Conduction Velocity and Intracellular Action Potentials of the Tunicate Heart

MAHLON E. KRIEBEL

From the Department of Zoology, University of Washington, Seattle, Washington 98150 and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Kriebel's present address is the Department of Anatomy, Albert Einstein College of Medicine, Yeshiva University, New York 10461

ABSTRACT The tubular heart of tunicates is composed of a single layer of myoendothelial cells. The direction of contraction reverses every few minutes. The conduction times in both directions are the same. Conduction velocity was greatest in the middle of the arms of the V-shaped heart and slowest in the apex. The greater the heart length, the greater was the conduction velocity. The Q_{10} of conduction velocity was 2-2.3. Removal of the raphe which attaches the heart to the pericardium and removal of a line of undifferentiated cells opposite the raphe did not change the conduction velocity or prevent the heart from reversing the direction of conduction. The median resting potential of 42 cells was −71 mv and the median action potential was 75 mv. At 20°C the duration of the action potential was 1.2 sec and the maximal rate of depolarization was 3-10 v/sec. An increase in the beat frequency produced by electrically stimulating the heart decreased the resting potential, rate of rise, the duration, and the overshoot of the action potential. The shape of the action potential was sometimes different in the two directions of conduction. The electrophysiological evidence indicates only one cell type and suggests that the mode of the spread of excitation is by local current flow from cell to cell.

INTRODUCTION

The heart of the tunicate, *Ciona*, is a long V-shaped tube with a pacemaker at each end. Pumping action is produced by a peristaltic wave starting at one end and travelling to the other. The direction of peristalsis reverses every minute or two, changing the direction of blood flow through the valveless circulatory system.

During morphogenesis, the primordial heart vesicle involutes on one surface and the two lips fuse together forming a *raphe*. The inner membrane develops into the tubular heart composed of a single layer of endothelial muscle cells. The outer membrane becomes the pericardium. The heart remains attached by the raphe along its entire length to the pericardium (Heine, 1903). Except

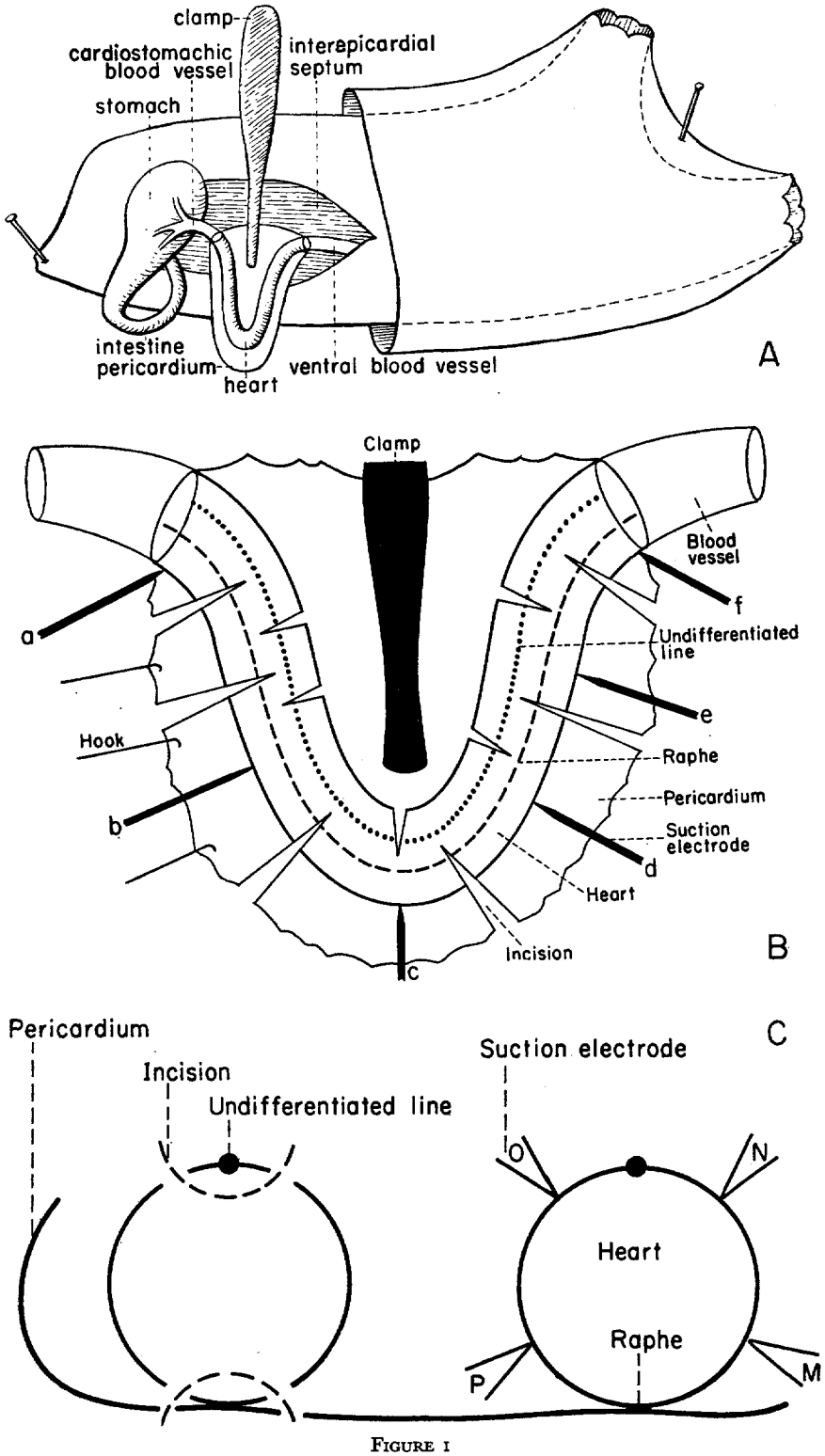
clamp
cardiostomachic blood vessel
interepicardial septum
stomach
intestine
pericardium
heart
ventral blood vessel
A
Clamp
Blood vessel
f
a
Undifferentiated line
e
Hook
Raphe
Pericardium
b
Suction electrode
d
Heart
Incision
c
B
Pericardium
C
Suction electrode
Incision
Undifferentiated line
O
N
Heart
Raphe
P
M

FIGURE 1

for a line of cuboidal cells two to five cells wide, termed the *undifferentiated line* and located opposite the raphe, there is only one cell type (70 μ long $\times$ 4 μ wide $\times$ 10 μ high) oriented at an angle of about 70° to the long axis of the heart (Heine, 1903; Millar, 1953; Schulze, 1964). Each cell contains a single myofibril (70 μ long $\times$ 2 μ wide $\times$ 3 μ high) which faces the lumen surface. Each cell bulges in the middle around one relatively large (4 μ diameter) nucleus located above the myofibril. The myofibrils of all the cells are compacted together to form a dense sheet one myofibril thick (Heine, 1903; Kawaguti and Ikemoto, 1958; Schulze, 1964). The apical surface of the tissue (opposite the lumen) resembles a layer of cuboidal or elliptical epithelial cells.

Almost all previous investigations of tunicate hearts have been concerned with the mechanism of reversal and little has been done to elucidate the electrophysiological properties of this simple chordate heart. Though the main objective of this communication is to describe the conduction velocity and action potentials in the myocardium, some observations are reported which have direct bearing on the mechanism of reversal.

METHODS

A. *Extracellular Recording*

The hearts of adult (6-10 cm long) *Ciona intestinalis* were exposed by removing the basal part of the tunic and then making a longitudinal incision through the body wall (Kriebel, 1964). The animals were placed in a seawater bath maintained at 10°, 15°, or 20°C. The triangular pericardium was clamped between the arms of the heart

---

FIGURE 1. Diagrams of *Ciona* hearts. A. Dissection showing a heart *in situ*. B. Isolated heart showing the position of the suction electrodes and incisions through the heart. Incisions were made only in those preparations used to determine whether the raphe and undifferentiated line are conduction systems. The raphe joins the lower surface of the heart to the lower surface of the pericardium (held in the clamp). The upper surface of the pericardium has been removed. The undifferentiated line of cells is on the top surface of the heart. Electrodes *a*, *c*, and *f* (or, *a*, *b*, *d*, and *f*) were used to measure the conduction velocity in each direction of conduction and were used to compare the conduction velocity in each arm of the heart (Table I). Electrodes *f*, *e*, *d*, and *b* were used to compare the conduction velocity in the end, middle arm, and apex of the heart (Table II). C. Cross-section through the two arms of the heart demonstrating two types of experiments. Left, suction electrodes placed at each end of the heart (*a* and *f*, in above diagram) were used to determine the conduction velocity before and after removing narrow 6 mm long strips of tissue containing either the raphe or the undifferentiated line or both (indicated by dashed semicircles) from each arm of the heart. Right, suction electrodes placed at *m*, *n*, *o*, and *p* were used to determine the orientation of the wave front of excitation to the long axis of the arm of the heart. The sequence of excitation was *m*, *n*, *o*, and *p* during contractions from left to right as seen in Fig. 1 B, and *p*, *o*, *n*, and *m* during contractions from right to left.

(Fig. 1). Hearts held in this manner continued to regularly reverse the direction of peristalsis for 3 or 4 days with little disturbance in circulation.

Small areas of raphe were drawn into suction electrodes (tip diameter about 100 μ). By connecting two electrodes to the same input terminal of a Tektronix 122 AC preamplifier up to four extracellular signals could be recorded on a dual channel Offner penrecorder. Provided the distance between the electrode tips was greater than 5 mm, the signals recorded by two connected electrodes appeared completely separated in time.

B. *Intracellular Recording*

Isolated hearts were flattened under a piece of nylon stocking against the bottom of a Plexiglass chamber containing seawater 1 mm deep.

The hearts were electrically stimulated via suction electrodes placed at each end. Glass microelectrodes filled with 3 M KCl (10-40 MΩ) were broken off below the taper and slipped over a 0.001 inch platinum-iridium wire to make floating electrodes (Woodbury and Brady, 1956). Intracellular action potentials were displayed on a Tektronix 502 oscilloscope, using a unity gain DC amplifier.

RESULTS AND DISCUSSION

A. *Conduction Velocity*

During 12 hr of continuous recording from hearts *in situ* or in isolation, no changes in conduction velocity, in the frequency of reversal, or in heart rate were detectable.

There were no extra systoles during normal reversals in which a pause occurred between the two pacemaker activities (Fig. 2 A). However, in collapsed hearts there was generally no pause during a reversal: for several seconds waves of contraction originated at both ends of the heart and they extinguished each other further along the heart with each beat (*pacemaker competition*). Artificial pacemaker competitions could be produced by driving the ends of a heart with stimulators set at slightly different frequencies; they were indistinguishable from natural pacemaker competitions. Thus it appears that in collapsed hearts both pacemakers were continuously active and that pacemaker competitions resulted from a slight shift in phase.

There is a third pacemaker region at the heart apex (the C center) but it was active only in hearts that were overdistended with blood. It was not active during normal reversals as reported by Mislin (1964).

Conduction velocities in both arms and in both directions of *in situ* and isolated hearts were about the same (Table I and Fig. 2 B). Hearts 15 mm long had a conduction velocity of about 6 mm/sec and larger (30 mm) hearts had a conduction velocity of about 12 mm/sec. The larger hearts have larger cells which may explain these differences in conduction velocity. It was noted that the visceral arm of the heart was sometimes as much as 2 mm longer than

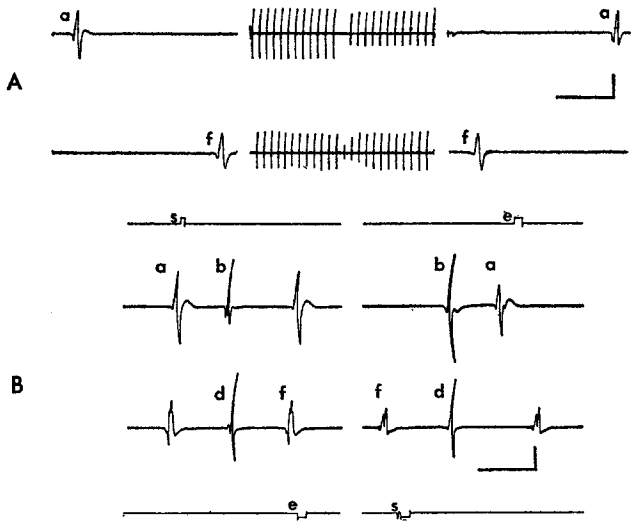

FIGURE 2. Extracellular action potentials from *Ciona* hearts. A. Action potentials from each end of a 22 mm long heart *in situ*. The direction of peristalsis was determined visually. Left, the wave of conduction started in the end of the heart recorded by the upper trace (*a*) (see Fig. 1 B for electrode placement). Calibration, 1 sec; 0.1 mv. Middle, a reversal in the direction of contraction is indicated by the change in the signal amplitude. Calibration, 25 sec; 0.1 mv. Right, the wave of conduction started in the end of the heart recorded by the lower trace (*f*). Calibration, 1 sec; 0.1 mv. 15°C. B. Action potentials from each end and each side of the apical region of the V-shaped heart during both directions of conduction. Signal marks on top and bottom traces indicate the visually observed start (*s*) and end (*e*) of peristalsis. See Fig. 1 B for electrode placement. Electrodes *a* and *b* (or *d* and *f*) were connected to one preamplifier. Calibration, 1 sec; 0.1 mv. 15°C. Left, the wave of peristalsis proceeded from electrodes *a* to *b* (10 mm apart) in one arm of the heart, then from *b* to *d* (1.8 mm apart) around the apex, and then from *d* to *f* (10 mm apart) in the second arm of the heart. Right, the wave of conduction started at electrode *f* and proceeded to electrode *a*. Note that the wave front changed direction in the apex region of the heart since it reached electrode *b* before it reached electrode *d*. However, conduction velocity between electrodes *a* and *f* in the two directions of conduction was the same. The difference in the shape of the signals recorded with different electrodes was due to different amounts of tissue pulled into the suction electrodes. The signal shapes sometimes varied in the two directions of conduction as shown by the signals recorded with electrode *b*. Electrodes with small tips with a minimal amount of tissue pulled into them gave the sharpest signals (as with electrodes *b* and *d*).

the branchial arm. This may account for the apparently greater velocity in the branchial arm reported by many earlier authors from visual observations (Skramlik, 1938; Krijgsman, 1956).

The middle portion of the arms conducted almost twice as fast as the end and apical regions (Table II). These middle segments of both arms conduct with the same velocity. The Q_{10} of conduction velocity as determined in single preparations was 2.0-2.3 (temperature range from 5° to 25°C). Conduction

time was the same whether the heart was overdistended with blood or collapsed. There was no change in conduction velocity within a range of 1 to 30 beats per minute.

TABLE I
CONDUCTION VELOCITIES IN *CIONA* HEARTS (MILLIMETERS PER SECOND)

Temperature	Length of heart*	Branchial to visceral contractions			Visceral to branchial contractions		
		Branchial arm	Visceral arm	Average	Visceral arm	Branchial arm	Average
°C	mm						
10	13	—	—	3.8	—	—	3.8
10	18	—	—	6.0	—	—	6.0
15	15	6.8	6.0	6.4	5.2	7.0	6.1
15	22	8.3	8.0	8.1	7.7	8.3	8.0
15	30	—	—	11.8	—	—	12.2
20	26	13.8	14.7	13.0	13.8	12.5	12.3

The figures were selected from the data of 18 hearts. Conduction velocity was determined in the branchial arm between electrodes *a* and *b*, in the visceral arm between electrodes *d* and *f*, and average velocity between electrodes *a* and *f* (for electrode placement, see Fig. 1 B). The error in measuring records was less than 0.1 mm/sec.

* The actual length of the heart was somewhat greater; this column represents the interelectrode distance to ±0.4 mm. Therefore, comparison of conduction velocities of different hearts is accurate only to the nearest millimeter per second.

TABLE II
CONDUCTION VELOCITY IN THREE 6 MM LONG SEGMENTS OF A 30 MM LONG HEART OF *CIONA*

Branchial to visceral contractions				Visceral to branchial contractions			
End segment	Middle of arm	Apex segment	All 3 regions	End segment	Middle of arm	Apex segment	All 3 regions
11.5	16.7	9.4	11.8	10.0	16.7	12.5	12.2

Velocity in millimeters per second. 15°C.

Segment at end of heart is between electrodes *e* and *f*, middle of arm segment is between electrodes *e* and *d*, apex region is between electrodes *d* and *b*, and all three regions are between electrodes *f* and *b* (for electrode placement see Fig. 1 B). Velocities are within 6%, since error in estimating interelectrode distance was 0.4 mm.

B. *Effect of Sectioning the Raphe on Conduction Velocity*

The frequency of contraction, the frequency of reversal, and the conduction velocities in both directions of conduction were determined for 1 to 4 hr in three *in situ* hearts. The raphe was then crushed for several millimeters. There was no detectable change in conduction velocities or blood flow.

In deflated, isolated hearts, the raphe, the undifferentiated line, or both, were removed for 6 mm in each arm of the heart (Fig. 1 C). There was little change in frequency and reversal period (reversals were by pacemaker com-

petition) and no change in conduction velocity. The velocity was not altered in four hearts after making 10 cross-sectional incisions through the raphe and undifferentiated line (Fig. 1 B). These results demonstrate that the raphe and undifferentiated line of cells are not required for conduction. This is in contradiction to previous suggestions (Krijgsman, 1956). This discrepancy may be ascribed to the use of different genera; however, it was found in *Ciona*, *Ascida callosa*, *Chelyosoma productum*, and *Corella willmariana* that conduction was sometimes blocked for several minutes if the heart was excessively stretched while cutting through the raphe.

Phase contrast studies of living pieces of myocardium showed that there is only one type of myoendothelial cell. This confirms histological work demonstrating the absence of nerves in the heart (Markman, 1958; Bone and Whitear, 1958) and Markman's observation that sectioning the raphe does not affect the wave of contraction. Thus the spread of excitation must be from one endothelial cell to the other.

C. *The Role of Raphe and Undifferentiated Line in Conduction (Fig. 1 B and C)*

Strips of tissue were isolated which were bisected by either the raphe or the undifferentiated line. Two suction electrodes were placed on the myocardium on each side of either the undifferentiated line or of the raphe, one for stimulation and one for recording. It was found that the wave of excitation could pass across the line of undifferentiated cells but not across the raphe.

D. *Orientation of Wave Front in the Middle of the Heart Arm*

The orientation of the wave front of excitation was determined by placing four electrodes around the middle segment of an arm of the heart (Fig. 1 C). In large hearts (2 mm diameter and 30 mm long) the leading edge of the wave front in both directions of conduction was found to be parallel to the long axis of the muscle cells. At the electrodes in Fig. 1 C the sequence of excitation in one direction of conduction was *m*, *n*, *o*, and *p* but *p*, *o*, *n*, and *m* in the opposite direction of conduction.

E. *Intracellular Action Potentials*

Various shapes of action potentials were found, showing from one to three phases of repolarization (Fig. 3). Action potentials from a single cell before and after a reversal were usually identical (Fig. 4 A) but occasionally they differed in shape (Fig. 4 B and C). The shape of action potentials recorded in one direction of conduction was constant up to 2 hr even after many natural and induced reversals (Table III). A difference in shapes of the action potentials in the two directions of conduction persisted even during high beat frequencies produced by electrical stimulation (Fig. 4 C and D).

The duration of the action potential at 15°C was about 1.6 sec (Table III).

Since the conduction velocity was 6.1 mm/sec in short hearts and 12.2 mm/sec in long hearts (Table I), the wave length of excitation was from 9.8 to 19.5 mm depending on the heart length.

Steps and notches were present on about 20% of the action potentials (Figs. 3 A; 4 B and C). Action potentials with prepotentials had an average maximal

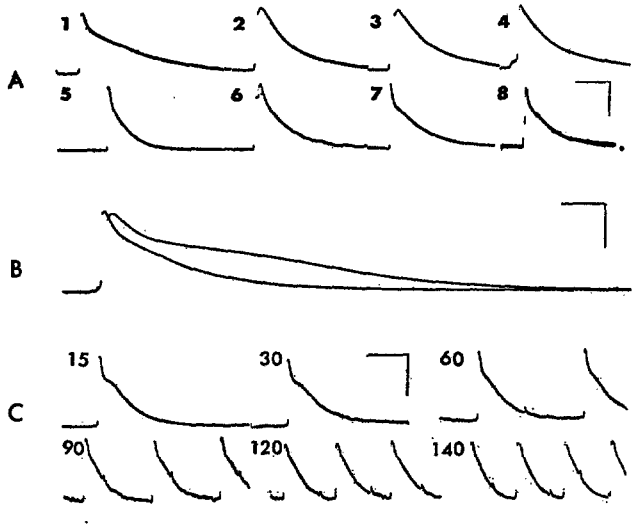

FIGURE 3. Intracellular action potentials. A. Variations of shape. 70% of the action potentials showed three phases of recovery (*1*) and the rest either showed one phase (*2*) or were intermediate (*5*) in shape. Action potentials *3*, *4*, *6*, *7*, and *8* show prepotentials. *3* and *4* are from the same cell before and after a reversal in the direction of conduction. Calibration, 40 mv; top row 0.2 sec; bottom row 0.4 sec. 20°C. B. Effect of temperature on shape of action potential. Action potentials from the same cell at 20° and 10°C are superimposed. Trace with the longer plateau is at 10°C. Calibration, 40 mv; 0.2 sec. C. Effect of an increase in frequency of contraction on the shape of the action potential. Frequencies of contraction are in beats per minute. Normal frequency of contraction at 20°C was 15 beats/min. The small 5 mv positive signals are stimulus artifacts. Calibration, 40 mv; 0.4 sec.

rate of rise of 3 v/sec whereas those without had an average maximal rate of rise of 10 v/sec (20°C). The durations and amplitudes of the action potentials were the same with or without prepotentials. A negative step sometimes occurred in a cell during one direction of conduction whereas a positive step occurred during the opposite direction of conduction. Also, different sizes of steps occasionally occurred during opposite directions of conduction (action potentials 3 and 4 in Fig. 3 A are from the same cell). Occasionally potentials of only 3-7 mv were observed even though the rest of the heart contracted.

It is suggested that the steps and notches of action potentials were not the result of injury to the recorded cell but were due to variations in the spread of

current into the cell (see next section). It is possible that the nylon stocking used to flatten the heart on the bottom of the chamber blocked some cells in one direction of conduction so that the symmetry of the wave front of excitation differed.

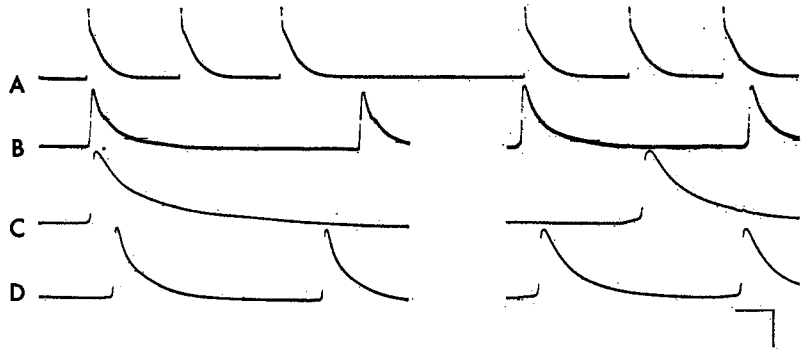

FIGURE 4. Selected records showing intracellular action potentials before and after beat reversal. Reversals occurred in the middle of the traces. The pause during the reversal in B, C, and D was long and therefore cut from the records. Calibration, 40 mv; A, 1 sec; B, 0.5 sec; C and D, 0.2 sec. A. The action potentials are similar before and after the reversal. B. The hump in the action potential (left trace) is absent after the reversal (right trace) and the step in the right trace is missing in the left trace. C. The long prepotential occurs only in the right trace. Frequency of contraction is 20/min. D. Same cell as C but the heart was stimulated to contract about three times faster by locally heating the pacemaker region. Note that the prepotential persisted.

TABLE III
DESCRIPTION OF ACTION POTENTIALS
OF *CIONA* HEART CELLS

Temperature, °C	10	15	20
No. of cells*	8	14	20
Resting potential, *mv*	68.7 (±12), 71	65.7 $\binom{+6}{-24}$, 72.0	67.0 (±12), 70
Action potential, *mv*	68.7 (±12), 71	68.7 $\binom{+6}{-24}$, 75.0	72.0 (±12), 75
Overshoot, *mv*	0 (±4)	3.0 (±5)	5.0 (±5)
Duration, *sec*	1.9 (±0.6), 1.8	1.6 (±0.4), 1.6	1.2 (±0.6), 1.2
Rise time‡, *v/sec*	5.0 (±2), 5	6.1 $\binom{+5}{-2}$, 5.5	7.0 $\binom{+5}{-4}$, 5
No. of cells§	8	8	8
Rise time, *v/sec*	5.0 (±2), 5	7.3 $\binom{+4}{-2}$, 7.0	10.6 (±1.6), 10.0

Under each temperature heading the left column represents the average followed by the range and median.

* These values include action potentials with prepotentials.

‡ Maximal rise time as measured from the middle of the upstroke.

§ These values exclude action potentials with prepotentials.

In conclusion, there appears to be only one electrophysiological cell type; however, the shape of the action potential may change with a change in the direction of the spread of excitation.

F. *Mechanism of Spread of Excitation*

The spread of excitation through the vertebrate myocardium is thought to be caused by either local current flow from excited cells into unexcited cells through areas of low membrane resistance (Woodbury, 1962) or by a release of transmitter (Sperelakis, 1963). *Ciona* hearts are of interest in this respect since the spread of excitation is from cell to cell. Since the wave front moves perpendicular to the long axis of the narrow (4 μ) cells, and since the average conduction velocity is 13 mm/sec there is only 0.3 msec available for the wave front to traverse one cell. Katz and Miledi (1965) have recorded a minimal synaptic delay in the frog sartorius of 0.4-0.5 msec and they concluded that the delay arises chiefly during the release of the transmitter after the arrival of the nerve impulse. In addition to the delays following depolarization of the presynaptic cell, Hagiwara and Tasaki (1958) have shown that the action potential in the presynaptic cell of the squid giant axon must reach 70% of its normal size before any transmitter is released. It would require 5 msec for a tunicate cell action potential to reach 70% of its final value. Assuming similar delays between tunicate heart cells, the time is too short to allow transmitter transmission.

Even if release, diffusion of transmitter, and transmitter action on the postsynaptic cell were instantaneous, an excited cell would have to release a hypothetical transmitter before it had depolarized by more than 3 mv since the maximal rate of depolarization was found to be only 10 v/sec and only 0.3 msec is available per cell. These calculations practically preclude the possibility of propagation by nonelectrical means in the tunicate heart. On the other hand, local current spread of cellular dimensions in the relatively large expanse of myocardium could account for the comparatively slow spread of conduction.

I wish to thank Drs. S. W. Kuffler and R. K. Josephson for their generosity in supplying equipment and space at the Marine Biological Laboratory.

I am indebted to Drs. E. Florey and J. W. Woodbury for their help in preparing the manuscript.

This work was done at the Marine Biological Laboratory, Woods Hole, Massachusetts, during tenure of a Neurophysiology Fellowship. It was supported in part by an Office of Naval Research Contract: NONR 1497 (00) to the Marine Biological Laboratory and by a training grant No. GM 1194 from the National Institute of General Medical Sciences.

This work represents part of a dissertation submitted to the University of Washington in partial fulfillment of the requirements for the Ph.D. degree in 1967.

Received for publication 19 April 1966.

BIBLIOGRAPHY

BONE, Q., and M. WHITEAR. 1958. A note on the innervation of the pericardium in *Ciona*. *Pubbl. Staz. Zool. Napoli.* **30**:337.

HAGIWARA, S., and I. TASAKI. 1958. A study of the mechanism of impulse transmission across the giant synapse of the squid. *J. Physiol., (London).* **143**:114.

HEINE, P. 1903. Untersuchungen über den Bau und die Entwicklung des Herzens der Salpen und der *Ciona intestinalis. Z. Wiss. Zool.* **73**:429.

KATZ, B., and R. MILEDI. 1965. The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proc. Roy. Soc. (London), Ser. B.* **161**:483.

KAWAGUTI, S., and N. IKEMOTO. 1958. Electron microscopy of the heart muscle from the sea squirt, *Ciona intestinalis. Biol. J. Okayama Univ.* **4**:93.

KRIEBEL, M. E. 1964. Studies on the cardiovascular physiology of the tunicate *Ciona intestinalis.* Master of Science Thesis. Department of Zoology, University of Washington.

KRIJGSMAN, B. 1956. Contractile and pacemaker mechanisms of the heart of tunicates. *Biol. Rev.* **31**:288.

MARKMAN, B. 1958. On the peripheral nervous system of ascidians. *Acta Zool., (Stockholm).* **39**:13.

MILLAR, R. H. 1953. *Ciona.* Liverpool Marine Biology Committee Memoirs on Typical British Marine Plants and Animals. J. S. Colman, editor. The University Press of Liverpool.

MISLIN, H. 1964. Über eine spontane Extrasystolie im Schrittmachersystem des Tunicatenherzens (*Ciona intestinalis* L.). *Experientia.* **20**:227.

SCHULZE, W. 1964. Zur Ultrastruktur des Herzschlauches von *Ciona intestinalis* L. *Experientia.* **20**:265.

SKRAMLIK, E. von. 1938. Über den Kreislauf bei den niedersten Chordaten. *Ergebn. Biol.* **15**:166.

SPERELAKIS, N. 1963. Additional evidence for high-resistance intercalated discs in the myocardium. *Circulation Res.* **12**:676.

WOODBURY, J. W. 1962. Cellular electrophysiology of the heart. *In* Handbook of Physiology. Sect. 2, Circulation. W. F. Hamilton and P. Dow, editors. The Waverly Press, Inc., Baltimore, Maryland. **1**:237.

WOODBURY, J. W., and A. J. BRADY. 1956. Intracellular recording from moving tissues with a flexibly mounted ultramicroelectrode. *Science.* **123**:100.