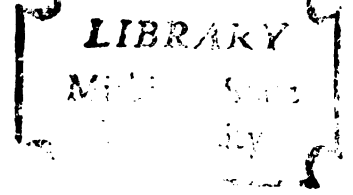


PHYSIOLOGICAL STUDIES ON
THE CROP-GIZZARD OF THE
EARTHWORM, LUMBRICUS TERRESTRIS

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
FRED CECIL DIVERS
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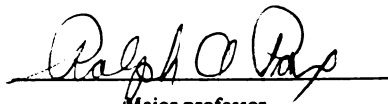
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ABSTRACT

PHYSIOLOGICAL STUDIES ON THE CROP-GIZZARD OF THE EARTHWORM, LUMBRICUS TERRESTRIS

By

Fred Cecil Divers

The earthworm crop-gizzard preparation has been reported to be a spontaneously active myogenic muscle preparation by previous workers who have examined the preparation by using either frog Ringer's or Pantin's earthworm saline as a bathing medium. Both of these earthworm salines differ considerably in ionic composition from the earthworm coelomic fluid. In the studies reported here the isolated and the intact crop-gizzard preparations have been re-examined with the use of a new earthworm saline, referred to as 6mM Ca saline, which is closer to the ionic composition of earthworm coelomic fluid than Pantin's earthworm saline.

Isolated crop-gizzard preparations bathed in Pantin's earthworm saline show spontaneous contractions which range in frequency from 2.6 ± 0.9 to 3.9 ± 0.8 contractions per minute. However, when isolated preparations are bathed in 6mM Ca saline no spontaneous contractions are observed.

The lack of spontaneous contractions in preparations bathed in 6mM Ca saline and the presence of such contractions in preparations bathed in Pantin's saline suggests that the presence or absence of spontaneous contractions in isolated preparations is due to the type of saline in which the preparations are bathed.

Intact crop-gizzard preparations bathed in Pantin's saline show increased levels of spontaneity while intact preparations bathed in 6mM Ca saline do not show such increases.

It appears likely that the increase in spontaneity of the intact and isolated crop-gizzard preparations bathed in Pantin's earthworm saline might be due to its low calcium concentration since intact preparations bathed in low calcium salines show the same spontaneous contractions seen in preparations bathed in Pantin's earthworm saline.

Spontaneous activity in the intact group-gizzard appears to be modulated by excitatory and inhibitory inputs from the central nervous system. The inhibitory pathway probably operates through the ventral nerve cord and the electrical stimulation of this pathway produces an average decline in the contraction rate of the crop-gizzard from 1.6 contractions per minute to 0.4 contractions per minute. At least one excitatory pathway originates in the brain and passes through the circum-esophageal connectives along the pharynx and the esophagus to the crop-gizzard. Electrical stimulation of this pathway produces an average increase in

the contraction rate of the crop-gizzard from 0.6 contractions per minute to 6.1 contractions per minute. The spontaneous activity also appears to be modulated by the amount of stretch exerted on the crop-gizzard preparation.

Electrical activity recorded from the intact crop-gizzard is closely correlated with the mechanical contractions. This observation suggests that the electrical activity might initiate these mechanical contractions in the crop-gizzard.

Experiments involving the removal of the brain from the animal show that all spontaneous activity is interrupted by this operation. This observation confirms the importance of the brain as the probable site of origin for the spontaneous activity seen in the intact crop-gizzard preparation.

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OF THE EARTHWORM, LUMBRICUS TERRESTRIS

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INTRODUCTION

General Properties of Smooth Muscle

In general smooth muscle occurs as part of complex tissues; frequently it is associated with secretory epithelium, often with nerve cells, nerve plexuses and endings. Smooth muscle cells are generally small, usually 2 to 5 μ in diameter. The activity of smooth muscle is sensitive to small changes in the environment and its response to external stimuli is often variable. Much smooth muscle is spontaneously active and its tone fluctuates from time to time. The physiological and pharmacological properties of smooth muscle also vary greatly depending upon; the type of organ from which it is taken, the age, physiological condition, sex, and the species. No two samples of smooth muscles from different organs are identical in their electrical and mechanical properties (Burnstock, Holman and Prosser, 1963).

The contractions seen in visceral type smooth muscle are often associated with the occurrence of one or more spike-type action potentials. In most spontaneously active smooth muscle the spikes arise from pacemakers which

may be localized or distributed throughout the smooth muscle. Bozler (1941) suggests that each smooth muscle cell of the gastro-intestinal tract possesses an inherent rhythmicity and that the results of the activity in pacemakers might be the asynchronous or synchronous firing of individual smooth muscle cells depending on such factors as excitability and the interaction between cells. Electrical records show that the pattern of the electrical activity varies for different smooth muscles and for the corresponding smooth muscles of different species. Electrical spikes may occur singly, in groups of two or three, or in rapid bursts separated by pauses in activity. Simple plateau-type action potentials without spikes or oscillations have been recorded from some types of smooth muscle. In many types of smooth muscle which have been investigated with intracellular electrodes, the largest spikes show an overshoot (Bülbring, 1962).

Generally speaking, most invertebrate smooth muscle is characterized by fluctuations in the membrane potential. These fluctuations are generally considered to be the basis for the rhythmical firing of action potentials and consequently, of the muscle activity. In smooth muscle sub-threshold activity appears to be of two kinds. In some cases it is nearly sinusoidal in appearance and is referred to as "slow wave" activity. In other cases the membrane potential decays slowly, depolarizes to a point where threshold is reached and an action potential is initiated.

The repolarization phase of the action potential restores the membrane potential to its original level (Burnstock, Holman and Prosser, 1963).

It has been fairly well established that the contraction of many invertebrate smooth muscle is associated and related to the electrical activity in the muscle (Bozler, 1941). It appears that the electrical activity activates the contractile mechanism in the smooth muscle. Not much is actually known about the mechanics of this process. There is sketchy evidence that both calcium and sodium are involved not only in the mechanism of contraction but also in the process which couples the action potential to the contractile apparatus. Much work is still needed in order to clarify the role of both calcium and sodium in the contraction of smooth muscle (Bozler, 1941).

Current scientific literature is rich in studies suggesting that the excitability of visceral smooth muscle can be modified by a variety of agents such as; transmitter substances, stretch, and various hormones. In general, those agents which depolarize the membrane lead to increased excitability and thus increased muscle contractions while those agents which hyperpolarize the membrane lead to a reduced excitability and reduced muscle contractions. Other agents may have a direct effect on the mechanism responsible for spontaneous activity without causing a change in resting potential (Laverack, 1963).

Most of the knowledge concerning the innervation of smooth muscle has come from pharmacological studies. Most of these studies suggest that smooth muscle is probably innervated by both excitatory and inhibitory nerve fibers. Excitatory nerves are thought to act by initiating junction potentials which sum and facilitate until a critical depolarization is reached. This depolarization leads to an action potential which produces the mechanical muscular contraction. The control of smooth muscle activity by nerves is largely by modulation of the resting potential (Sperlakis and Prosser, 1959).

There have been relatively few studies made on invertebrate smooth muscle preparations. These include: the esopagus of Arenicola (Whitewar, 1953), stomach activity in prosobranch gastropods (Fretter and Graham, 1962), the foregut of Aplysia (Yong, 1925), the radular muscles of Buccinum (Fang and Mattisson, 1958) and the crop-gizzard of Lumbricus (Wu, 1939; Ambache et al., 1945).

The present study will focus on the earthworm crop-gizzard.

The Anatomy of the Crop-Gizzard

The earthworm crop-gizzard located between segments 13 to 19 is part of the alimentary canal of the earthworm. The crop is a thin walled sac-like organ which is located anterior to the more muscular, thick walled gizzard. When the crop of an earthworm is opened under saline, folded

yellowish epithelium is forced out through the cut as if under pressure. The folds have the appearance of grooves and are arranged in longitudinal rows. The grooves in the crop probably serve to regulate the speed at which the contents of the crop pass into the gizzard (Arthur, 1963). Materials caught up in the channels between the folds will pursue a longer path in the maze before being released into the gizzard. It is thought that the spontaneous movements of the crop may aid in the mixing of the contents of the crop (Guardabassi, 1957; Roots, 1956).

The crop epithelium is separated from the underlying circular muscle by distinct connective tissue and muscle strands. The latter originate as fibers from the more compact circular muscle band (Arthur, 1963). The epithelial cells are of the columnar type having a striated border and covered with a cuticle. The blood vessels which supply the crop are longitudinally arranged beneath the epithelial layer.

The muscles of the gizzard are thick and are arranged in three bands beneath a thin peritoneal layer. The outer longitudinal band is about 0.01 mm thick, the middle layer of radially arranged muscles are 10 times thicker than the outer muscle band. The innermost layer of circular muscles is 0.85 mm thick. Scattered groups of nerve cells have been found between the muscle layers of the earthworm crop-gizzard by several workers (Ambache et al., 1945; Smallwood, 1923; Dawson, 1920 and Hess, 1925).

Columnar epithelial cells line the lumen of the crop-gizzard. These cells are covered by a thin layer of cuticle. There is an opening between the crop and the gizzard which can be occluded by a band of circular muscle which acts as a sphincter muscle that allows the contents of the crop to pass into the gizzard in small quantities (Roach, 1968).

Spontaneous Mechanical Activity in the Crop-Gizzard

The earthworm crop-gizzard contracts rhythmically as shown by Mines (1907) and by Wu (1937, 1939). These spontaneous mechanical contractions have been shown to persist for as many as 24 hours in isolated preparations bathed in frog Ringer's saline. The wave of contraction starts in the esophagus and gradually moves down into the crop-gizzard. The contraction waves of the crop-gizzard appear to be akin to the peristaltic waves seen in the human digestive tract. There is usually a short time lag between the contraction of the crop and that of the gizzard. This time lag is quite variable from one preparation to another (Collier, 1939).

Bayliss and Starling (1900) observed that the wave of contraction in the earthworm crop-gizzard is followed by a wave of relaxation. This contraction-relaxation process is referred to as the "contraction cycle" of the crop-gizzard. This cycle occurs two to three times a minute

in isolated crop-gizzard preparations which are bathed in frog Ringer's saline.

The Pharmacology of the Crop-Gizzard

The pharmacology of the earthworm crop-gizzard has been described by Wu (1939), Ambache et al. (1945), Millott (1943), and Blaschko et al. (1937).

The earthworm crop-gizzard is quite sensitive to Ach the effect being detectable down to 1×10^{-11} M. With high concentrations there is a sharp tone rise and the rhythmic contractions are abolished. With lower concentrations the tone rise is less pronounced and the rhythmic contractions persist with diminished amplitude and increased frequency. There is a graded series of responses over a wide range of different concentrations.

Eserine, in 1: 500,000 concentration causes a slight rise in tone, an increase in frequency and a decrease in amplitude. The effect of eserine is much like that of Ach (Wu, 1939).

The action of Ach on tone is not augmented by eserine even after hours of exposure to eserine but there is a slight increase in both the frequency and the amplitude of the contraction of the crop-gizzard when exposed to eserine and Ach together.

The action of Ach is abolished by atropine, 1: 1,000. By itself in the same concentration atropine has

a slight exciting action on the earthworm crop-gizzard preparation.

The crop-gizzard is inhibited by adrenaline in concentrations above a threshold which varies considerably in different preparations. If the application is repeated the inhibition becomes more marked. Below that threshold concentration the effect of adrenaline is variable; sometimes there is no effect, but often there is an exciting effect. Usually inhibition occurs in concentrations down to 1: 100,000 and excitation down to 1: 1,000,000 (Ambache et al., 1945).

Ergotoxine in concentrations of 1: 50,000 abolishes the inhibitory action of high adrenaline concentrations on the crop-gizzard. Applied in the absence of adrenaline, ergotoxine has no action on the crop-gizzard preparation at these concentrations (Millott, 1943).

Low concentrations of ephedrine increase the action of adrenaline on certain vertebrate tissues. In the earthworm crop-gizzard there is a parallel between the actions of ephedrine and eserine. Ephedrine in a concentration of 1: 10,000 causes a slight inhibition of the crop-gizzard. The action of ephedrine by itself resembles that of adrenaline, much as the action of eserine by itself resembles that of Ach. Possible the effect of ephedrine is due to a potentiation of an adrenaline-like substance already present in the crop-gizzard.

Choline chloride in concentrations of 1: 100,000 tends to slow the rhythm of the crop-gizzard but the effect is sometimes quite variable and unpredictable (Blaschko et al., 1937).

The action of histamine on the earthworm crop-gizzard is also variable. In some cases there is no clear-cut effect on tone or frequency in concentrations up to 1: 100,000. In some preparations lower concentrations produce either a definite inhibition of rhythm and tone, or excitation. Other preparations show a conflict between the two actions, showing alternating excitation and inhibition under the influence of histamine (Ambache et al., 1945).

The action of excess potassium and calcium has also been studied. Various amounts of KCl or CaCl₂ were added to a frog Ringer's saline before it was applied to the crop-gizzard preparation. Excess potassium at first excites and then in higher concentrations inhibits the contractions of the crop-gizzard preparation. Excess calcium causes a slowing of the rhythm, loss of tone and in extremely high concentrations causes complete stoppage of the contractions in the relaxed state.

Crop-gizzard examined at room temperatures, warm preparations, with the use of cocaine in concentrations of 1: 200 showed an increase in the amplitude of the spontaneous contractions. The inhibitory effect of cocaine becomes manifest only with higher concentrations. An arrest of

rhythmic activity is produced by increasing the concentration of cocaine to 1: 100. At this concentration the preparation becomes insensitive to Ach, indicating an impairment of the muscle fibers.

Nicotine, in concentrations of 1: 100, differs from cocaine in its action on crop-gizzard preparations in that it causes cessation of peristaltic activity without loss of excitability to Ach. Nicotine abolishes the excitatory effect of barium at a concentration of 2×10^{-4} M.

The effects of potassium-free and calcium-free frog Ringer's have been studied. The crop-gizzard reacts very similarly to a lack of calcium or potassium. There is a noticeable disturbance in the rhythm and the frequency of the contractions of the crop-gizzard. Lack of potassium combined with an increase in calcium concentration leads to a rapid twitching uncoordinated beat (Wu, 1939).

Chloretone in all concentrations completely abolishes the rhythmic spontaneous contractions of the earthworm crop-gizzard (Ambache et al., 1945).

OBJECTIVES

There have been relatively few studies performed on the physiology of the earthworm crop-gizzard. Those studies which have been made are not recent ones. More important, all of the previous studies on the earthworm crop-gizzard have examined only the isolated preparation with the use of either Pantin's earthworm saline, a commonly used earthworm saline, or frog Ringer's saline. Both of these saline solutions differ significantly from the coelomic fluid of the earthworm in terms of ionic composition.

The primary objective of this study was to re-examine some of the physiology of both the isolated and the intact crop-gizzard in light of a new earthworm saline which is more comparable in terms of ionic composition with the earthworm coelomic fluid. Initially, the isolated preparation was examined with the use of Pantin's saline and the new saline in order to determine if there are any differences in the responses of the crop-gizzard to these two earthworm salines. These experiments were repeated using the intact preparation in order to determine if the

intact preparation responds differently to the two saline solutions. These results should indicate whether or not the spontaneous rhythms reported by previous workers for isolated crop-gizzard preparations are indeed "normal." These results might also suggest whether or not the crop-gizzard activity is neurogenic or myogenic in origin.

Another objective of this study was to investigate the origin and the control of the spontaneous activity in the crop-gizzard. This required an investigation of the electrical activity of the intact preparation as well as an investigation of the influence of the central nervous system on the activity of the crop-gizzard. These investigations involved the electrical stimulation of the brain and the ventral nerve cord as well as ablation experiments. The results of these experiments should give some indications of the role of the central nervous system in the control and the modulation of the spontaneous activity in the crop-gizzard.

Intralumenal pressure studies were also made in order to determine whether or not the amount of stretch within the lumen of the crop-gizzard can serve to modulate the spontaneous activity in the intact organ.

The findings from these experiments should provide a better understanding of the spontaneity of the earthworm crop-gizzard.

MATERIALS AND METHODS

Procurement and Maintenance of Experimental Animals

Adult earthworms, Lumbricus terrestris, obtained from a commercial supplier (Wholesale Bait Company, Hamilton, Ohio) or collected locally were used in these experiments. Before use these animals were stored in Buss-Bedding (Buss Manufacturing Company, Lanark, Illinois), in a cold room maintained at temperatures between 12 to 15 C°.

Preparation of Animals for Study

Prior to dissection the animals were pinned out in a paraffin tray and covered with earthworm saline. The crop-gizzard was carefully dissected from the animal and immediately placed in fresh earthworm saline. These crop-gizzard preparations were used in experiments involving isolated preparation studies.

In those experiments involving intact studies, the animals were pinned in a paraffin try and covered with earthworm saline. A short slit was made in the dorsal body wall between segments 13 to 19. This slit was extended laterally on both sides so as to produce an "oval-window"

opening directly over the crop-gizzard. The exposed crop-gizzard could then be observed visually and both mechanical and electrical recordings could be made while the crop-gizzard was still intact.

Except where noted, during all of these dissections and at all times thereafter the crop-gizzard preparation was covered with fresh applications of earthworm saline which was maintained at room temperature (20 to 22 C°).

Prior to mechanical and electrical recordings from the intact crop-gizzard, all of the body segments posterior to the clitellum were removed. This operation eliminated all excessive body movements while the recordings were being made. Preliminary experiments indicated that this operation did not affect the "normal" functioning of the intact crop-gizzard.

In those experiments involving the stimulation of the brain, the dorsal body wall was opened along the midline so that both the crop-gizzard and the brain were exposed.

Mechanical Recordings

The mechanical activity of both the isolated and the intact crop-gizzard preparation was recorded by means of a micro-displacement myograph transducer (Linear Core F-50, E and M Instrument Company, Houston, Texas) and displayed on a physiograph (Narco Biosystems Incorporated, Houston, Texas).

Electrical Stimulation and Recording

Electrical stimulation, consisting of square-wave pulses, was delivered to the brain and the ventral nerve cord by means of a Grass stimulator (Model S-4, Grass Instrument Company, Quincy, Massachusetts) through a suction type stimulating electrode.

The electrical activity of the crop-gizzard was recorded by means of a suction electrode which was applied lightly to the surface of the crop-gizzard with a minimal amount of suction. The electrical activity was amplified by means of a Grass preamplifier (Model P-15, Grass Instrument Company, Quincy, Massachusetts) and displayed either on an oscilloscope (Model 502A, Tektronix Company, Chicago, Illinois) or on a physiograph (Narco Biosystems Incorporated, Houston, Texas).

Intraluminal Pressure Studies

The intraluminal pressure of the crop-gizzard was increased or decreased by either injecting known amounts of saline into the lumen or withdrawing known amounts of materials from the lumen. This operation was accomplished with a syringe and needle which could be carefully inserted into the lumen of the crop-gizzard with the use of a micro-manipulator. The use of a calibrated syringe allowed for an accurate measurement of the amount of material which was either added to or withdrawn from the lumen of the crop-gizzard.

Physiological Salines

In these experiments two types of physiological saline solutions were used; Pantin's earthworm saline (Pantin, 1948) and a new earthworm saline which, solely for the sake of convenience, I will refer to as 6mM Ca earthworm saline. The ionic composition of these two earthworm salines is shown in Table 1. The 6mM Ca earthworm saline is closer to the actual ionic composition of the earthworm coelomic fluid than Pantin's earthworm saline. One can see that there is quite a large variation in the concentrations of the various ions found in these two earthworm salines. The concentrations and ionic composition of the 6mM Ca earthworm saline is based upon an analysis of earthworm coelomic fluid which was made by Kamemoto et al. (1962).

Table 1. Ionic compositions of Pantin's and 6mM Ca earthworm salines.

Ions	Pantin's Saline (mM/l)	6mM Ca Saline (mM/l)
Na	135.0	77.0
K	2.7	4.0
Ca	1.8	6.0
Mg	0.4	1.0
Cl	142.0	43.0
SO ₄	0.4	26.0
PO ₄	1.0	. .
Tris	. .	2.0
Sucrose	. .	55.0
mOsm	210.0	167.0
pH	7.4	7.4

RESULTS

Mechanical Activity in the Isolated Crop-Gizzard Preparation

The initial experiments of this study were designed to compare the mechanical activity of the isolated crop-gizzard preparation when exposed to Pantin's earthworm saline and to 6mM Ca saline.

Initially, 12 isolated preparations were examined with the use of Pantin's earthworm saline. Bathed in this saline, all 12 isolated crop-gizzard preparations showed spontaneous mechanical activity. These mechanical contractions were recorded and displayed on physiograph records. Figure 1 shows a typical record of the type of responses that were seen. One can note the rhythmic nature of the contractions as well as the relatively constant amplitude of these contractions in this record. However, the frequency and the amplitude of the contractions showed variability from one isolated preparation to another. Table 2 shows the variability in the mean frequency of contractions of the 12 isolated preparations which were examined using Pantin's earthworm saline. Contraction rates shown in the table were determined by counting the

Figure 1. Physiograph record showing the spontaneous mechanical contractions of an isolated crop-gizzard preparation which was bathed in Pantin's earthworm saline. Calibration: amplitude = 2.5 g. Time markes = 1.0 Min.

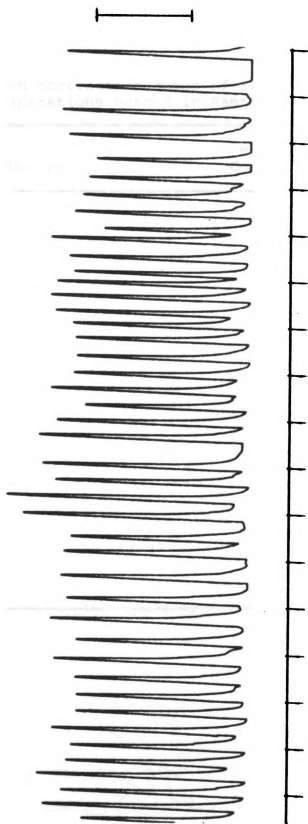


Figure 1

Table 2.--Mean contraction rates of isolated crop-gizzard preparations bathed in Pantin's earthworm saline.

Preparation Number	Mean Contraction Rate Contractions/Min $\pm$ S.D.
1	3.2 $\pm$ 0.6
2	3.4 $\pm$ 0.5
3	2.8 $\pm$ 0.5
4	3.2 $\pm$ 0.6
5	3.2 $\pm$ 0.4
6	3.5 $\pm$ 0.5
7	3.9 $\pm$ 0.9
8	3.3 $\pm$ 0.5
9	2.6 $\pm$ 0.9
10	3.3 $\pm$ 0.5
11	2.8 $\pm$ 0.6
12	3.1 $\pm$ 0.6

number of contractions for a 30 minute time period for each preparation and then determining the mean and the standard deviation of these contractions. The mean frequency of the contractions for the 12 preparations ranged from 2.8 ± 0.5 to 3.9 ± 0.8 contractions per minute. The average contraction frequency was 3.1 contractions per minute for the 12 preparations. The time intervals between the spontaneous contractions of the crop-gizzard are quite variable with a range of 10 to 60 seconds being observed within single preparations. The average tension developed by these contractions was 3.5 g.

Perfusion of the Isolated Crop-Gizzard Preparation

In an effort to determine some of the sources of variability seen in the previous studies a series of experiments examining the effects of perfusion were performed. A cannula was inserted into each end of the preparation so that saline flowed from a suspended container into the inlet cannula, through the lumen of the preparation and out through the outlet cannula. The rate of saline flow was controlled with a pinch clamp inserted on the Tygon tubing between the suspended saline container and the inlet cannula.

Table 3 shows the mean results of five experiments. The perfusion rate was measured in drops per minute flowing out of the outlet cannula. Prior to the perfusion of the isolated preparation the average rate of contraction was

Table 3. Average numbers of contractions per minute for isolated crop-gizzard preparations perfused at various rates with Pantin's earthworm saline.

Preparation Number	Perfusion Rate Drops/Min				
	0	10	20	30	40
1	2	4	5	7	0
2	4	4	6	8	0
3	2	3	5	6	0
4	3	5	8	9	0
5	2	3	5	8	0
$\bar{x}$	2.6	3.8	5.8	7.6	0.0

2.6 contractions per minute. When the preparations were perfused at rates of 10, 20, 30, and 40 drops per minute the average contraction rates were 3.8, 5.8, 7.6, and 0.0 respectively. When the perfusion rate was increased to 40 drops per minute no contractions were observed in the isolated preparation. Because of the perfusion arrangement used the major effect produced by changes in the perfusion rate was a stretch of the crop-gizzard walls. Thus a perfusion rate of 40 drops per minute caused a great deal of distension of the preparation and this probably accounts for the lack of activity at this perfusion rate. At the lower perfusion rates it is clear that as the intraluminal pressure is increased the contraction rate of the isolated preparation also increases.

Thus, it is obvious that the mechanical activity of the isolated preparation can be modulated by the amount of intraluminal stretch to which the preparation is subjected.

Responses of Isolated Crop-Gizzard
Preparation to Alternate Changes
Between Pantin's and 6mM Ca
Earthworm Salines

In the first group of experiments in this series, eight isolated crop-gizzard preparations were bathed in 6mM Ca earthworm saline. Bathed in this earthworm saline, none of the eight preparations showed any spontaneous mechanical contractions.

In order to show that the spontaneity of the isolated crop-gizzard is affected differently by Pantin's earthworm saline and 6mM Ca earthworm saline, six preparations were exposed to alternate changes between Pantin's saline and 6mM Ca saline. Figure 2 shows average results for the six isolated preparations for each 5 minute time period covering a 150 minute total time span. Saline changes were made after each 30 minute time period. During those time periods when the preparations were being bathed in Pantin's earthworm saline spontaneous contractions were recorded. The number of spontaneous contractions ranged from 3 to 12 contractions per 5 minute time period. When the preparations were changed to 6mM Ca earthworm saline no spontaneous contractions were recorded.

From these experiments it can be concluded that the presence of spontaneous contractions when the isolated

Figure 2. Average number of contractions per 5 minute for six isolated crop-gizzard preparations which were bathed alternately in Pantin's earthworm saline and 6mM Ca earthworm saline. The preparations were bathed in Pantin's earthworm saline from 30 to 65 minutes and from 100 to 115 minutes. During all other times the preparations were bathed in 6mM Ca earthworm saline.

preparations are bathed in Pantin's earthworm saline and the lack of contractions in 6mM Ca earthworm saline is due to the saline. The lack of spontaneous contractions in the isolated crop-gizzard preparation when bathed in 6mM Ca saline, which is closer to the composition of earthworm coelomic fluid, and the presence of such contractions in Pantin's earthworm saline suggests that the presence or absence of spontaneous contractions in isolated preparations is due to the type of saline in which they are bathed.

Mechanical Activity in the Intact Crop-Gizzard Preparation

The intact crop-gizzard preparation was examined in order to determine the contraction rate when the preparation was bathed in its own coelomic fluid. Also the two saline solutions were tested and compared with the "normal" situation. In this way one could determine whether Pantin's earthworm saline or the 6mM Ca earthworm saline gave responses nearer the "normal" response. In addition the intact preparation was examined to see what the contraction pattern was when the crop-gizzard preparation had its neural connections intact. This could not be determined using the isolated preparation. By using the intact crop-gizzard preparation one can also compare the effects produced by the salines to those produced by the electrical stimulation of various parts of the central nervous system.

In six initial experiments intact crop-gizzard preparations were tested without the use of any earthworm

saline. In order to prevent drying of the crop-gizzard a drop of mineral oil was placed over the slit which was made in the body wall for observation of the crop-gizzard. Under these conditions the contractions of the intact preparation occurred at very irregular time intervals. These time intervals ranged from 30 seconds to 5 minutes, the average time between spontaneous contractions being 1.5 minutes. The mean number of contractions for six preparations bathed with the drops of mineral oil was 2.3 ± 0.3 contractions per 5 minute time period. This rate was less than one-fifth that seen in isolated preparations which were bathed in Pantin's earthworm saline. The average tension developed in these contractions was 1.5 g.

Six intact crop-gizzard preparations were also bathed in the Pantin's earthworm saline. Under these conditions the preparations also showed irregular patterns of contractions. The time intervals between contractions for intact preparations bathed in Pantin's earthworm saline, however, were considerably shorter than those observed in the preparations which were bathed in mineral oil. The average time interval between the spontaneous contractions was 30 seconds. It should be noted that the average time interval between contractions in the intact preparation bathed in Pantin's earthworm saline was the same as it was for isolated preparations which were bathed in Pantin's earthworm saline. The mean contraction rate for those preparations bathed in Pantin's earthworm saline

was 14.0 ± 4.5 contractions per 5 minute time period; a rate also nearly equal that seen in isolated preparations bathed in Pantin's earthworm saline. The average tension developed in these contractions was 2.5 g.

In preparations bathed in 6mM Ca earthworm saline, the spontaneous contractions also occurred at irregular time intervals. For six preparations the time intervals between contractions ranged from 40 seconds up to 5 minutes, the average time interval between contractions being 2.0 minutes. The mean frequency of the contractions was 2.6 ± 0.5 contractions per 5 minute time period; a rate nearly equal to that seen in intact crop-gizzard preparations bathed in mineral oil. The average tension developed by these contractions was 1.6 g.

Figure 3 shows typical results obtained from three preparations used in this series of experiments. Each point on this figure was determined by counting the total number of contractions during each 5 minute time period. One can note that the preparation which was bathed in Pantin's earthworm saline (the closed circles on the figure) had a markedly higher rate of contraction than did the preparation which was bathed in mineral oil (the squares on the figure). In 6mM Ca earthworm saline (the open circles on the figure) the rate of contraction closely approximates that seen in preparations bathed in mineral oil.

Observations on the intact crop-gizzard preparation without the use of any type of earthworm saline suggests

Figure 3. Graph showing the number of contractions per 5 minute for three different intact crop-gizzard preparations bathed in mineral oil (dark squares), 6mM Ca earthworm saline (open circles) and Pantin's earthworm saline (dark circles).

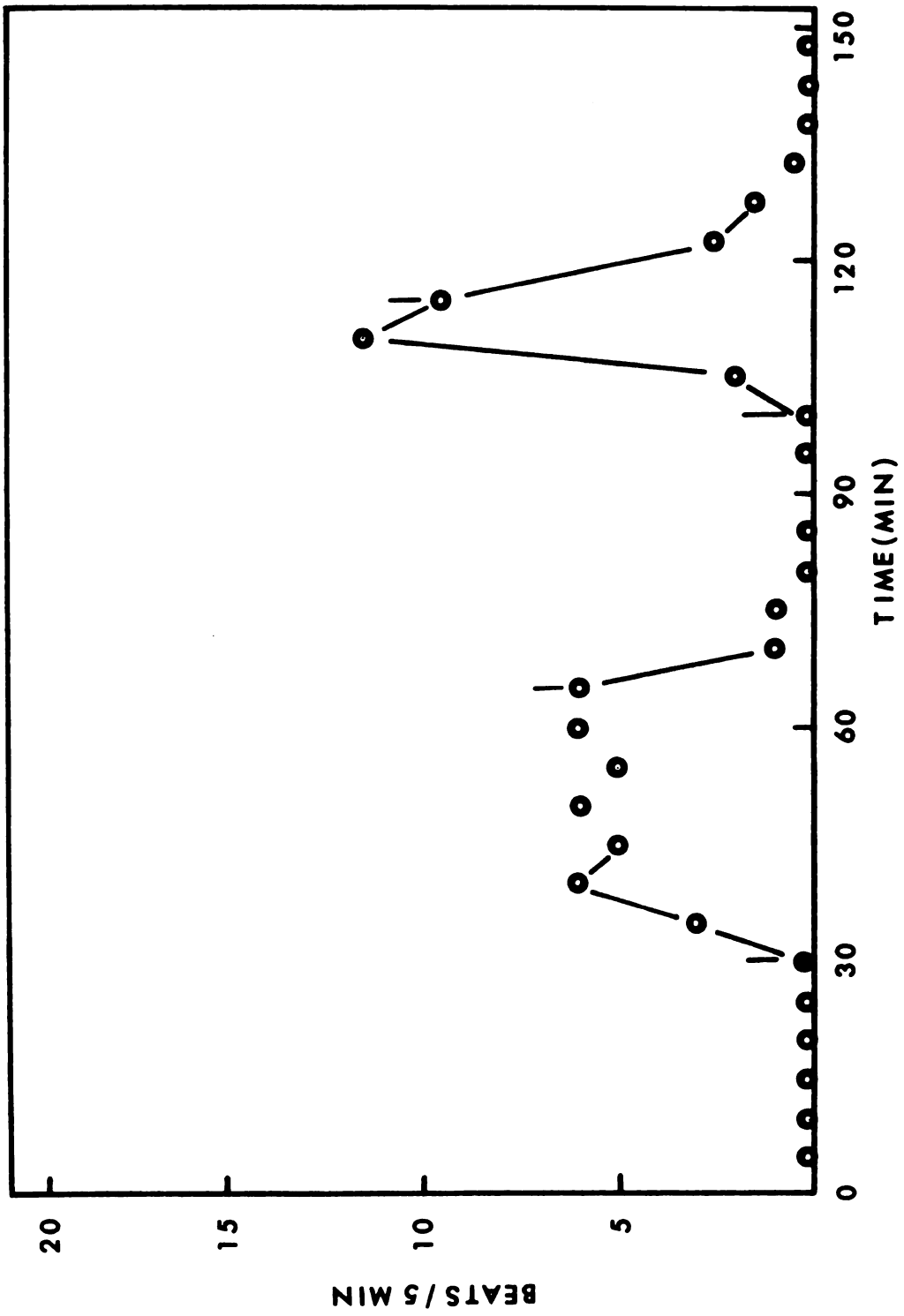


Figure 3

that the spontaneity seen under these conditions is probably "normal." When intact preparations are bathed in 6mM Ca earthworm saline a similar spontaneity is seen. However, when these preparations are bathed in Pantin's earthworm saline, a substantial increase in the rate of contraction is seen. This suggests that the increase in the rate might be due to the effects of the saline.

When isolated preparations are bathed with the 6mM Ca earthworm saline no spontaneous contractions were seen. When isolated preparations were bathed with Pantin's earthworm saline spontaneous contractions were observed. From these observations it seems likely that the use of Pantin's earthworm saline either: (1) induces spontaneity in the isolated crop-gizzard; or (2) increases the degree of spontaneity in the intact crop-gizzard preparation above the "normal" level.

The "normal" spontaneity seen in the intact crop-gizzard preparation occurs at a different rate from the spontaneity induced in the isolated crop-gizzard preparation by the use of Pantin's earthworm saline. This may be due to a lack of modulating influences of the central nervous system caused by the removal of the crop-gizzard preparation from the body of the animal.

The Effects of Different Calcium
Concentrations on the Spontaneity
of the Crop-Gizzard

Many workers who have studied spontaneous nerve and muscle activity believe that the calcium level of the external bathing medium is a key factor in nerve and muscle activity. Therefore, the effects of different calcium concentrations were tested on the intact crop-gizzard preparation. In these experiments only the calcium concentration of 6mM Ca earthworm saline was changed; all other ionic concentrations as well as the osmotic concentration were kept at concentrations equal to that of the earthworm coelomic fluid. The osmotic concentration was adjusted by adding sucrose to the saline.

Table 4 shows the average results of 20 experiments where the intact crop-gizzard preparation was bathed in earthworm salines which had different calcium concentrations. This table shows that the average rate of contraction is higher in the salines which had the lower calcium concentrations and the contraction rate is lower in the salines which had the higher calcium concentrations. At a calcium concentration near that present in Pantin's earthworm saline the rate of contraction is nearly equal that seen in Pantin's earthworm saline.

The results of these experiments confirm the reports of other workers who have shown that the level of spontaneity appears to be directly related to the level of external calcium present in the bathing medium. Also from

Table 4. The effects of various calcium concentrations on the contraction rate of the intact crop-gizzard.

Calcium Concentration of the saline (mM/l)	Average Contraction Rate Contractions/Min ± S. D.
1	6.2 ± 0.4
3	4.1 ± 0.2
6	2.3 ± 0.2
12	1.0 ± 0.3
18	0.3 ± 0.2

these experiments it appears that the calcium ion is of primary importance since even though there are major differences between Pantin's earthworm saline and the saline I have used here with respect to a number of other ions, lowering of the calcium concentration to that in Pantin's earthworm saline produces a contraction rate equal to that seen in Pantin's earthworm saline.

Stimulation of the Brain

Brain stimulation experiments were performed in order to determine whether or not the spontaneous mechanical activity in the intact crop-gizzard preparation could be modified by these stimulations. The results should establish whether or not an excitatory pathway exists between the brain and the intact crop-gizzard. These experiments should also indicate whether or not the brain is the beginning of this excitatory pathway. Stimulation

of this proposed pathway should give some indications of the influence of the central nervous system on the spontaneous activity in the intact crop-gizzard.

In these experiments the brain of the earthworm was electrically stimulated by means of a suction type stimulating electrode approximately 100 μ in diameter at its tip. This electrode was applied to the dorsal surface of the brain with a minimum amount of suction. Stimulations consisted of square wave pulses delivered at a rate of 1/second for 1 minute (60V, 5 msec.). Mechanical activity was recorded with a myograph transducer and displayed on a physiograph. Prior to the stimulations a base-line rate of contraction was determined for each preparation studied and this rate was compared to that obtained during the stimulation of the brain.

The contraction rates of the intact crop-gizzard observed during the stimulation of the brain of 5 separate animals are shown in Table 5. As seen in this table there is a substantial increase in the contraction rates of the crop-gizzard preparation during the period of stimulation. This increase in contraction rate ranged from 3.5 to 6.4 contractions per minute during the period of stimulation. The average increase in the contraction rate during the stimulations for the 5 preparations was 4.8 contractions per minute; a rate nearly ten times higher than the rate prior to stimulation. The average increase in tension

Table 5. Comparison of the contraction rate of the crop-gizzard prior to and during brain stimulation.

Preparation Number	Mean Rate Prior to Stimulation Cont./Min ± S.D.	Mean Rate During Stimulation Cont./Min ± S.D.	Change In Rate Cont./Min
1	0.6 ± 0.2	4.1 ± 0.1	+ 3.5
2	0.5 ± 0.1	6.1 ± 0.1	+ 5.6
3	0.6 ± 0.1	7.0 ± 0.2	+ 6.4
4	0.5 ± 0.1	4.4 ± 0.1	+ 3.9
5	0.5 ± 0.2	5.0 ± 0.3	+ 4.5

developed by the intact preparations during the period of stimulation was 1.0 g.

In order to be sure that the increase in the contraction rate of the crop-gizzard was due only to the stimulation of the brain and not from the passive spread of current along the body wall from the stimulating electrode, the circumesophageal connectives around the brain were cut in several preparations prior to the stimulation of the brain. The cutting of these connectives around the brain stopped all spontaneous activity in the crop-gizzard and stimulation of the brain after the connectives were cut failed to produce any spontaneous contractions in the crop-gizzard. From these results it appears that at least one excitatory pathway passes by way of the brain via the circumesophageal connectives to the crop-gizzard and that the electrical stimulation of this excitatory pathway

produces an increase in the mechanical activity in the crop-gizzard. These results also suggests that the brain might also be the site of origin of the spontaneous mechanical activity seen in the intact crop-gizzard preparation.

The Cutting of the Ventral Nerve Cord

In order to more precisely determine the pathway by which activity in the crop-gizzard is modulated by the nervous system a series of experiments were performed to determine whether the cutting of the ventral nerve cord affects the level of spontaneity in the intact crop-gizzard. The cutting of the ventral nerve cord was made two segments anterior to the crop-gizzard. Prior to cutting of the ventral nerve cord, base-line rates of contractions were determined for each of the preparations studied. Six preparations studied showed an overall decline in the contraction rate after the ventral nerve cord was cut. The average decrease in the contraction rate was 0.6 contractions per minute. Table 6 shows that the spontaneous activity in the crop-gizzard is probably modulated by the activity of the ventral nerve cord. Thus it appears likely that at least one other pathway of neural control of the crop-gizzard is through the ventral nerve cord. The decline in activity after ventral nerve cord cutting would seem to indicate that an excitatory pathway to the crop-gizzard had been interrupted. However, it is equally

Table 6. Effect of stimulation of the ventral nerve cord on the contraction rate of the intact crop-gizzard.

Preparation Number	Rate Before Cord Was Cut Cont./Min ± S.D.	Rate After Cord Was Cut Cont./Min ± S.D.	Rate During Stim Cord Cont./Min ± S.D.
1	1.4 ± 0.4	0.8 ± 0.2	0.4 ± 0.1
2	0.4 ± 0.1	0.2 ± 0.3	0.0 ± 0.0
3	2.8 ± 0.4	2.0 ± 0.3	0.6 ± 0.1
4	1.0 ± 0.2	0.4 ± 0.1	0.2 ± 0.1
5	2.4 ± 0.3	1.8 ± 0.4	0.6 ± 0.2

possible that cutting of the ventral nerve cord released an inhibitory pathway so that the net result seen was a decline in the activity in the crop-gizzard. These results seem to indicate that the pathway leading from the ventral nerve cord to the intact crop-gizzard could be either excitatory or inhibitory.

The Stimulation of the Ventral Nerve Cord

In order to more clearly demonstrate the nature of this pathway electrical stimulation of the ventral nerve cord was done to determine its effect on the spontaneous activity in the intact crop-gizzard. As in the previous series of experiments, base-line rates of contractions were determined before the stimulations were begun. Prior to the stimulations the ventral nerve cord was cut two segments anterior to the crop-gizzard. The cut end of the ventral nerve cord nearest the crop-gizzard was sucked into the end of a suction electrode and stimulated. Stimulations consisted of square wave pulses delivered at a rate of 1/second for 1.0 minute (60V, 5 msec). Mechanical contractions were recorded with a myograph transducer and displayed on a physiograph.

Table 6 shows the results of 5 experiments in which the rates of contraction of the crop-gizzard were compared prior to the cutting of the ventral nerve cord, after the cutting of the ventral nerve cord and during stimulation of the ventral nerve cord. The average contraction rate prior

to the cutting of the ventral nerve cord for 5 preparations was 1.6 contractions per minute. After the ventral nerve cord was cut the contraction rate average declined to 1.0 contractions per minute. During the stimulation of the ventral nerve cord the contraction rate average declined further to 0.4 contractions per minute.

Since stimulation of the ventral nerve cord produces a greater decline in the contraction rate than cutting of the ventral nerve cord does, it appears likely that there is an inhibitory pathway in the ventral nerve cord which innervates the crop-gizzard and produces a decline in the contraction rate of the crop-gizzard when electrically stimulated.

In a final group of experiments in this series, the effects of brain stimulation was studied after the ventral nerve cord was cut two segments anterior to the crop-gizzard. In four preparations the average contraction rate before the ventral nerve cord was cut was 1.4 contractions per minute. After the ventral nerve cord was cut, the average contraction rate for these four preparations declined to 0.7 contractions per minute. Stimulation of the brain after the ventral nerve cord had been cut could still produce an increase in the contraction rate of the crop-gizzard, the average rate of contraction seen during the stimulation of the brain being 1.0 contractions per minute. Figure 4 presents a summary of the results obtained from the stimulation and ablation experiments.

Figure 4. Changes in contraction rate of intact crop-gizzard preparations as a result of: (a) stimulation of the brain, (b) stimulation of the ventral nerve cord, and (c) the cutting of the ventral nerve cord. The zero line represents an average base-line rate of 8.0 contractions per 5 minute. The preparations were bathed in 6mM Ca earthworm saline.

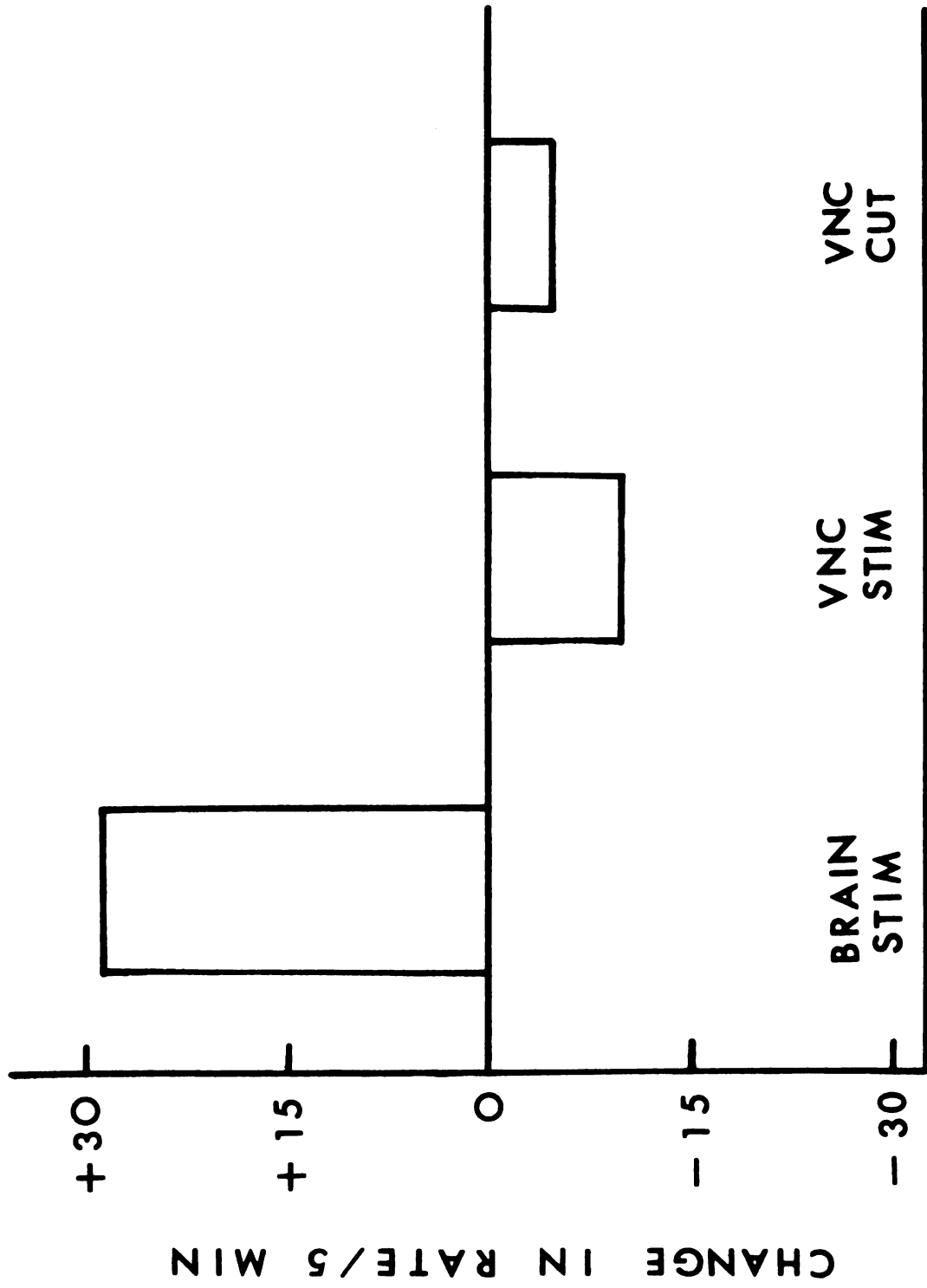


Figure 4

The results of these experiments seem to indicate that: (1) an excitatory pathway originating in the brain goes through the circum-esophageal connectives to the crop-gizzard, (2) the excitatory pathway does not go through the ventral nerve cord but must leave the ventral nerve cord anterior to the point where the ventral nerve was cut, (3) an inhibitory pathway appears to go down the ventral nerve cord, and (4) cutting of the ventral nerve cord must cause increased inhibitory activation either by the release of the inhibitory pathway upon cutting or because of spontaneous activity in the pathway caused by damage of cutting the ventral nerve cord.

It would appear that the level of spontaneity seen in "normal" intact crop-gizzard preparations is probably the result of both excitatory and inhibitory inputs from the central nervous system. Electrical stimulation of either the excitatory or the inhibitory pathways modulates the spontaneous activity in the earthworm crop-gizzard.

Ligation of the Oesophagus

From the previous experiments it appears that there exists an excitatory nerve pathway to the crop-gizzard from the brain through the circum-oesophageal connectives but that these pathways do not follow the ventral nerve cord to the crop-gizzard. Instead they must leave the ventral nerve cord at some point anterior to the crop-gizzard and follow some other route to the crop-gizzard. The most probable

other route would be the walls of the pharynx and the oesophagus.

In four separate experiments the oesophagus was ligated tightly two segments anterior to the crop-gizzard with a cotton thread. The purpose of these experiments was to determine the course of the excitatory pathway after it leaves the brain. Base-line rates of contractions averaged 1.2 contractions per minute prior to the ligation of the oesophagus. After the ligation of the oesophagus the average rate of contraction of the crop-gizzard declined to 0.6 contractions per minute. In two cases where the removal of the ligature was successful, the contraction rate of the crop-gizzard returned to its original rate. Cutting of the oesophagus two segments anterior to the crop-gizzard caused a complete cessation of the spontaneous activity in the preparation. Ligation of the intestinal area posterior to the crop-gizzard had no effect on the contraction rate of the crop-gizzard.

The results of these experiments suggests that at least one pathway of excitation to the crop-gizzard leads along the wall of the oesophagus in its route to the crop-gizzard.

Removal of the Brain

In an attempt to more clearly demonstrate the importance of the brain for the excitatory pathway to the crop-gizzard, in six animals the brain was removed.

Base-line rates of contractions were determined before the brain was removed. The average contraction rate for the six preparations was 1.5 contractions per minute. Removal of the brain caused a complete cessation of all rhythmic activity in the crop-gizzard. These results confirm the importance of the brain as the site of origin of excitatory pathways to the crop-gizzard.

Cutting of the Septa Around the Crop-Gizzard

In this series of experiments the septa around the crop-gizzard were cut in an effort to determine if these structures are involved in the excitatory or inhibitory pathways leading to the crop-gizzard. In four experiments base-line rates of contractions were determined before the septa around the crop-gizzard were cut. The average contraction rate was 1.4 contractions per minute before the septa were cut. After the septa were cut all spontaneous contractions in the crop-gizzard stopped immediately. From these results two important questions are raised: (1) do the excitatory and inhibitory pathways pass through the septa before they innervate the crop-gizzard? (2) do the intact septa provide stretch on the preparation and cause it to contract? These experiments do not provide a definite answer to either of these questions. However, the possibility exists that the cessation of activity in the crop-gizzard may have been due to an interruption of an

excitatory pathway or the release of an inhibitory pathway caused by the cutting of the septa. A decrease in the amount of stretch on the crop-gizzard caused by the cutting of the septa is an equal possibility. Therefore, the role of the septa in the excitatory or the inhibitory pathway to the crop-gizzard remains unclear.

The results of the previous groups of experiments would seem to indicate that the spontaneous activity in the crop-gizzard is modulated by excitatory nerves which originate in the brain and pass along the pharynx to the crop-gizzard. This pathway appears to leave the ventral nerve cord anterior to the crop-gizzard. An inhibitory pathway appears to pass along the ventral nerve cord in its route to the crop-gizzard. Thus, it appears that the crop-gizzard receives a dual innervation which modulates the activity seen in this organ.

The Effects of Intraluminal Pressure on the Spontaneity of the Crop-Gizzard

The purpose of these experiments was to examine the effects of either increasing or decreasing the intraluminal pressure within the intact crop-gizzard on the spontaneous contractions in the preparation. These experiments were designed to show the effects of stretch on the spontaneous contractions of the crop-gizzard. The intraluminal pressure was increased by injecting a known amount of saline into the lumen of the intact preparation. The

intraluminal pressure was decreased by withdrawing a known amount of material from the lumen of the intact preparation.

The average base-line rate of contraction for 5 preparations prior to increasing the intraluminal pressure was 1.6 contractions per minute. Immediately after 0.5 ml of earthworm saline was injected into the lumen of these preparations, the average contraction rate increased to 2.4 contractions per minute. Removal of the 0.5 ml of material from the lumen returned the contraction average to its original value.

In another series of experiments the effects of increased intraluminal pressure were observed in three intact preparations. In these experiments 1.0 ml of earthworm saline was injected into the lumen. The average base-line rate of contraction prior to the injection of the saline was 1.5 contractions per minute. Immediately after the saline was injected, the average contraction rate of these preparations increased to 3.6 contractions per minute. When the fluid was removed from the lumen of the crop-gizzard the contraction rates returned to their original values.

In three preparations 1.5 ml of earthworm saline was injected into the lumen. Under these conditions the lumen became quite distended and no contractions were seen. This may have been due to the fact that the preparation was too weak to contract against this large amount of pressure.

When the pressure was reduced by removing 1.5 ml of material from the lumen, contractions were again observed in all three preparations.

In another series of experiments the effect of decreased intraluminal pressure was studied. These experiments were designed to show the effects of decreasing the amount of stretch on the intact crop-gizzard preparation. The average base-line rate of contraction for three preparations prior to decreasing the amount of intraluminal pressure was 1.2 contractions per minute. When 0.5 ml of material was withdrawn from the lumen of these preparations, the average contraction rate declined to 0.6 contractions per minute and the contractions were weak and very irregular. When 1.0 ml of material was withdrawn from the lumen of two different preparations, the spontaneous contractions stopped completely. When the original material was re-injected into the lumen, the spontaneous contractions were observed again.

From these observations it is obvious that increasing the intraluminal pressure in the intact crop-gizzard preparation modifies the contraction rate of the preparation, there being an increase in the contraction rate with increased intraluminal pressure up to a point. Likewise a decrease in the intraluminal pressure in the crop-gizzard produces a decrease in the contraction rate of the intact preparation. Thus, it appears that in

addition to the modulation of activity exerted by the central nervous system the amount of tension on the walls of the crop-gizzard can also modulate the activity in the crop-gizzard preparation.

Figure 5 shows the results of an experiment where the intraluminal pressure was alternately increased and decreased in an intact crop-gizzard preparation. In this figure the intraluminal pressure was increased (upward arrows) by adding 0.5 ml and 1.0 ml of saline at 5 and 15 minutes. The intraluminal pressure was decreased (downward arrows) by removing 0.5 and 1.0 ml of material from the lumen at 10 and 20 minutes. The points were determined by counting the total number of contractions during each 5 minute time period. One can note that during those periods when the intraluminal pressure was increased, the contraction rate of the crop-gizzard showed an increase and during those periods where the intraluminal pressure was decreased there was a decline in the contraction rate of the crop-gizzard.

Electrical Activity in the Intact Crop-Gizzard

In order to determine more precisely the nature of the electrical activity in the crop-gizzard, the electrical activity of the intact crop-gizzard bathed in 6mM Ca saline was recorded (Figure 6). Electrical activity was recorded from various spots on either the surface of the crop or the gizzard. All of the electrical activity recorded during

Figure 5. The effects of intraluminal pressure changes on the contraction rate of the intact crop-gizzard. The intraluminal pressure was increased (upward arrows) by injecting 0.5 ml and 1.0 ml of earthworm saline into the lumen of the preparation at 5 and 15 minutes. Intraluminal pressure was decreased (downward arrows) by withdrawing 0.5 ml and 1.0 ml of materials from the lumen at 10 and 20 minutes respectively.

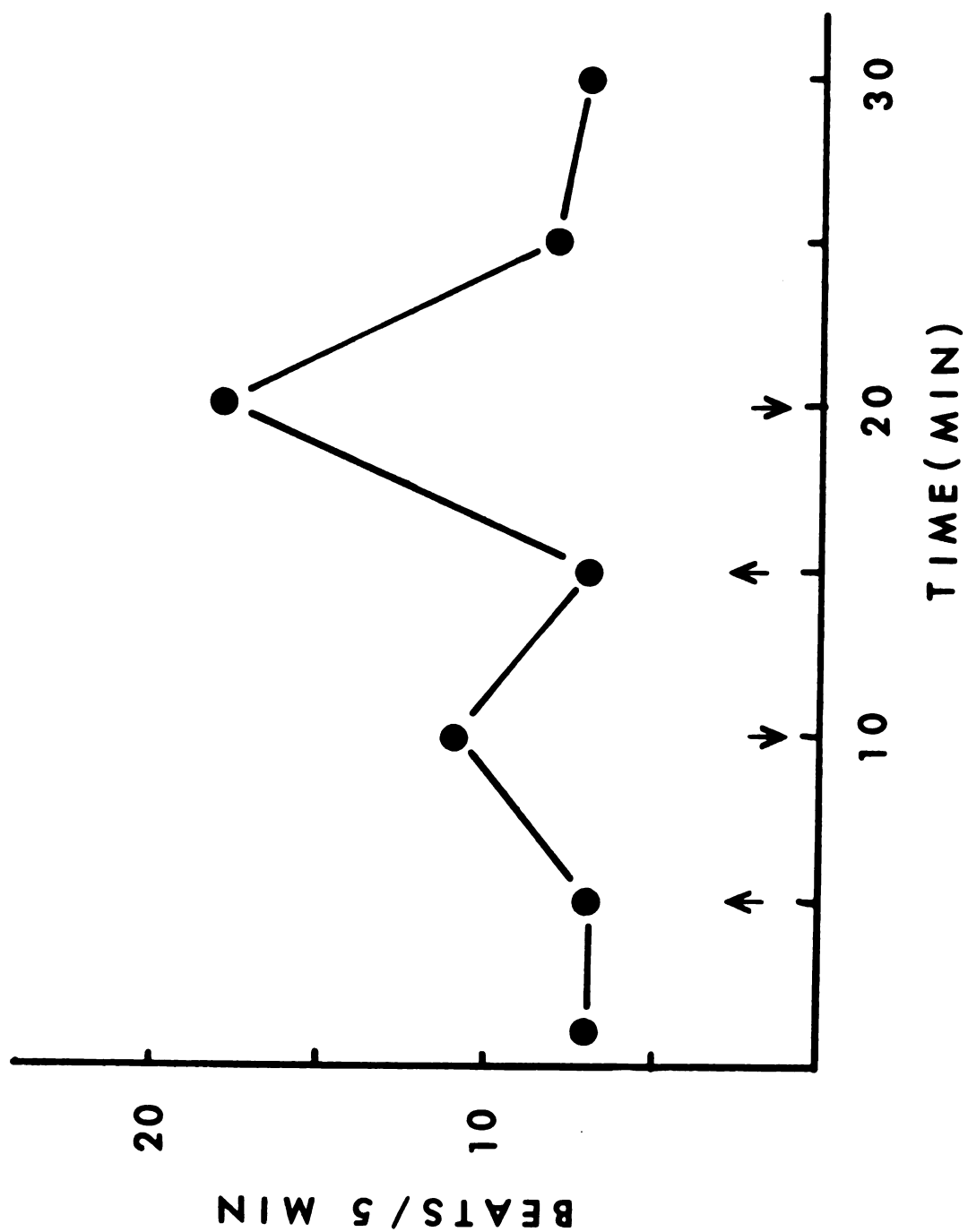


Figure 5

Figure 6. Physiograph recording of the electrical activity recorded from the dorsal surface of the earthworm crop. The intact preparation was bathed in 6mM Ca earthworm saline. Calibrations: 250 μ V, 15 seconds.

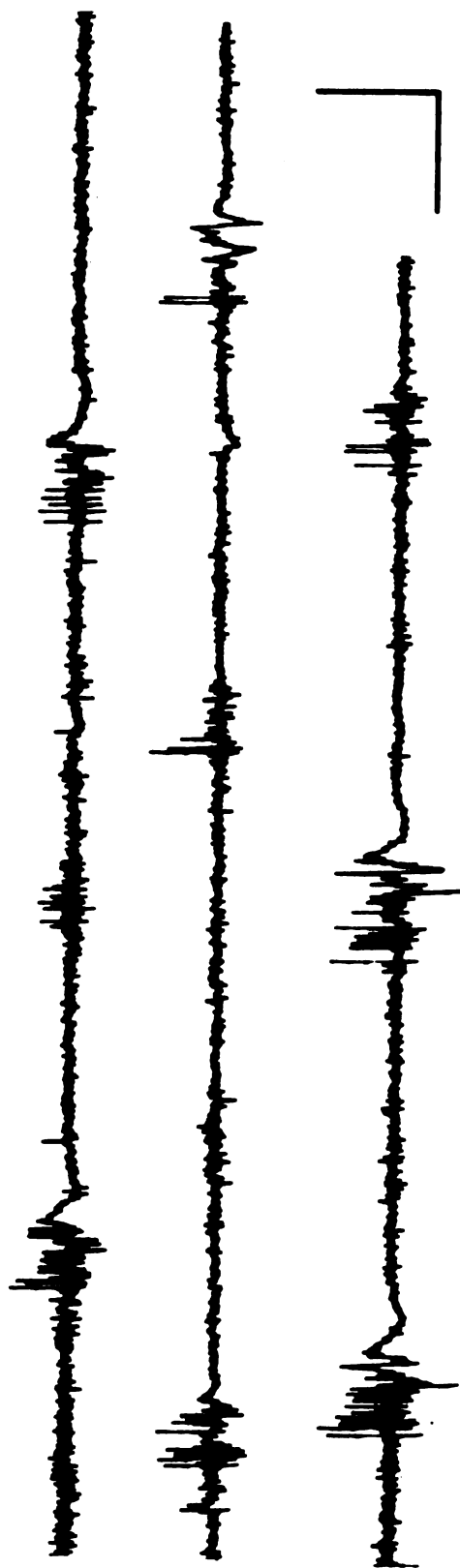


Figure 6

these experiments was recorded by means of a suction type recording electrode, approximately 100 μ in diameter at the tip. Therefore, all activity recorded represents external potentials.

The electrical activity consists of short bursts of potentials which are often separated by periods of electrical inactivity. These inter-burst periods were variable within the same preparation and from one preparation to another. The individual electrical bursts consisted of a complex waveform lasting anywhere from 10-30 seconds. Generally, there is an initial fairly large potential which is followed by a variable number of smaller slow potentials. The initial larger potentials ranged in size from 100-300 μ V and the smaller potentials ranged from 50-150 μ V in size.

Correlation Between the Electrical and Mechanical Activity in the Intact Crop-Gizzard

In six experiments the mechanical and the electrical activity were recorded simultaneously from the intact crop-gizzard preparation. The purpose of these experiments was to determine whether or not the electrical activity recorded from the intact preparation showed any correlation with the mechanical activity. The electrical activity was recorded by means of a suction electrode and the mechanical activity was recorded by using a myograph transducer and a physiograph.

Figure 7 shows a typical record of the type of results which were obtained in these experiments. From this record one can see that the electrical bursts and the mechanical contractions are closely correlated and that the electrical bursts precede the mechanical contractions. The electrical activity preceded the mechanical activity from 5.0 to 10.0 seconds. Thus, it appears that the electrical activity triggers the mechanical contractions of the intact earthworm crop-gizzard.

Figure 7. Physiograph recording of the electrical and mechanical activity from the intact crop-gizzard. The electrical activity is shown in the upper trace and the mechanical activity is shown in the lower trace. The preparation was bathed in 6mM Ca earthworm saline. Calibrations: 250 μ V (upper trace), 2.5 g (lower trace). Time marks = 1.0 minute.

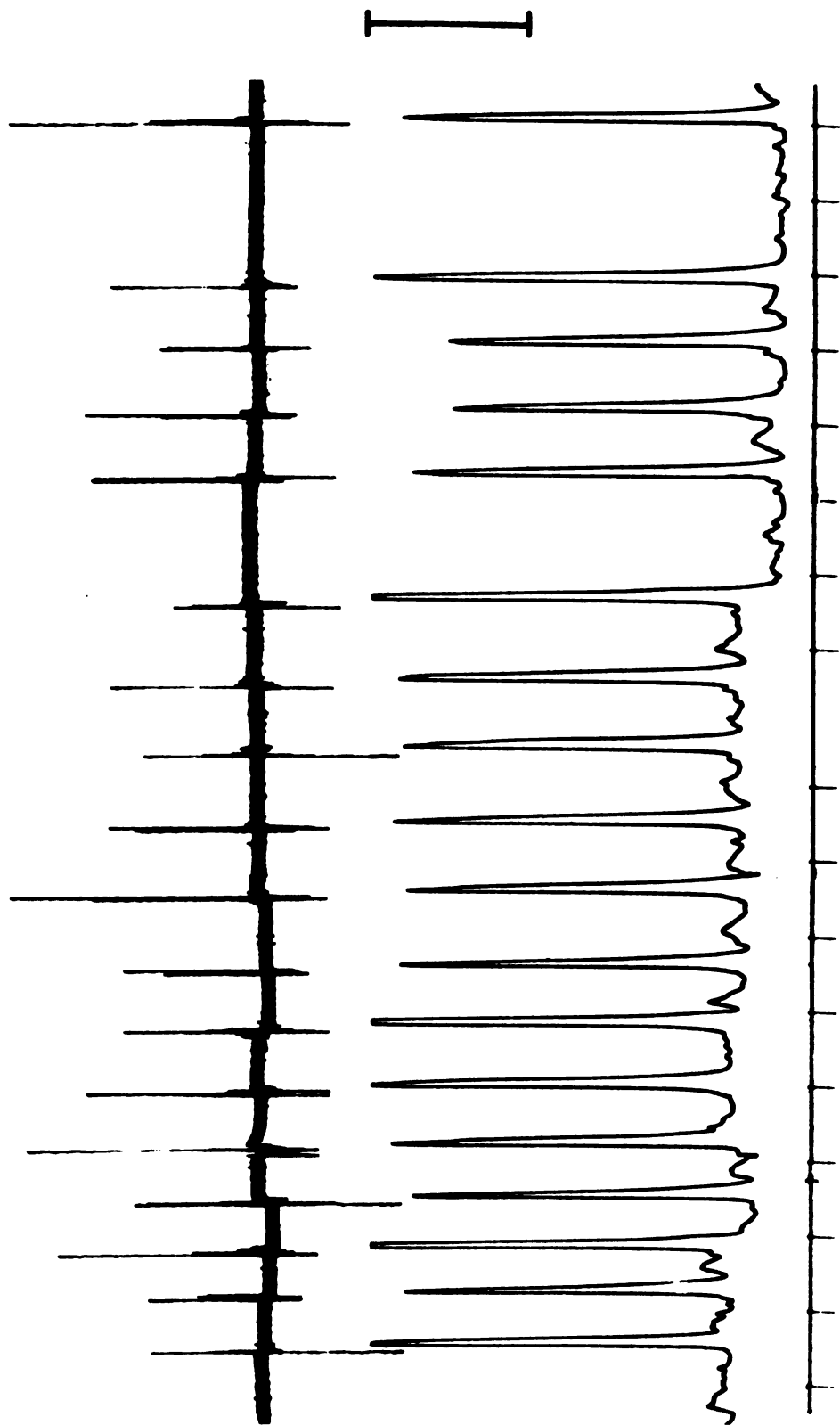


Figure 7

DISCUSSION

The Effects of Different Saline Solutions on the Spontaneity of the Earthworm Crop-Gizzard

Prior experiments dealing with the isolated earthworm crop-gizzard have shown that this preparation is spontaneously active when bathed either in frog Ringer's saline or Pantin's earthworm saline (Wu, 1939; Ambache et al., 1945; Millott, 1944). The initial results of this study confirm these results of previous workers. However, there is a marked difference between the ionic composition of both frog Ringer's saline and Pantin's earthworm saline and that of the earthworm coelomic fluid.

Table 7 shows a comparison between the major ions of the new 6mM Ca earthworm saline, Pantin's earthworm saline and the earthworm coelomic fluid as reported by Lockwood (1961). One can note the close similarity between the ionic concentrations of the earthworm coelomic fluid and the 6mM Ca earthworm saline. There are several differences between Pantin's earthworm saline and that of the earthworm coelomic fluid. One major difference is that the calcium ion concentration of earthworm coelomic

Table 7. Concentrations of the major ions found in earthworm coelomic fluid, 6mM Ca earthworm saline and Pantin's earthworm saline.

Ion	Pantin's Saline (mM)	Coelomic Fluid (mM)	6mM Ca Saline (mM)
Na	135.0	75.6	77.0
K	2.7	4.0	4.0
Ca	1.8	5.9	6.0
Cl	142.0	42.8	43.0

fluid is over three times higher than it is in Pantin's earthworm saline. Sodium, potassium and chloride concentrations are also quite different.

In contrast to the activity in isolated crop-gizzards bathed in Pantin's earthworm saline, isolated crop-gizzard preparations bathed in the 6mM Ca earthworm saline do not show spontaneous contractions. The first question, then, which must be answered is: which is "normal," the rhythmic activity of the isolated preparation bathed in Pantin's earthworm saline or the non-rhythmic activity seen in the isolated preparations bathed in the new 6mM Ca earthworm saline?

The results of the intact preparation studies give some indications of what might be the "normal" contraction pattern of the isolated crop-gizzard preparation. These results show that the contraction rate of the crop-gizzard is nearly the same for preparations bathed with no saline

and for preparations bathed in the new 6mM Ca earthworm saline. When the intact preparation was bathed in the Pantin's earthworm saline there was a substantial increase in the contraction rate. These observations suggests that no mechanical activity is "normal" for the isolated crop-gizzard preparation and that the new 6mM Ca earthworm saline is more appropriate for use in earthworm physiological studies.

As mentioned above the new 6mM Ca earthworm saline differs from Pantin's earthworm saline in a number of respects. Calcium, sodium, potassium and chloride concentrations are all different. The results obtained from changing the calcium concentrations while maintaining the other ionic concentrations constant for intact preparations suggests that the spontaneous contractions seen in the isolated crop-gizzard preparations are due primarily to the low calcium concentration of Pantin's earthworm saline and not to the other ions.

Several workers who have studied spontaneous nerve and muscle activity believe that calcium levels in the external bathing medium are critical in spontaneous nerve and muscle activity (Brink, 1954; Lehman, 1937; Kuffler, 1944). Feng and Shen (1937) observed an increase in the spontaneous contractions of a frog sciatic-gastronemius preparation upon a reduction in the calcium level of frog Ringer's saline. These investigators were able to demonstrate an increase in the amount of Ach released

under low calcium conditions. It was suggested that under low calcium conditions the brake for the release of Ach is removed and there is an increased liberation of Ach which triggers the increased mechanical contractions observed under low calcium conditions.

Data from A-fibers in the sciatic nerve of the frog shows that the membrane stability decreased markedly when the concentration of ionized calcium is reduced below 0.8 mM in the external bathing saline (Brink, Bronk and Larrabee, 1946). These investigators also observed that a decrease in the external calcium concentration lowers the threshold of excitability of frog sciatic nerve fibers. As the threshold of excitability falls the nerve becomes unstable and discharges a sequence of spontaneous impulses. If the nerve is soaked in isotonic NaCl to remove more calcium, the spontaneous impulses are discharged at a frequency of 10 to 30 per second. If the NaCl is replaced with sodium citrate which reduces the calcium level even more, the frequency of discharge is increased to 100 to 200 per second. When the fiber is returned to normal frog Ringer's saline the spontaneous nerve impulses stop completely.

Roeder (1941) observed that by reducing the level of calcium in crayfish saline spontaneous electrical and mechanical activity resulted. This was the same affect observed when the potassium level of the saline was increased five times its normal level. These studies also

showed that high calcium and low potassium produce a decrease in the spontaneous activity in the crayfish nerve cord. The explanation for these effects indicates that the potassium-calcium relationship probably represents a case of antagonism. The effect obtained by reducing the calcium is probably due to the action of increased potassium release from the tissue due to the low calcium conditions. It is generally believed that an increase in calcium concentration protects the tissue against high potassium release. An increase in potassium release from the tissue probably causes a sustained depolarization of the cell membrane which initiates the mechanical contractions.

Further evidence (Hodgkin, 1958; Bozler, 1962) suggests that the increased excitability seen in nerve and muscle cells which are bathed in low calcium bathing fluids depends on an increase in sodium conductance. It is possible that during the action potential sodium is carried into the cell by a carrier mechanism and that in the resting cell under normal calcium conditions this carrier is blocked by calcium. Removal or reduction of calcium in the external medium allows for sodium conductance which triggers the increased mechanical activity.

Evidence that the use of Pantin's earthworm saline increases the spontaneity in the crop-gizzard was obtained in the studies using intact preparations. However, when these preparations were bathed in no saline or the new 6mM Ca earthworm saline no increase in spontaneity was observed.

It appears likely that the increase in spontaneity seen with the use of Pantin's earthworm saline may have been due to the saline itself. These results seem to be in agreement with those of other workers who have observed similar increases in spontaneity of preparations which have been bathed in low calcium bathing solutions. Therefore, it is concluded that the increase in spontaneity observed with the use of Pantin's earthworm saline is induced by the use of a low calcium saline.

Origin of the Spontaneity in the Intact Crop-Gizzard

Previous studies have indicated that the spontaneous mechanical and electrical activity seen in the isolated crop-gizzard is endogenous to the crop-gizzard and is myogenic in origin (Ambache et al., 1945). From the results of the previously discussed saline experiments it is clear that no spontaneity is seen in isolated preparations when bathed in a saline approximating coelomic fluid in ionic composition. Only in intact preparations was spontaneity obtained. Two possibilities seem plausible. The lack of spontaneity in the isolated crop-gizzard preparation might indicate that stretch is lost when the preparation is removed from the animal or that necessary neural inputs are interrupted during the process of isolation. Experiments done on both isolated and intact preparations have shown that the amount of stretch applied to the crop-gizzard has a marked affect on the activity. Other experiments,

however, indicate that stretch is not the only essential component necessary for the production of rhythmic contractions and that neural inputs are also necessary. When the circum-esophageal connectives are cut or the brain removed all spontaneous activity in the crop-gizzard stops, even though there has been no change in the stretch being applied. Thus, it seems likely that the origin of the rhythmic activity is in the central nervous system and not in the crop-gizzard preparation itself.

Though the previous experiments indicate a central nervous origin for the rhythmic contractions, the oscillatory nature of the electrical potentials recorded from the intact crop-gizzard suggest that there is a neurogenic component to the activity. However, these oscillatory potentials may represent the input from the central nervous system. Histological studies of the earthworm crop-gizzard have shown the presence of nerve cells spread throughout the muscle layers of the crop-gizzard (Hess, 1925; Ambache et al., 1945; Smallwood, 1926). It seems conceivable that the electrical activity I have recorded from the crop-gizzard might originate in these nerve cells.

Millott (1943) painted a 5% novacaine preparation on the surface of the intact crop-gizzard and immediate relaxation and cessation of all rhythmic activity resulted. These results indicate that the cessation of spontaneous activity is due to the impairment of nervous function,

since novacaine is known to act very selectively on nervous tissue rather than on muscle tissue.

From these data it seems that the only clear conclusion which may be drawn is that the crop-gizzard is dependent on input from the central nervous system for development of rhythmic contractions. Whether this input impinges directly on the muscle fibers themselves or only indirectly through intermediary nerve cells are questions that this study have not answered.

Modulation of Activity in the Crop-Gizzard

In addition to the apparent dependence of the crop-gizzard on the central nervous system for rhythmic activity, the activity of the intact crop-gizzard appears also to be modulated by both excitatory and inhibitory nerves which emerge from the central nervous system. Stimulation of the brain results in a substantial increase in the contraction rate of the intact crop-gizzard. This observation suggests that an excitatory pathway was activated which probably originates in the brain. The cutting of the circum-esophageal connectives around the brain produces a cessation of all rhythmic activity in the crop-gizzard, suggesting that the excitatory pathway passes through these connectives after leaving the brain. The cutting of the pharynx anterior to the crop-gizzard also produced a cessation of all rhythmic activity in the crop-gizzard. This observation suggests an interruption of the excitatory

pathway to the crop-gizzard. Thus, it appears that an excitatory pathway leaves the brain and passes along the pharynx to the crop-gizzard. Whether this excitatory pathway is identical to the one responsible for initiation of activity in the crop-gizzard is a question which the present experiments have not answered.

The evidence that there is an inhibitory pathway to the crop-gizzard is stronger. The cutting of the ventral nerve cord produces a decline in the contraction rate of the crop-gizzard. The most obvious explanation for this decline in rate would be that an excitatory pathway to the crop-gizzard had been severed. If this were the case then one would expect that stimulation of the cut end of the cord would cause restoration of the contraction rate. This does not occur. Instead stimulation of the nerve cord results in an even further decline in the contraction rate of the crop-gizzard. This suggests that an inhibitory pathway probably operates through the ventral nerve cord.

Perfusion studies involving the isolated and the intact crop-gizzard preparations show that the spontaneous rhythmic activity is increased by the stretch provided by increasing the rate of perfusion. Decreasing the amount of stretch on the preparation by removing some of the crop-gizzard contents produces a decline in the contraction rate of the preparation. These observations suggest that the spontaneous rhythmic activity in the earthworm

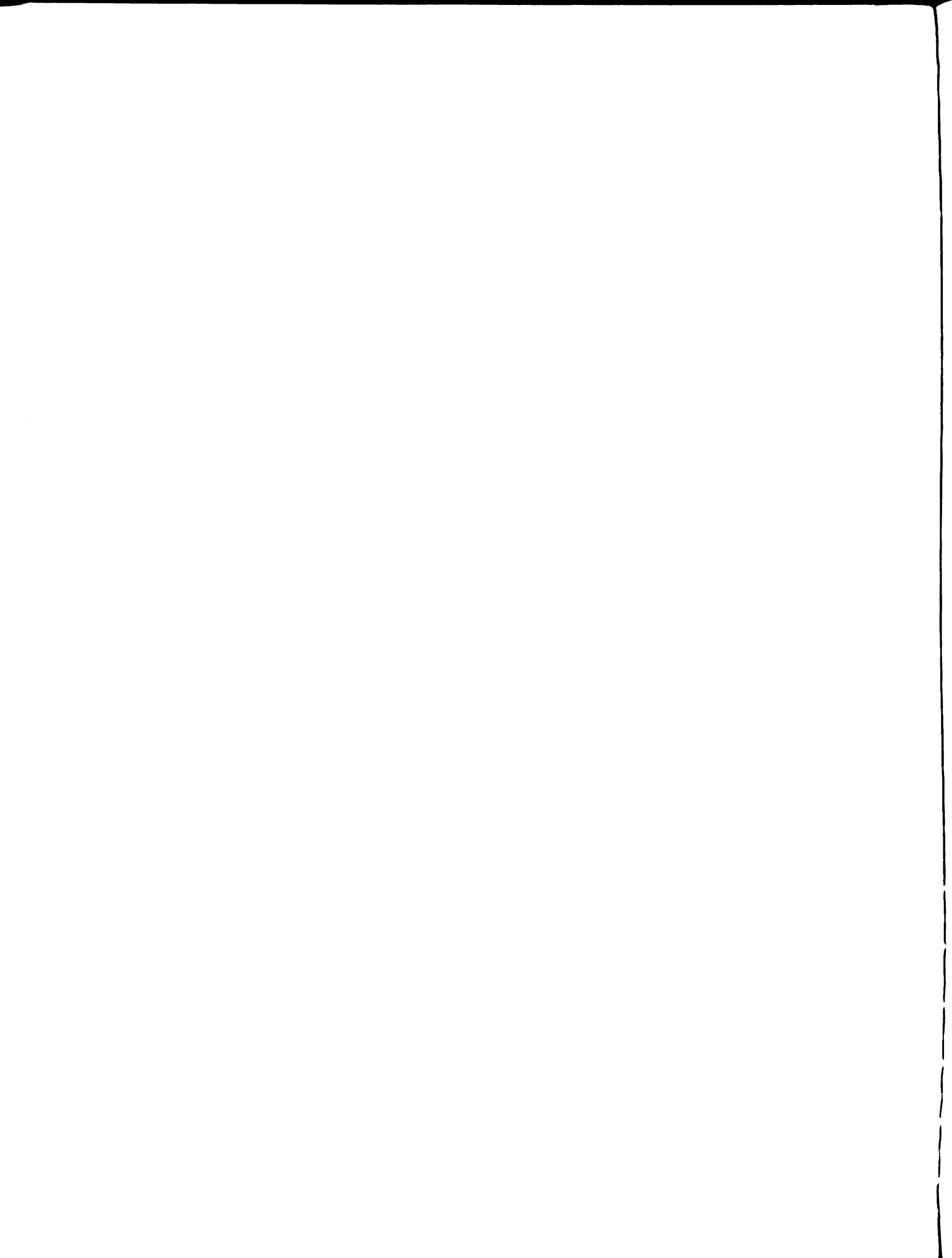
crop-gizzard is also modulated by the amount of stretch exerted on the preparation.

Ten Carte (1924) observed that Helix stomach activity is directly proportional to the degree of filling of the organ. The experiments of this study show that the earthworm crop-gizzard contracts best when partially distended. The effect of stretching produces an increase in the contraction rate of the crop-gizzard, thus suggesting local reflex modulation of the rhythmic activity in the preparation. This proposed local reflex modulating system probably operates through a nerve network. There is an extensive array of nerve cells within the walls of the crop-gizzard which could provide the basis for such a stretch reflex system.

Millot (1937) suggested that the spontaneous peristaltic activity of Jorunna gut is under reflex modulation, particularly as the spontaneous activity is augmented by increasing the internal gut pressure. Pricking the gut wall with a sharp instrument also causes a temporary increase in the peristaltic activity of the intact gut. These observations suggested that the gut activity is under local reflex modulation.

On the basis of the results obtained from these experiments it would appear that the spontaneous rhythmic activity of the intact earthworm crop-gizzard is modulated by the activity of both excitatory and inhibitory nerves

which emerge from the central nervous system as well as local reflex modulation.



SUMMARY

1. Studies presented here have re-examined some of the physiology of the earthworm crop-gizzard with the use of a new 6mM Ca earthworm saline which is comparable to earthworm coelomic fluid.
2. Isolated crop-gizzard preparations do not show spontaneous activity when bathed in 6mM Ca earthworm saline compared to the spontaneous activity seen in isolated preparations bathed in Pantin's earthworm saline.
3. It is probable that the use of Pantin's earthworm saline induces spontaneous activity in the isolated crop-gizzard and increases the level of spontaneity in the intact crop-gizzard.
4. The effects produced by the use of Pantin's earthworm saline are similar to those produced by the use of other low calcium earthworm salines.
5. The spontaneous activity of the intact crop-gizzard is modulated by excitatory and inhibitory inputs from the central nervous system as well as by

the amount of stretch exerted on the crop-gizzard preparation.

6. The electrical and the mechanical activity in the crop-gizzard are closely correlated. The electrical activity appears to initiate the mechanical contractions.
7. The brain is the most probable site of origin for the spontaneous activity seen in the intact crop-gizzard.

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