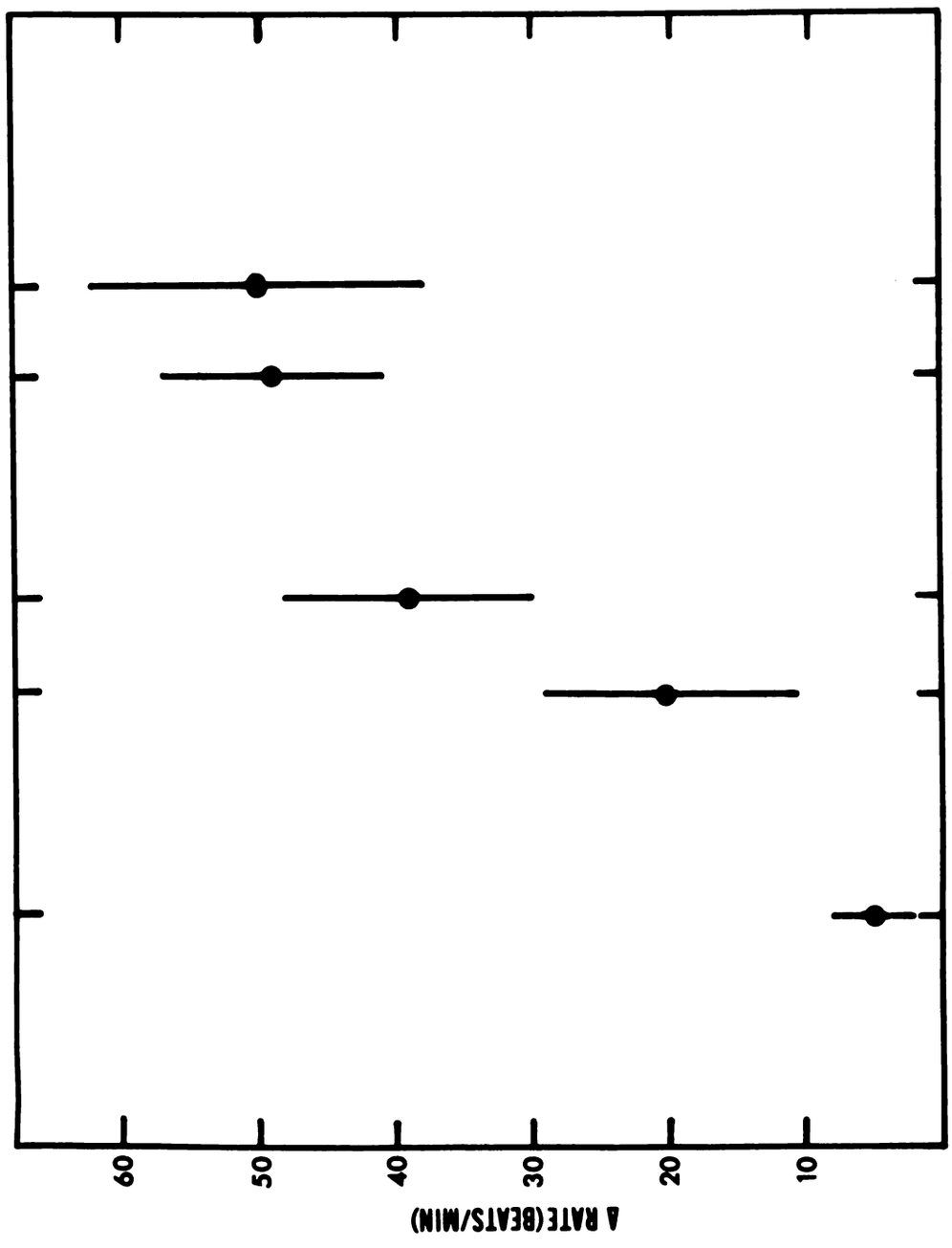


Figure 23

...eats. Vertical lines represent ...

Figure 24. Dose-response curve for the rate effect of norepinephrine on the heart. Each point represents the mean response to seven replications made on at least four different hearts. Vertical lines represent one standard deviation.

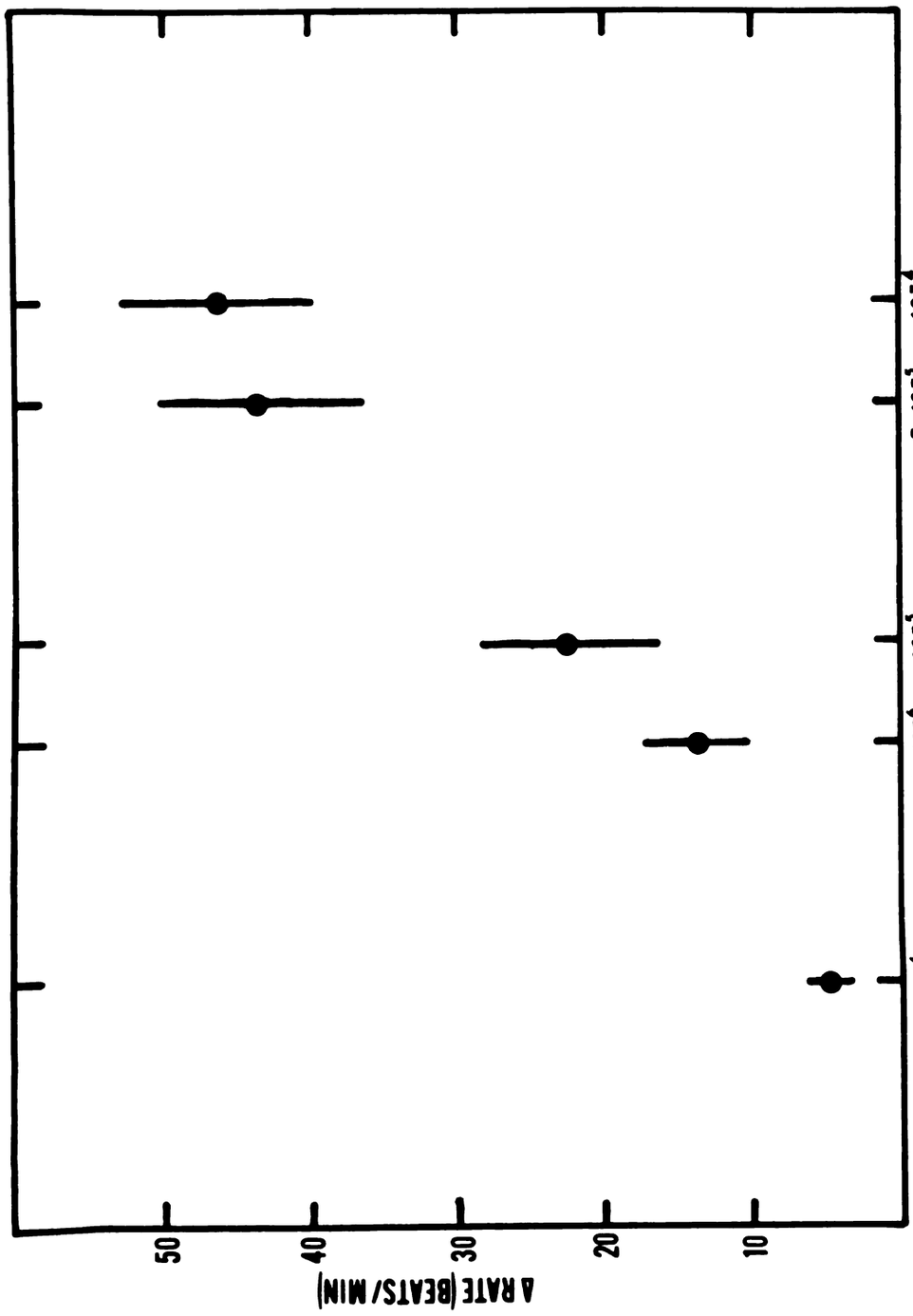

Δ RATE (BEATS/MIN)
50
40
30
20
10
CONCENTRATION (M)
10^{-6}
$5x10^{-6}$
10^{-5}
$5x10^{-5}$
10^{-4}

Figure 24

Table 4. Effects of epinephrine and norepinephrine on heartbeat amplitude

Concentration (M)	Mean Heartbeat Amplitude Change In Mg (Range)	Mean Increase In Tonus In Mg (Range)	Number of Tone Increases	Mean Time In Tetany In Sec (Range)	Number Showing Tetany	Total N
			Epinephrine			
1×10^{-6}	10 (-10 to 25)	0	0	0	0	7
5×10^{-6}	30 (10 to 55)	10 (0 to 40)	2	0	0	7
1×10^{-5}	55 (30 to 135)	40 (0 to 40)	4	0	0	7
5×10^{-5}	65 (30 to 115)	80 (0 to 220)	6	0 (0 to 2)	1	7
1×10^{-4}	85 (30 to 175)	100 (30 to 200)	7	1 (0 to 3)	2	7
			Norepinephrine			
1×10^{-6}	0 (-10 to 10)	0	0	0	0	7
5×10^{-6}	10 (-10 to 35)	0	0	0	0	7
1×10^{-5}	15 (0 to 55)	10 (0 to 30)	4	0	0	7
5×10^{-5}	30 (0 to 95)	45 (0 to 80)	6	1 (0 to 2)	3	7
1×10^{-4}	45 (15 to 95)	90 (40 to 100)	7	2 (0 to 3)	5	7

Site of Catecholamine Action

Epinephrine and norepinephrine, in concentrations up to 1×10^{-4} M, produce no recordable changes in the myocardial tonus of deganglionated hearts. Figures 25 and 26 show the effects of epinephrine and norepinephrine on cardiac ganglion electrical activity. Both of these drugs produce an increase in ganglion burst frequency as well as an increase in the amount of activity in each burst.

Amino Acids

A number of amino acids have been reported to be active on the hearts of decapod crustaceans and L. polyphemus (Florey, 1957; Pax and Sanborn, 1967a). In this study, gamma aminobutyric acid, glycine, d and l-glutamic acid and d and l-aspartic acid were investigated as to their effects on the E. marxi heart.

Response of the Heart to Gamma Aminobutyric Acid (Gaba)

Gaba produces an immediate decrease in the rate and amplitude of the heartbeat. Although the maximum inhibitory effect of Gaba usually occurs immediately after application, sometimes the maximum effect is not seen until several seconds later. This is particularly true at lower concentrations. In any case, several seconds after the maximum effects of Gaba are seen, the heart rate begins to return to the pretreatment rate, but it never completely recovers

Figure 25. Effect of epinephrine on cardiac ganglion electrical activity.

A: in saline

B: immediately after 5×10^{-5} M epinephrine.

C: same as B, except one minute later.

D: in saline just after epinephrine removed.

E: same as D, except five minutes later.

F: same as D, except 10 minutes later.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

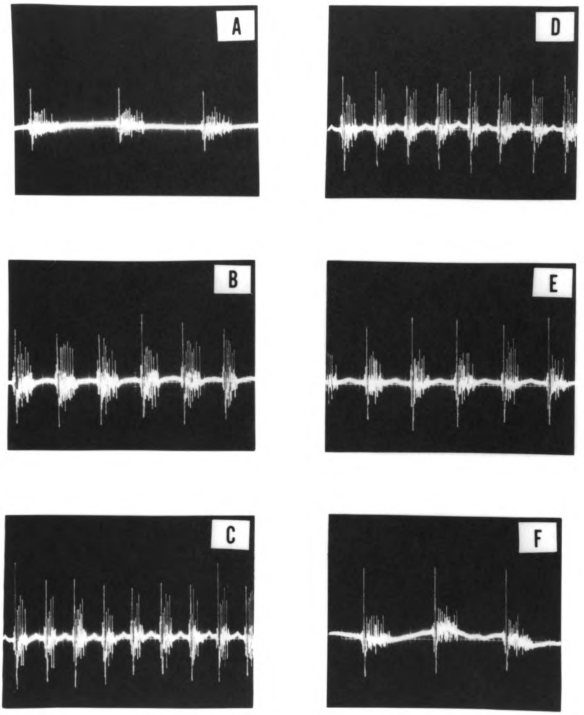


Figure 25

Figure 26. Effect of norepinephrine on cardiac ganglion electrical activity.

A: in saline.

B: immediately after 5×10^{-5} M norepinephrine.

C: same as B, except one minute later.

D: in saline just after norepinephrine removed.

E: same as D, except five minutes later.

F: same as D, except 10 minutes later.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

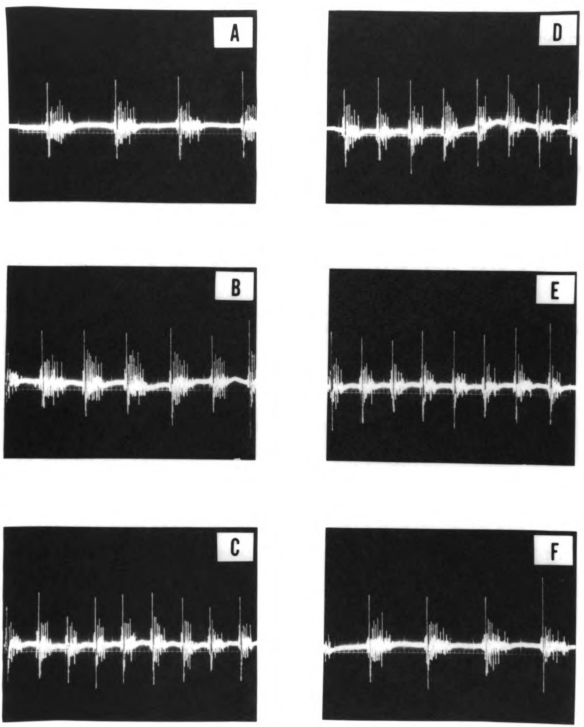


Figure 26

as long as Gaba is on the heart. Concurrent with the recovery in rate, and even more striking, the amplitude of the heartbeat begins to increase until it exceeds the heartbeat amplitude seen before the beginning of the Gaba treatment. Occasional disruptions in the synchrony of the contractions are also seen. Upon removal of Gaba from the heart, a further increase in the beat amplitude is often seen before the response declines. Recovery to pretreatment levels usually occurs within six minutes. Figure 27 shows the response of an isolated heart to treatment with 5×10^{-4} M Gaba.

Figure 28 is a dose-response curve for Gaba. The dose required to produce a consistent decrease in rate of four beats/min or more was 1×10^{-5} M or slightly greater.

The effect of Gaba on the amplitude of the heartbeat is shown in Table 5. Each value represents the maximum effect of at least five applications made on at least four different hearts. The threshold dose required to produce a change in the heartbeat amplitude was about 5×10^{-5} M. The biphasic nature of the amplitude response to Gaba is readily apparent in the table. Each column of numbers was obtained by averaging the values obtained for the first 10 heartbeats that occurred at the beginning of each of the three minutes that the drug was on the heart. In the first minute the average response was a decrease in beat amplitude, but in the second and third minutes the amplitude was larger than that of the control heartbeats.

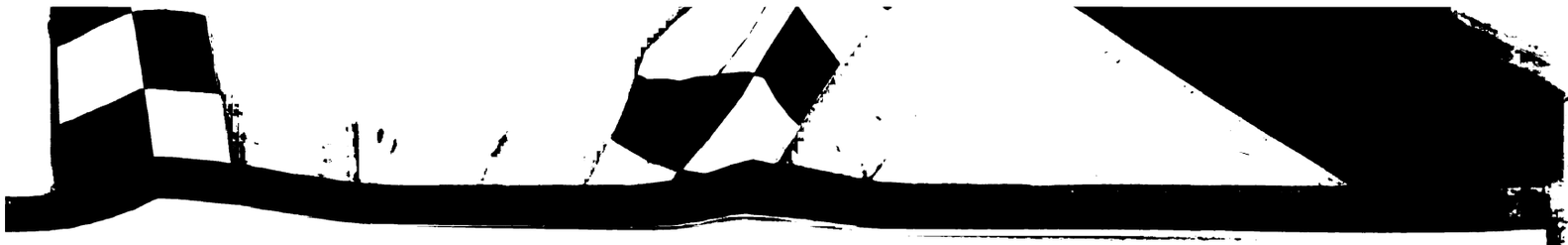
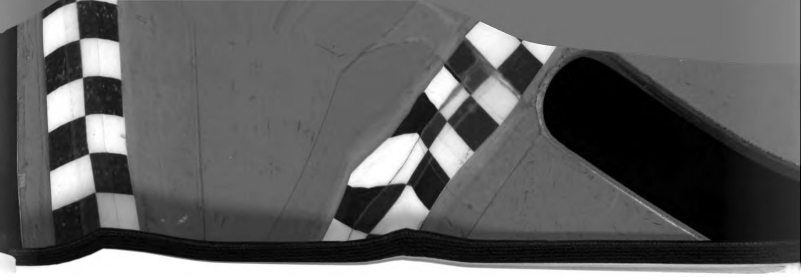


Figure 27. Effect of Gaba on the heart. Continuous record from top left to bottom right. Saline removed at first arrow and 5×10^{-4} M Gaba applied at second arrow. Gaba removed at third arrow and saline applied at fourth arrow.

Aplitude cal: 10 mg/mm.

Time cal: 1/sec.



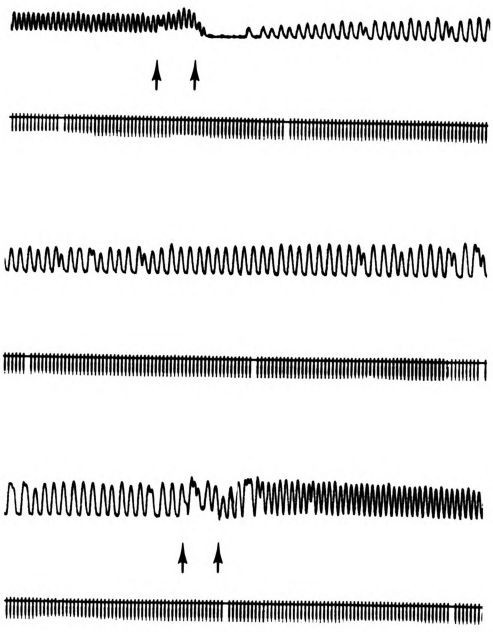


Figure 27

Figure 28. Dose-response curve for the rate effect of Gaba on the heart. Each point represents the mean response to at least five replications made on at least four different hearts. Vertical lines represent one standard deviation.

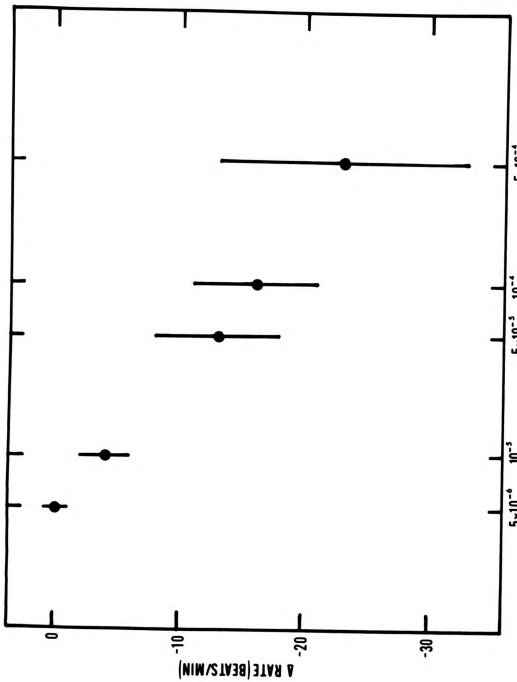

0
-10
-20
-30
Δ RATE (BEATS/MIN)
5x10⁻⁶
10⁻⁵
5x10⁻⁵
10⁻⁴
5x10⁻⁴
CONCENTRATION (M)

Figure 28

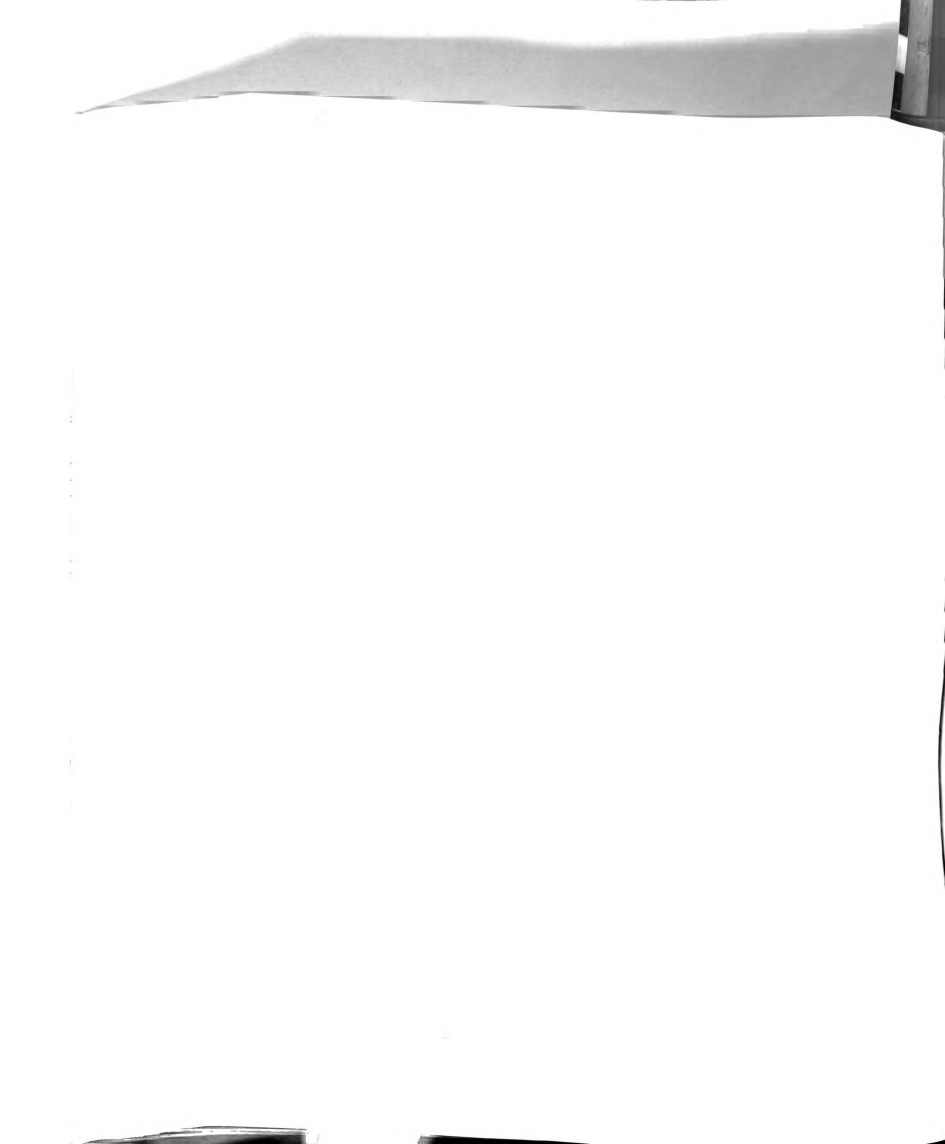


Table 5. Effect of gamma aminobutyric acid on heartbeat amplitude

Concentration (M)	Mean Change In Aplitude In First Minute (Mg)	Mean Change In Amplitude In Second Minute (Mg)	Mean Change In Amplitude In Third Minute (Mg)
5×10^{-6}	0	0	0
1×10^{-5}	0	0	0
5×10^{-5}	-5	+10	+15
1×10^{-4}	-20	+25	+35
5×10^{-4}	-30	+15	+45

Picrotoxin and Gaba

Picrotoxin blocks the action of applied Gaba on the decapod heart (Florey, 1957), but not on the heart of L. polyphemus (Pax and Sanborn, 1967a). To determine if picrotoxin blocks the effect of applied Gaba in the E. marxi heart, four hearts were treated from five to 30 minutes with 5×10^{-4} M picrotoxin and then with 5×10^{-4} M picrotoxin plus Gaba in concentrations from 5×10^{-5} to 5×10^{-4} M.

Picrotoxin alone had only a slight effect on the heart which consisted of a gradual decline in heartbeat amplitude that occurred over a period of several minutes. Little change in the heart rate was seen.

Regardless of the duration of the picrotoxin treatment, pircotoxin does not block the action of applied Gaba on the heart. Figure 29 shows the response of an isolated heart to Gaba alone, to picrotoxin alone and to combined Gaba and picrotoxin treatments.

Site of Action of Gaba

Gaba does not produce any recordable change in myocardial tonus when applied to deganglionated hearts. When applied to intact hearts, it reduces the electrical activity of the ganglion (Figure 30). Immediately after application, Gaba produces spordic firing of the ganglion cells, along with occasional bursts of prolonged duration and reduced amplitude (Figure 30B). After about 30 seconds,

Figure 29. Effects of Gaba and picrotoxin on the heart.

A: saline removed at first arrow and 5×10^{-4} M Gaba applied at second arrow.

B: same as A, except 5×10^{-4} M picrotoxin was applied at second arrow.

C: picrotoxin removed after a 15 minute treatment at first arrow and 5×10^{-4} M Gaba plus 5×10^{-4} M picrotoxin applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

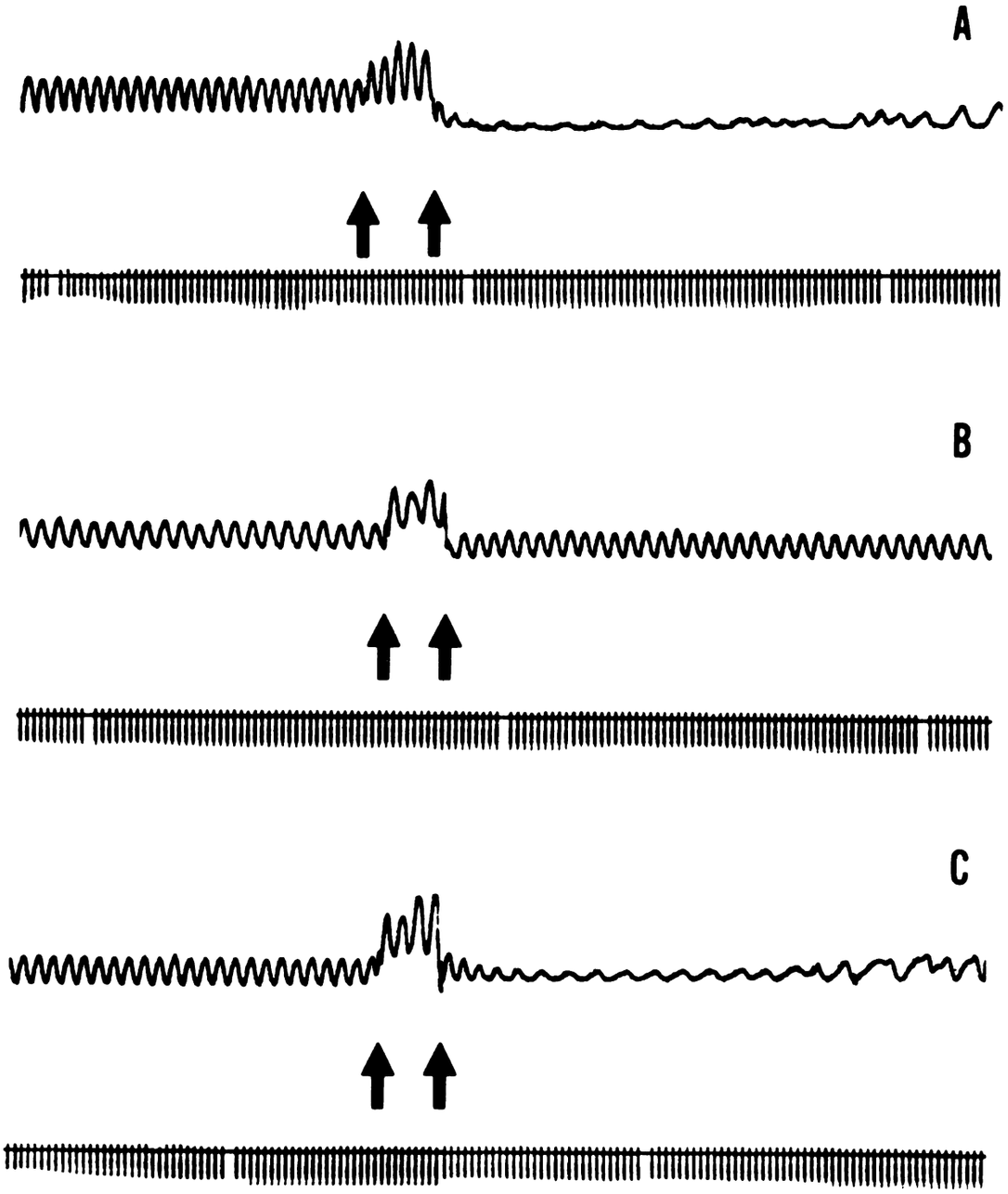


Figure 29

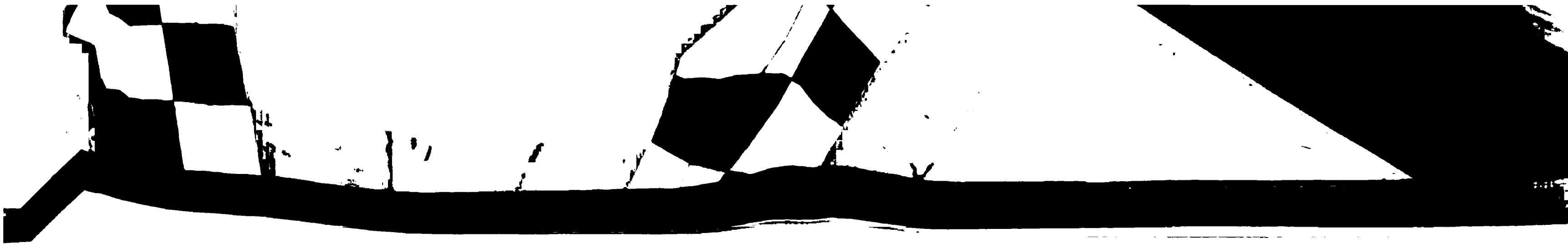


Figure 30. Effects of Gaba and picrotoxin on cardiac ganglion electrical activity.

A: in saline.

B: just after application of 5×10^{-4} M Gaba.

C: same as C, except 30 seconds later.

D: in saline 45 seconds after Gaba removed.

E: same as D, except five minutes later.

F: in 5×10^{-4} M picrotoxin.

G: in 5×10^{-4} M Gaba plus 5×10^{-4} M picrotoxin, after five minute picrotoxin treatment.

H: five minutes after Gaba and picrotoxin removed and saline applied.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

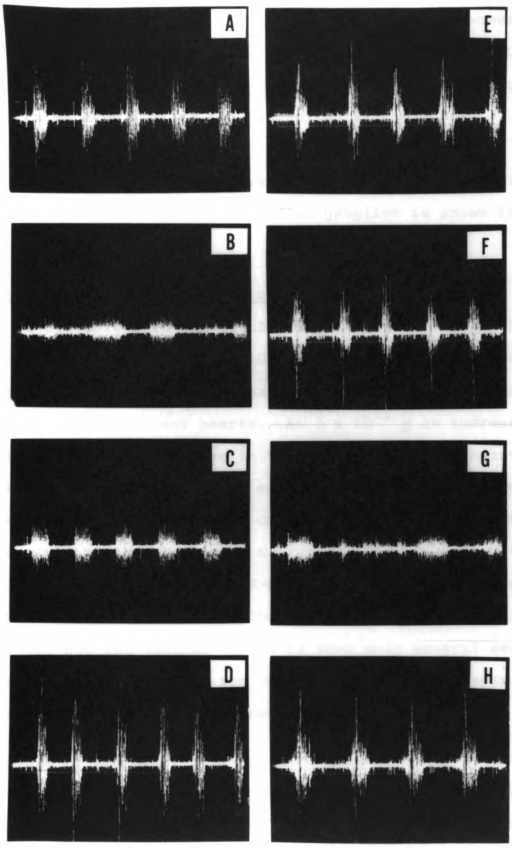


Figure 30

coordinated bursts at an increased rate appear. However, the amplitude of each burst is still reduced (Figure 30C). About 45 seconds after removal of Gaba the burst amplitude is greater than that of the control burst amplitude (Figure 30D).

The inability of picrotoxin to block the effects of Gaba on the activity of the cardiac ganglion is shown in Figure 30G.

Response of the Heart to d-Glutamic Acid

d-Glutamic acid has little effect on the E. marxi heart in concentrations up to 1 x 10^{-4} M (Table 6). Each value in the table represents at least two replications made on at least two different hearts. At 5 x 10^{-4} M an increase in the rate and a decrease in the amplitude of the heartbeat occurs. At 1 x 10^{-3} M these effects are even more pronounced, often resulting in such weak contractions that the heart rate could not be accurately measured. The effects of two different concentrations of d-glutamate on the heart are shown in Figure 31A and B. The effect of d-glutamate is immediate, but the maximum effect is seen only several seconds after application. Upon removal of the drug the response quickly declines to pretreatment levels.

Response of the Heart to l-Glutamic Acid

l-Glutamic acid has no appreciable effect on the heart in concentrations up to 1 x 10^{-4} M (Table 6). At 1 x 10^{-4} M,

Table 6. Effects of various amino acids on the heart

Concentration (M)	Mean Rate Change (Beats/Min)	Mean Amplitude Change (Mg)	N
	d-Glutamic Acid		
1×10^{-5}	2	0	2
1×10^{-4}	4	0	3
5×10^{-4}	12	-5	3
1×10^{-3}	--	-20	3
	l-Glutamic Acid		
1×10^{-5}	1	0	2
1×10^{-4}	3	10	3
5×10^{-4}	3	30	3
1×10^{-3}	6	35	3
	d-Aspartic Acid		
1×10^{-5}	1	0	2
1×10^{-4}	2	0	2
5×10^{-4}	2	10	4
1×10^{-3}	5	15	4
	l-Aspartic Acid		
1×10^{-5}	2	0	2
1×10^{-4}	2	0	3
5×10^{-4}	5	10	4
1×10^{-3}	7	20	4
	Glycine		
1×10^{-5}	1	0	2
1×10^{-4}	2	0	3
1×10^{-3}	4	5	3

Figure 31. Effects of d and l-glutamic acid on the heart.

A: saline removed at first arrow and 1 x 10^{-3} M d-glutamic acid applied at second arrow.

B: same as A, except 5 x 10^{-4} M d-glutamic acid applied at second arrow.

C: same as A, except 1 x 10^{-3} M l-glutamic acid applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

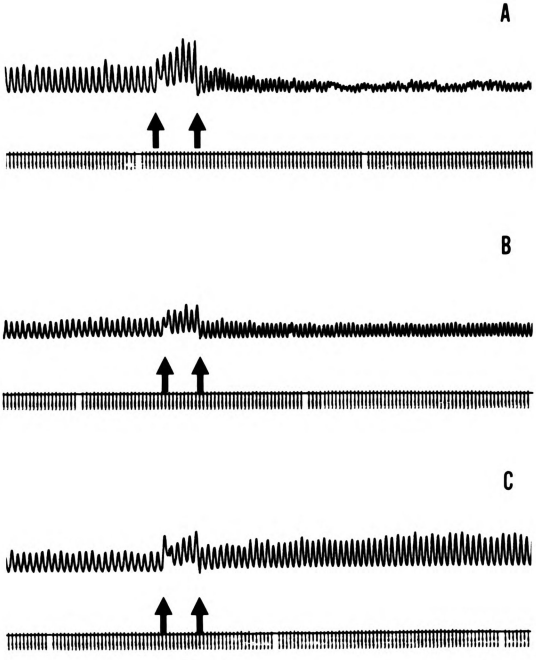
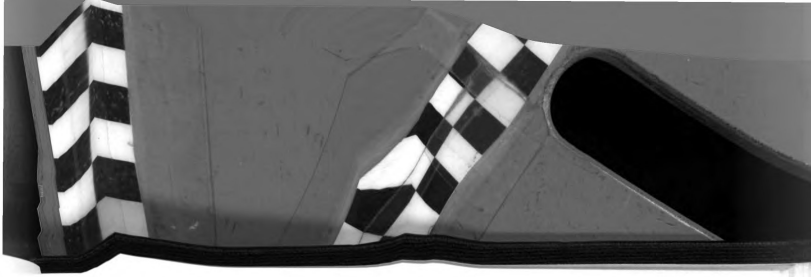


Figure 31



a slight increase in the heart rate and beat amplitude occurs. A more pronounced increase in heartbeat amplitude is seen at concentrations of 5×10^{-4} and 1×10^{-3} M, although marked increases in the rate are not observed. The effect of l-glutamic acid on an isolated heart is shown in Figure 31C.

Response of the Heart to Other Amino Acids

d and l-Aspartic acid and glycine produce only slight increases in the heart rate and beat amplitude in concentrations from 1×10^{-5} through 1×10^{-3} M. The effects of these amino acids on the heart are shown in Figure 32 and Table 6.

Site of Action of Amino Acids Other Than Gaba

Of the amino acids tested, d-glutamate was the only one other than Gaba which had an effect on the electrical activity of the cardiac ganglion. The response of the ganglion to d-glutamate consisted of an increase in the rate of ganglionic bursting, a decrease in the burst amplitude and a general appearance of asychronous firing (Figure 33).

When applied to deganglionated hearts, all of the amino acids with the exception of l-glutamate produced no recordable changes in myocardial tonus in concentrations up to 1×10^{-3} M. l-Glutamate evoked sustained contractions of the myocardium when administered in concentrations of 1×10^{-4} M to 1×10^{-3} M. The increased tonus produced by

Figure 32. Effects of d and l-aspartic acid and glycine on the heart.

A: saline removed at first arrow and 1 x 10^{-3} M l-aspartic acid applied at second arrow.

B: same as A, except 1 x 10^{-3} M d-aspartic acid applied at second arrow.

C: same as A, except 1 x 10^{-3} M glycine applied at second arrow.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

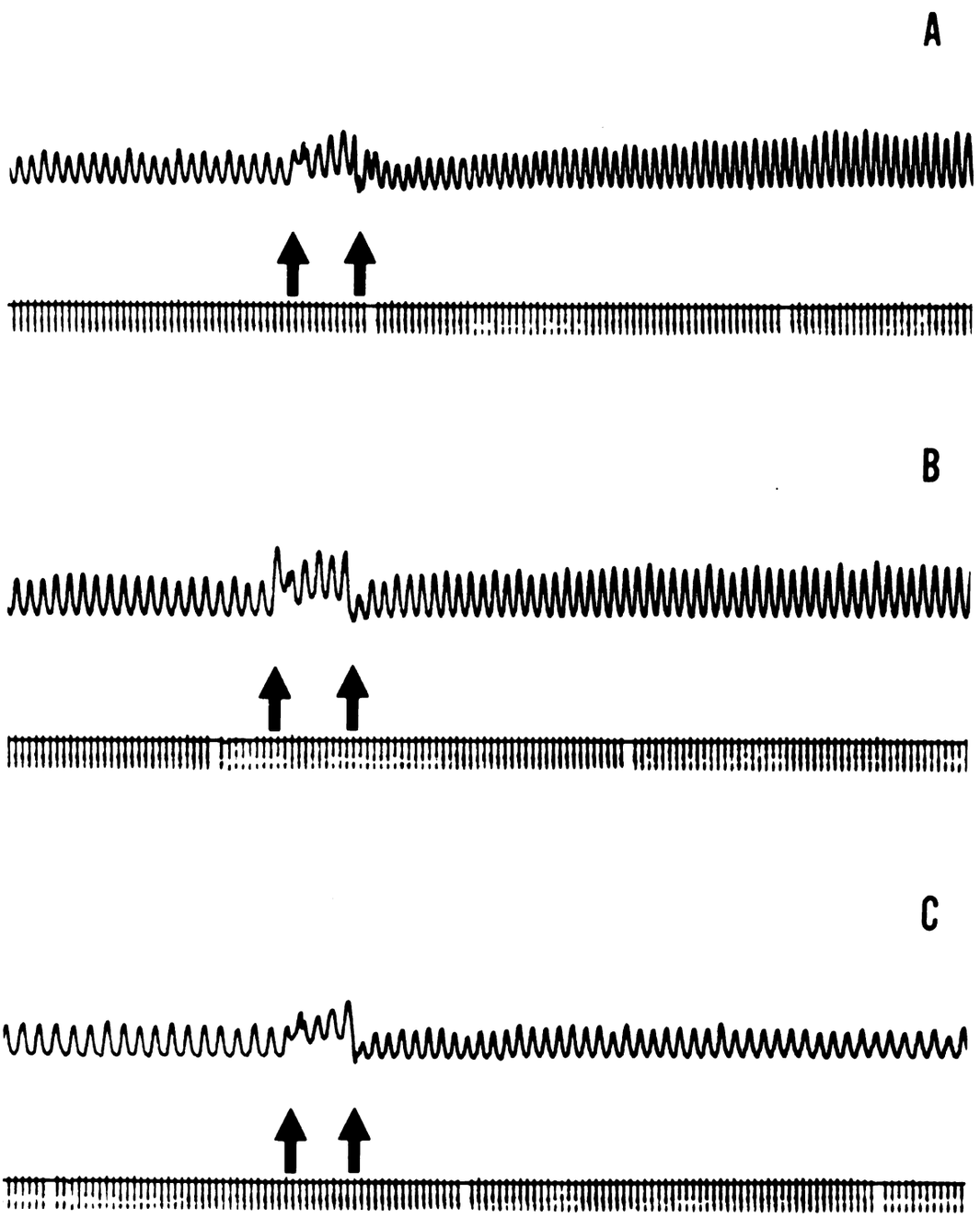


Figure 32

Figure 33. Effect of d-glutamic acid on cardiac ganglion electrical activity.

A: in saline.

B: 30 seconds after application of 1×10^{-3} M d-glutamic acid.

C: same as B, except one minute later.

D: in saline just after d-glutamic acid removed.

E: same as D, except three minutes later.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

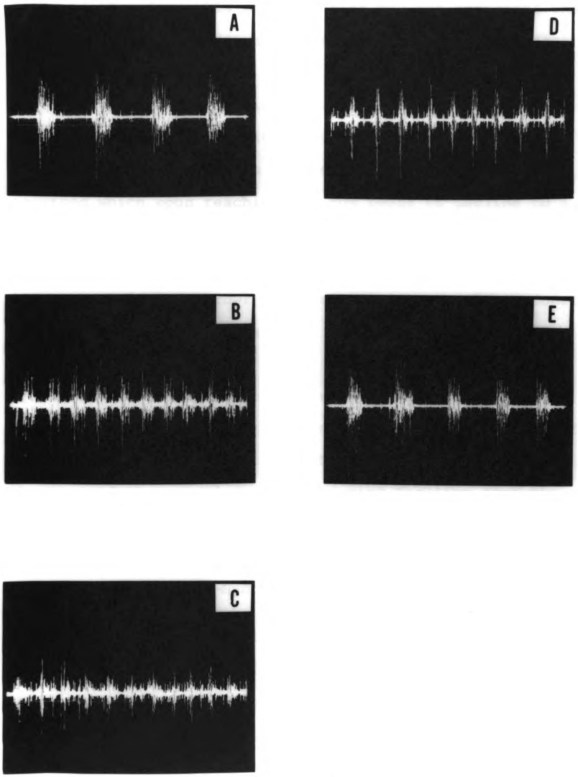


Figure 33

1×10^{-4} M l-glutamate was barely recordable by the methods used and therefore this concentration may not represent the actual threshold concentration required to produce changes in myocardial tonus.

A sustained contraction induced by 5×10^{-4} M l-glutamate is shown in Figure 34. At the second arrow, l-glutamate was applied to the heart. A rapid contraction occurred which upon reaching maximum began to decline to a point about one-third that of the maximum. The contraction lasted for as long as l-glutamate was on the heart and upon its removal (third arrow) and the addition of saline (fourth arrow) the contraction gradually returned to the control baseline.

A number of smaller oscillatory contractions are also seen in the record after treatment with l-glutamate and during the sustained contraction. They persisted throughout the rest of the experiment, even during the time that the heart was bathed only in saline.

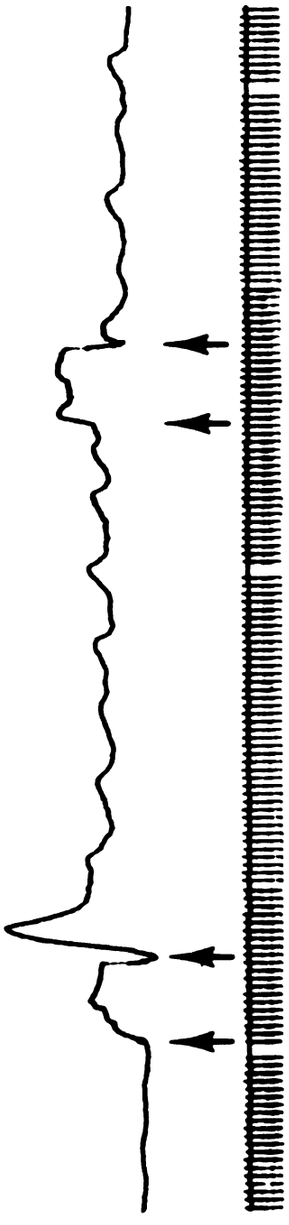
5-Hydroxytryptamine

5-Hydroxytryptamine (5-Ht) produces acceleration of the decapod crustacean heart (Kerkut and Price, 1964; Cooke, 1966) and inhibition of the *Limulus* heartbeat (Pax and Sanborn, 1967b). Since this chemical has opposite effects on these neurogenic hearts it would be interesting to see what effect, if any, 5-Ht has on the neurogenic *E. marxi* heart.

Figure 34. Effect of l-glutamic acid on the myocardial tonus of a deganglionated heart. Saline removed at first arrow and 5 x 10^{-4} M l-glutamic acid applied at second arrow. l-glutamic acid removed at third arrow and saline applied at fourth arrow.

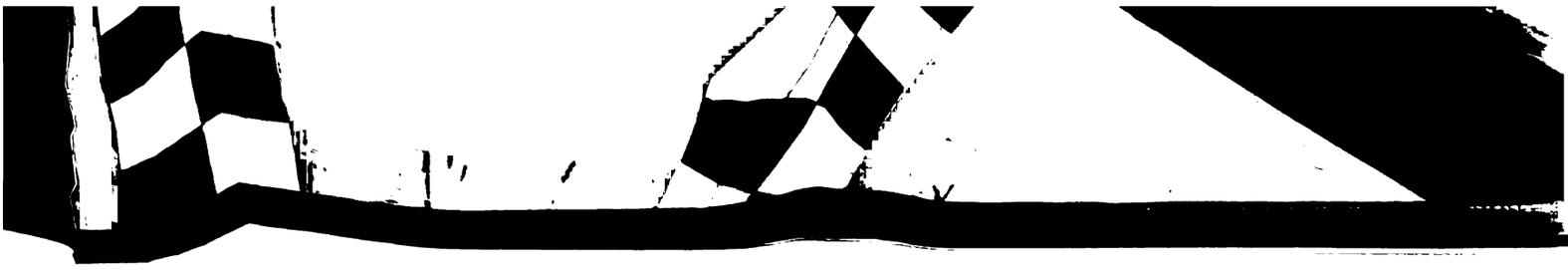
Amplitude cal: 5 mg/mm.

Time mark: 1/sec.



Time mark: 1/sec.

Figure 34



Concentrations of 5-Ht ranging from 1×10^{-6} M to 5×10^{-3} M were applied to four different isolated hearts. Little change in the rate and amplitude of the contractions were seen at concentrations of 5-Ht up to 5×10^{-4} M, although occasional small increases in heart rate and beat amplitude occurred. At 1×10^{-3} M, two of the hearts responded with an average increase in rate of five beats/min and a slight increase in the beat amplitude. The other two hearts responded to 1×10^{-3} M 5-Ht with a decrease in heart rate averaging seven beats/min and a pronounced decrease in the amplitude of the heartbeat.

The most striking response to 5-Ht was seen at a concentration of 5×10^{-3} M. This response is shown for one heart in Figure 35. Immediately upon addition of 5-Ht, the amplitude of the heartbeat was reduced to the point where it was barely recordable. At the same time, the heart rate was reduced. Heart rates could be measured at this time, but only with difficulty because of the tremendous decrease in beat amplitude. In Figure 35, the heart rate was decreased by 13 beats/min. The amplitude decrease continued until the drug was removed. A few seconds after this was done, a pronounced increase in the beat amplitude occurred which was approximately twice that of the control beat amplitude. This amplitude increase was only transitory and after a few minutes in saline the beat amplitude reached that seen before treatment with 5-Ht.

Figure 35. Effect of 5-Ht on the heart. Continuous record from top left to bottom right. Saline removed at first arrow and 5 x 10^{-3} M 5-Ht applied at second arrow. 5-Ht removed at third arrow and saline applied at fourth arrow. Fifth and sixth arrows denote a saline wash.

Amplitude cal: 10 mg/mm.

Time mark: 1/sec.

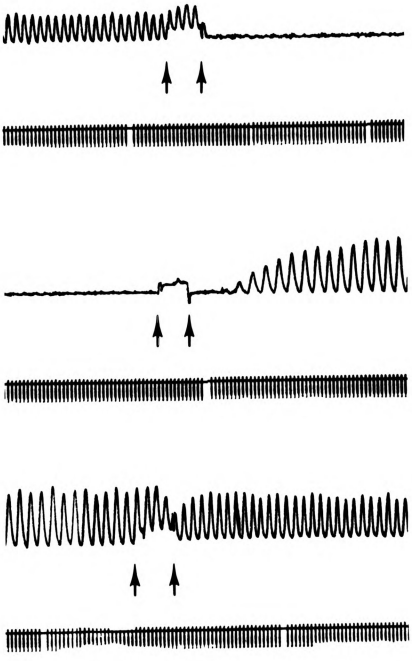
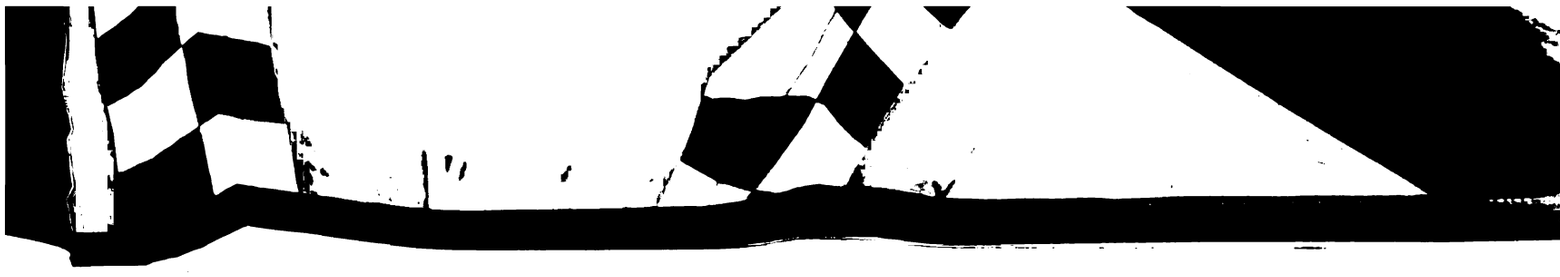


Figure 35



The site of 5-Ht action was investigated by applying 5×10^{-3} M 5-Ht to deganglionated hearts for a period of five minutes. No recordable effects on the myocardium were observed.

When 5×10^{-3} M 5-Ht was applied to the heart while monitoring cardiac ganglion electrical activity, a striking decrease in the amplitude of the ganglionic bursts was seen, with only a slight decrease in the burst rate. This result is shown in Figure 36. Although the burst amplitude was greatly reduced, coordinated bursts still occurred.

Figure 36. Effect of 5-Ht on cardiac ganglion electrical activity.

A: in saline.

B: immediately after 5×10^{-3} M 5-Ht applied.

C: same as B, except one minute later.

D: five minutes after 5-Ht removed.

Amplitude cal: 16 uV/mm.

Time cal: 160 msec/mm.

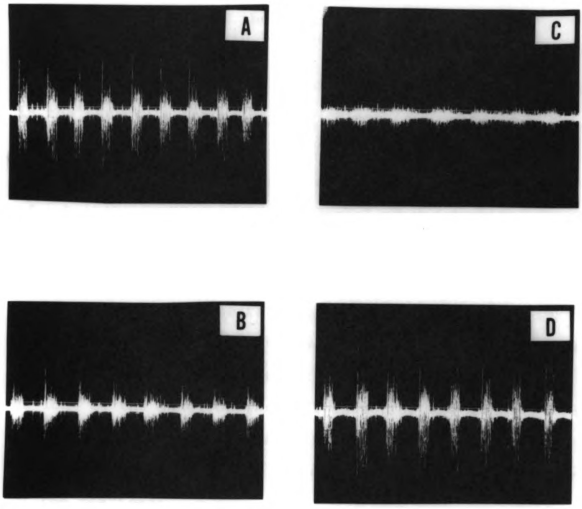


Figure 36

DISCUSSION

Role of the Cardiac Ganglion in the Heartbeat

The results obtained in this study show that heartbeat in the tarantula Eurypelma marxi Simon is neurogenic. The electrical activity of the cardiac ganglion present on the heart precedes all other heart activity during the course of a heartbeat. In addition, removal of the ganglion results in the immediate cessation of the heartbeat. The role of the ganglion in the coordination of the unit contractions of the heart was demonstrated by the experiments in which the heart was separated into two parts. Here, muscle cells which were separated by the cut still functioned as a unit as long as the ganglion was left intact on the heart. From these results it can be stated that the cardiac ganglion in E. marxi functions both in the genesis and the coordination of the heartbeat.

In relationship to other arthropod hearts, the heart of E. marxi possesses functional characteristics similar to the hearts of scorpions, the Merostomata and the higher crustaceans. In thses animals, as in E. marxi, a cardiac ganglion is present which functions to initiate and control

contractions of the heart (Carlson, 1904a,b; Brown, 1964a,b; Zwicky and Hodgson, 1965; Abbott et al., 1969). On the other hand, the heart of E. marxi is functionally quite unlike that of those insects in which sufficient studies have been performed to permit comparisons. Heartbeat in the moth Hyalophora cercropia and the cockroach Periplaneta americana is myogenic. In H. cercropia, there are no cardiac ganglion cells on the heart and pacemaker activity resides in the myocardial cells. Pacemaker action potentials are conducted throughout the myocardium, causing it to contract in a wave-length fashion (McCann, 1964, 1965). Heartbeat in P. americana is myogenic even though cardiac ganglion cells are present. Like H. cercropia, pacemaker potentials in P. americana arise in the myocardium, but unlike the moth, coordination of the heartbeat is at least partly achieved by the paired lateral cardiac ganglia so that the entire heart contracts simultaneously and not in a wave-length fashion (Miller, 1968).

Origin of the Heartbeat of Spiders in General

On the basis of the results of this study and the information previously reported, it seems very likely that heartbeat in all spiders is neurogenic. A cardiac ganglion is known to occur in 14 different families of spiders (Sherman et al., 1969). Electrocardiograms have been

recorded from members of five different families and in each case the electrocardiogram recorded was that characteristically obtained for neurogenic hearts (Rijlant, 1933; Sherman and Pax, 1968). Furthermore, microelectrode recordings of the active heart muscle cell electrical response have been made from hearts of two different spider families and in both instances these reponses were essentially the same as those recorded from other neurogenic hearts (Sherman and Pax, 1969; and this study).

Summary of Pharmacological Studies

In this study several drugs were tested for their effects on a tarantula heart. Table 7 provides a listing of the drugs used as well as a summary of their effects on the E. marxi heart. Although none of these chemicals can be considered proven to have a physiological role on the basis of this investigation, some of them deserve further study as to their possible involvement in chemical transmission in the E. marxi heart. The following sections will deal with the justification for further studying some of the chemicals tested here as well as the reasons for discounting a physiological role for the others.

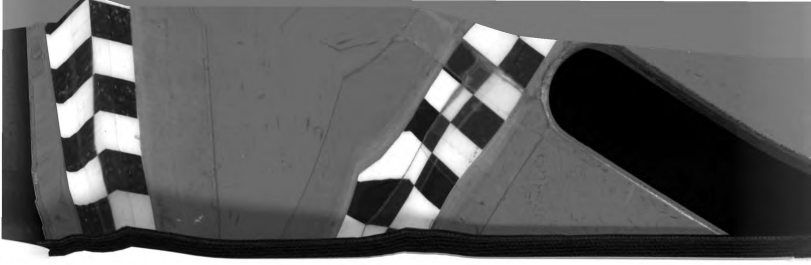
Response of the Heart to Cholinergic Compounds

Acetylcholine, methacholine and nicotine produce a marked increase in the rate and amplitude of the heartbeat of

Table 7. A summary of the effects of various compounds on the heart

Compound	Rate	Amplitude
Acetylcholine (Ach)	++	++
Eserine	+	+
Ach - eserine	+++	+++
Atropine	0	+
Ach - atropine	0	0
d-Tubocurarine	0	0
Ach - d-tubocurarine	+	+
Hexamethonium	0	0
Ach - hexamethonium	+	++
Methacholine	+	++
Methacholine - atropine	0	0
Methacholine - hexamethonium	+	++
Nicotine	++	+
Nicotine - hexamethonium	+	0
Nicotine - atropine	++	0
Epinephrine	+++	+++
Norepinephrine	+++	+++
Gamma aminobutyric acid (Gaba)	--	--,++
Picrotoxin	0	0
Gaba - picrotoxin	--	--,++
5-Hydroxytryptamine	-	--
Glycine	0	0
l-Glutamic acid	0	+
d-Glutamic acid	+	-
l-Aspartic acid	0	0
d-Aspartic acid	0	0

+ denotes increase
0 denotes little change
- denotes decrease



E. marxi by evoking an increase in the electrical activity of the cardiac ganglion. Thus cholinergic sensitive neurons are present in the cardiac ganglion of E. marxi.

In systems where Ach has a physiological role, such as the mediation of nerve transmission, an enzymatic mechanism is present for the termination of the action of Ach. Several lines of evidence suggest that such an enzymatic mechanism exists in the cardiac ganglion of E. marxi. Since eserine is known to inhibit cholinesterase activity, the enhancement of the effects of Ach on the heart by eserine indicates that a cholinesterase is present in the ganglion. This possibility is strengthened by the observation that the response to Ach is greatest immediately after its administration to the heart, and that the response declines even though Ach is still present on the heart. This decline in response could be explained by the presence of an enzyme for the rapid hydrolysis of Ach. Furthermore, the presence of cholinesterase could also account for the rapid recovery of the ganglion upon removal of the applied Ach.

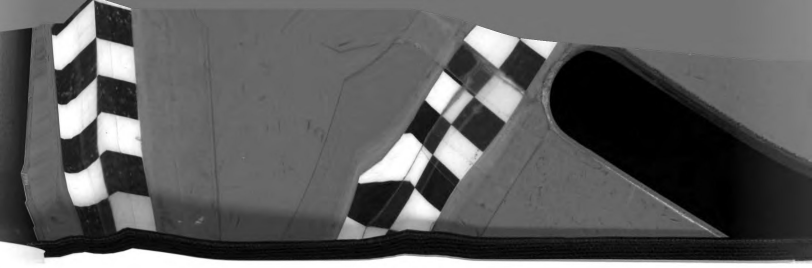
In many well-established cases of Ach mediation of nerve transmission, atropine, hexamethonium and d-tubocurarine may block the actions of Ach (Koelle, 1965b). When the E. marxi heart was treated with these blocking agents, the pronounced excitatory effects of Ach were considerably reduced.

From the above considerations it can be stated that cholinergic sensitive neurons are present in the cardiac

ganglion of E. marxi, that a cholinesterase is probably present which terminates the actions of Ach and that the responses of these cholinergic sensitive neurons can be at least partially blocked. Further information about the cholinergic sensitive neurons in the ganglion was obtained by the experiments involving nicotine and methacholine.

The response of the heart to nicotine is characteristically different from the response to methacholine at comparable concentrations. Nicotine evokes a pronounced increase in the heart rate, but seldom any marked increase in the amplitude of the heartbeat. On the other hand, methacholine elicits a large increase in the heartbeat amplitude and only a small increase in the heart rate. Since the principal effects of nicotine and methacholine are characteristically different, it is possible that these chemicals are acting on different sites in the cardiac ganglion.

Two different cholinergic sites occur in the vertebrate peripheral nervous system. These sites are distinguished on the basis of their responses to certain cholinergic stimulating and blocking compounds. One type of receptor site is activated by nicotine and blocked by hexamethonium and is called the nicotinic receptor. The other receptor is activated by methacholine and blocked by atropine and is called the muscarinic receptor (Koelle, 1965b).



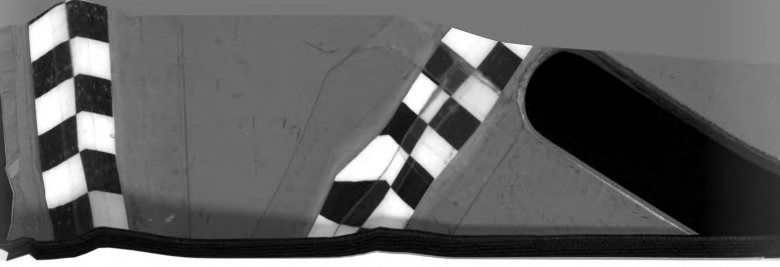
Effects similar to these were obtained from the E. marxi heart. Here, atropine effectively blocked all actions of methacholine on the heart, while the response to nicotine was either unaffected or only partially diminished by atropine. On the other hand, hexamethonium reduced the response to nicotine, but did not alter the response to methacholine.

Further evidence for the presence of two different cholinergic sensitive receptor sites in E. marxi was obtained from the experiment in which nicotine and methacholine were applied to the heart simultaneously. When this was done their effects on the heart summed to produce a response greater than that seen to each alone. Furthermore, upon atropine treatment the response to methacholine was apparently selectively blocked, while that to nicotine was unaltered when all three were applied to the heart at the same time.

The results obtained from the experiments involving nicotine and methacholine can be explained by assuming that they are acting on two different sites in the cardiac ganglion. The activation of one site leads to an increase in the heart rate, while activation of the other produces an increase in the amplitude of the contractions. Although it is not known whether functionally different types of neurons are present in the cardiac ganglion of spiders, two functionally different kinds of nerve cells do occur in the lobster cardiac ganglion (Maynard, 1955; Hartline, 1967).

There are nine neurons in this cardiac ganglion; five large cells (200 micra) and four smaller ones (80 micra) (Alexandrowicz, 1932). The smaller cells initiate and control the rate of bursting of the ganglion and are therefore called the pacemakers, while the larger cells (termed followers) are driven by the smaller cells and function as the motor output to the heart muscle. The smaller cells determine the heart rate, while the output from the larger cells determines the strength of the contractions. If such functionally distinct neurons are also present in the cardiac ganglion of spiders, nicotine could be acting principally on the pacemaker cells and methacholine principally on the follower cells. If indeed this is actually the case, then the pacemaker cells would possess mainly nicotinic properties and the follower cells mainly muscarinic properties.

Although this hypothesis may account for the main effects of nicotine and methacholine on the heart, it is not supported by the experiments involving Ach and the various blocking agents. If the increased heart rate produced by Ach is achieved by the activation of nicotinic receptor sites, then hexamethonium and d-tubocurarine should greatly reduce the rate response to Ach. However, these nicotinic blocking agents only partially block the rate response to Ach (about a 50% reduction). Furthermore, atropine, a muscarinic blocking agent, is more effective in blocking the rate response to Ach than are d-tubocurarine and hexamethonium. Thus, all



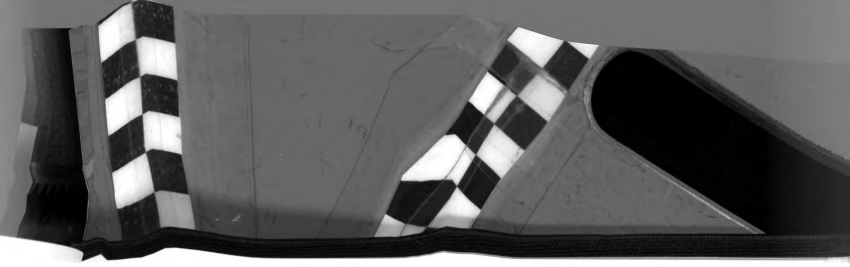
of the responses to cholinergic compounds shown by the E. marxi heart cannot be grouped into purely nicotinic and muscarinic categories.

An alternate explanation for the responses shown to the cholinergic compounds is that the receptor sites activated by Ach are different from those activated by nicotine and methacholine. Thus, heterogeneous cholinergic sites may be present on each of the functional cell types in the cardiac ganglion and the response seen to different combinations of cholinergic compounds would thus be a function of the interactions between the various cholinergic sites.

Effect of Ach on Other Spider Hearts

In the only other pharmacological study on spider hearts Kadziela and Kokociński (1965) reported that Ach, when injected into the abdomen of Tegenaria atrica, produced a decrease in the heart rate and a decrease in the amplitude of the heartbeat. These results are exactly opposite to those obtained in the present study from isolated hearts of E. marxi.

The possibility that this difference is due to species differences cannot be eliminated, but it seems more likely that it is due to the totally different manner in which the two studies were made. Kadziela and Kokociński injected whole abdomens with Ach, while isolated hearts were used in the present study. The use of an in vivo heart preparation



in spiders has serious disadvantages. By injecting Ach into the abdomen, an equal distribution of the drug throughout the abdomen cannot be assumed. Thus, the actual concentration reaching the heart is not known. Unfortunately, Kadziela and Kokociński did not stipulate the point at which the drug injections were made into the abdomen. Another serious problem involved in using whole abdomen preparations is that the volume of the drug solution injected could produce a large increase in the internal abdominal pressure. Since the spider abdomen is essentially a closed system after a ligature has been tied around the pedicel, as was done in their study, a small rise in internal volume could produce a large increase in internal pressure around the heart. This could account for the decrease in the amplitude of the heartbeat that is evident in their records. This could be the case even though Kadziela and Kokociński state that "test injections did not produce any change in the action of the heart," since they did not specifically state what the test injections consisted of.

Response of the Heart to Epinephrine and Norepinephrine

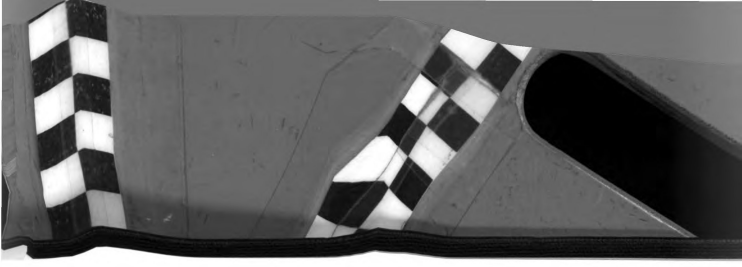
Epinephrine and norepinephrine, like Ach, produce a marked increase in the rate and amplitude of the E. marxi heartbeat. However the time course of the response to these catecholamines is quite different than that for Ach. Unlike Ach,

once the maximum response to epinephrine and norepinephrine is reached there is little change in rate for as long as these drugs are on the heart and upon their removal, the response only gradually diminishes. These differences between the response to Ach and the catecholamines used in this study could be explained on the basis that a different mechanism is present for terminating the actions of epinephrine and norepinephrine than for Ach. The lack of a rapid enzymatic process for terminating the actions of catecholamines has been reported for other animals (Koelle, 1965b) and this may also be the case for the heart of E. marxi.

Although this study has shown that catecholamine sensitive neurons are present in the cardiac ganglion of E. marxi, little can be said as to whether or not catecholamines have a physiological role in this heart.

Effect of Epinephrine on Other Spider Hearts

The effect of epinephrine on the heart of T. atrica was also studied by Kadziela and Kokociński (1965). They reported that epinephrine produced an increase in the heart rate and a decrease in the amplitude of the beat. In the present study, epinephrine also caused an increase in the heart rate, but the heartbeat amplitude was also increased. This difference in the effect of epinephrine on the amplitude of the heartbeat in these spiders could be explained



by the different methods employed in the two studies. As discussed previously for Ach, the injection of a drug solution into the abdomen of a spider may produce a large increase in the pressure outside the heart, which in turn could produce a decrease in the amplitude of the heartbeat.

Response of the Heart to Other Compounds

Of the remaining compounds tested, only d and l-glutamate, Gaba and 5-Ht have marked effects on the E. marxi heart. Since the effects of d-glutamate and 5-Ht occur only at relatively high concentrations, it seems unlikely that these substances have a physiological role in the E. marxi heartbeat.

l-Glutamic acid is the only compound used in this study which could be involved in neuromuscular transmission in the E. marxi heart, since all of the other substances used have no recordable effect on deganglionated hearts.

The biphasic amplitude response of the E. marxi heart to Gaba possibly could be due to metabolism of Gaba to produce l-glutamate. Since l-glutamate produces an increased heartbeat amplitude due to its action on the myocardium, the production of l-glutamate after the application of Gaba could explain the increased heartbeat amplitude. However this possibility seems unlikely in view of the effects of Gaba on the electrical activity of the cardiac ganglion. If the increased heartbeat amplitude seen upon Gaba treatment

is due to the action of l-glutamate on the myocardium, then Gaba should only produce a decrease in the amplitude of the ganglionic bursts. However, the amplitude response of the ganglion to Gaba is also biphasic.

An alternate explanation for the biphasic amplitude response to Gaba is that through some means of synaptic potentiation, the initial reduction in beat amplitude gives way, after several seconds, to an increased beat amplitude. The prolonged ganglionic bursts seen in electrical recordings obtained several seconds after the application of Gaba tends to support the possibility that such synaptic potentiation occurs. The possibility that some type of synaptic potentiation occurs due to Gaba was also suggested by Maynard (1958) to account for certain characteristics of the response to Gaba shown by the lobster cardiac ganglion. A microelectrode analysis of the mechanisms of action of Gaba on the cardiac ganglion nerve cells should provide an explanation for the biphasic amplitude response.

Possible Sites and Mechanisms of Drug Actions

Although the pharmacological studies presented here were undertaken with the aim of surveying the effects of a number of different neuropharmacological agents on a spider heart, it seems instructive at this point to speculate as to the possible specific locations and mechanisms of drug actions

on the E. marxi heart. One mechanism of drug action by which changes in heart rate and heartbeat amplitude can be brought about is the alteration of synaptic processes to produce either an increase or a decrease in synaptic activity. Since the organization of the neural elements involved in cardiac function in E. marxi have yet to be elucidated, the location of synapses is unknown. However, there are a number of places where synapses are likely to occur, based on the assumption that the E. marxi heart is structured in a manner similar to that of other neurogenic hearts. Synapses may occur between cardioregulatory nerves from the central nervous system and the nerve cells in the cardiac ganglion, between afferent stretch receptor fibers from the heart musculature and the nerve cells in the ganglion, between the nerve cells in the ganglion and the heart muscle cells and between the various nerve cells in the ganglion themselves.

Another possible mechanism of drug action where changes in the heart rate could be produced involves the generation of pacemaker activity in the cardiac ganglion. If the generation of pacemaker potentials in the cardiac ganglion involves an intracellular action of one or more chemicals, a drug induced alteration in the pacemaker generation process would produce a change in the heart rate.

In this study, the principle actions of the drugs used were mediated on the neural elements of the cardiac ganglion, with the notable exception of l-glutamic acid. Even though

these drugs produced no recordable changes in myocardial tonus when applied to deganglionated hearts, the possibility remains that some or all of these drugs may produce subtle changes in the heart muscle cell membranes which are not directly reflected in a change in myocardial tonus. Such changes could produce a response greater than that seen due to drug effects on the neural elements alone.

Comparative Pharmacology of Neurogenic Hearts

Studies into the effects of various compounds on neurogenic hearts have been performed on three groups of arthropods; L. polyphemus (Merostomata), decapod crustaceans and scorpions. A number of the chemicals that have been investigated for their effects on the neurogenic hearts listed above have also been studied as to their actions on the E. marxi heart. This makes possible a comparison between the results obtained for E. marxi and those for the other neurogenic hearts.

Effects of Cholinergic Compounds

With the exception of scorpions, Ach, nicotine and methacholine produce a marked increase in the heart rate of neurogenic hearts due to their actions on the cardiac ganglion (Carlson, 1906; Welsh, 1939a,b; Davenport, 1941, 1942; Garrey, 1942). In these hearts, eserine potentiates the action of Ach, and except in L. polyphemus, the effect of Ach

is blocked by atropine. In scorpions, Ach produces a decrease in heart rate and atropine does not block this Ach effect (Zwicky, 1968). While eserine is reported to potentiate the action of Ach on the heart of one scorpion (Kanungo, 1957), it does not potentiate the effect of Ach on the heart of another (Zwicky, 1968).

A physiological role for Ach in neurogenic hearts has yet to be demonstrated. Conflicting results have been reported for the presence of Ach in the cardiac nerves of crustaceans (Welsh, 1939a; Florey, 1958, 1963). Although atropine blocks and eserine potentiates the effects of applied Ach on the decapod crustacean heart, atropine and eserine have been reported to be without effect on the actions of the cardioacceleratory nerves in decapods (Florey, 1963).

In L. polyphemus, cholinesterase has been reported present in the cardiac ganglion (Smith and Glick, 1939), but there have been no investigations into the presence of Ach in the cardiac nerves. Furthermore, there have been no studies into the effects of eserine and atropine on the actions of the cardioacceleratory nerves on the L. polyphemus heart.

In scorpions, Zwicky (1968) found that eserine enhanced the effects of the cardioinhibitory nerves in Urodacus novaehollandiae and on this basis concluded that cardiac inhibition in this scorpion is cholinergic. However, due to the lack of potentiation of the action of Ach by eserine,

Zwicky concluded that cardiac inhibition is mediated by a choline ester other than Ach.

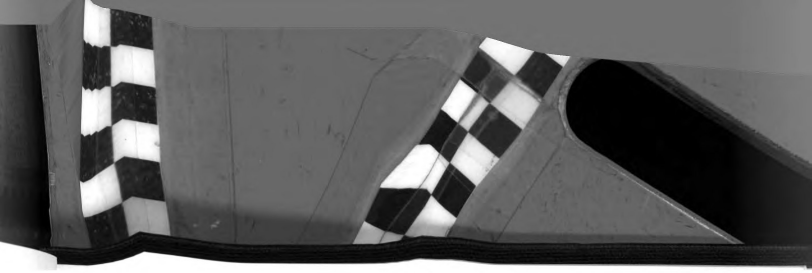
Effects of Catecholamines

Epinephrine produces a pronounced increase in the heart rate of L. polyphemus (Garrey, 1922), a scorpion (Kanungo, 1957) and decapod crustaceans (Bain, 1929; Welsh, 1939b). Other than the fact that epinephrine has a marked excitatory effect on these hearts, no additional information exists for a physiological role for catecholamines in neurogenic hearts.

Effects of Gaba

In addition to E. marxi, Gaba also has an inhibitory effect on the hearts of L. polyphemus (Pax and Sanborn, 1967a; Abbott et al., 1969) and decapod crustaceans (Florey, 1957; Maynard, 1961) and this effect is due to the action of Gaba on the cardiac ganglion.

A physiological role for Gaba has been suggested for decapod crustaceans by Florey (1957) and Maynard (1961) on the basis of the similarity between the actions of exogenous Gaba and the cardioinhibitory nerves on the heart. Pax and Sanborn (1967a) suggested that Gaba is probably not the chemical mediator of the L. polyphemus cardioinhibitory nerves, since picrotoxin blocks the actions of the inhibitory nerves, but not the effects of applied Gaba.



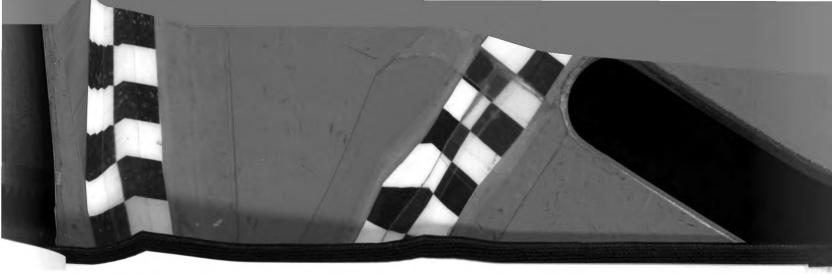
Effects of 5-Ht

5-Ht, not particularly effective on the heart of E. marxi, has a pronounced inhibitory effect on the heart of L. polyphemus and this effect is due to its actions on the cardiac ganglion (Burgen and Kuffler, 1957; Pax and Sanborn, 1967b). Since 5-Ht and stimulation of the cardio-inhibitory nerves produce similar effects on the heart of L. polyphemus, Pax and Sanborn (1967b) suggested that either 5-Ht or a related compound may be the chemical mediator of cardioinhibition in this animal.

In contrast to the inhibitory effects of 5-Ht on the heart of L. polyphemus, 5-Ht has an excitatory effect on the hearts of decapod crustaceans (Florey and Florey, 1954; Maynard and Welsh, 1959; Kerkut and Price, 1964; Belamarich and Terwilliger, 1966). Since 5-Ht and closely related compounds are present in the pericardial organs of decapod crustaceans and since these compounds mimic the actions of the pericardial organs on the heart, a 5-Ht-like compound is believed to be the neurohormone released by these organs in crustaceans (Carlisle, 1956; Kerkut and Price, 1964; Cooke 1966; Belamarich and Terwilliger, 1966).

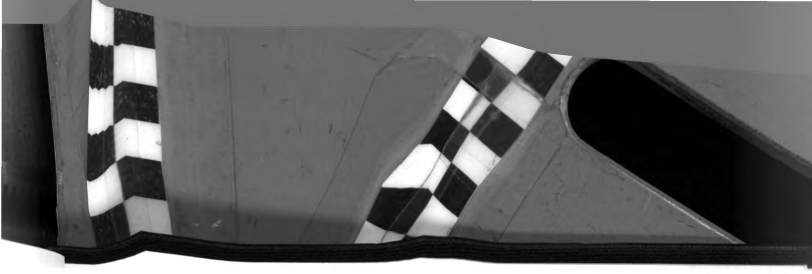
Effects of l-Glutamic Acid

l-Glutamic acid, as in E. marxi, has little effect on the heart rate of L. polyphemus (Pax and Sanborn, 1967a; Abbott et al., 1969). However, it produces sustained contractions of deganglionated hearts of both L. polyphemus



(Abbott et al., 1969) and E. marxi. To my knowledge, l-glutamate has not been tested on deganglionated hearts of crustaceans.

l-Glutamic acid is the only substance reported to have a direct effect on the myocardial cells of neurogenic hearts, although numerous other chemicals have been tested in this regard (Carlson, 1906; and this study).

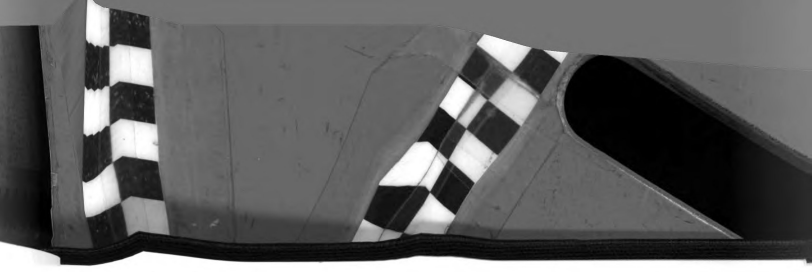


SUMMARY

The studies presented here provide a basis by which the heart of the spider Eurypelma marxi Simon can be compared to other arthropod hearts. The results obtained concerning the electrophysiology of the heart indicates that it is neurogenic like that of L. polyphemus, scorpions, and many of the higher crustaceans and that the electrophysiology of the E. marxi heart is very similar to that of the above forms. The pharmacological studies show that this heart is similar to the hearts of L. polyphemus and decapod crustaceans with respect to its responses to acetylcholine, catecholamines and gamma aminobutyric acid, but differs from the scorpion heart with respect to its responses to acetylcholine and also from the heart of decapod crustaceans with respect to its responses to 5-hydroxytryptamine.

These studies also provide a framework from which further investigations into spider cardiac physiology can be undertaken. Now that the role of the cardiac ganglion has been demonstrated, detailed studies into the origin and regulation of cardiac ganglion activity can be instituted. Furthermore, since a number of drugs have been found to produce marked changes in heart activity, investigations

into the mechanisms of drug action as well as into the physiological involvement of drugs in spider cardiac function can be conducted on the *E. marxi* heart.



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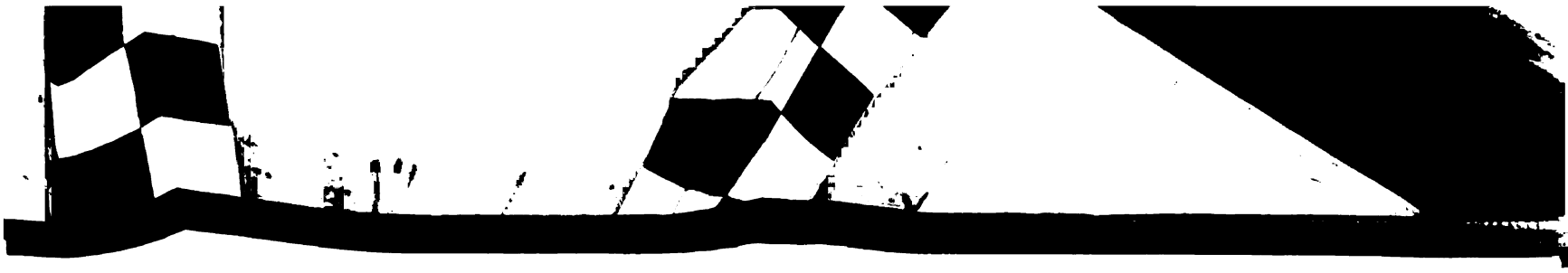

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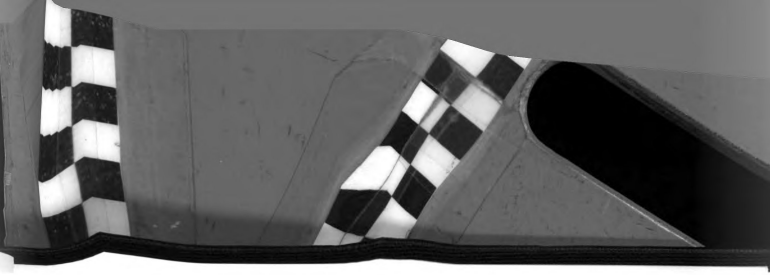

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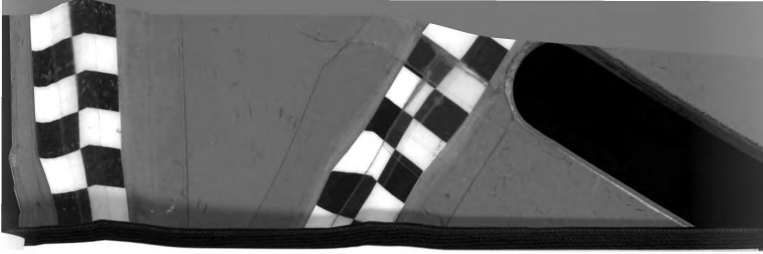

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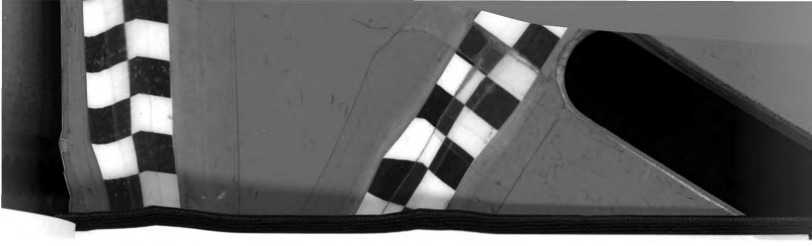

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