studied (13 were immature and 17 were undergoing estrus cycles). The animals were killed by cervical dislocation; the ovaries were removed, dissected free of fat and oviducts, chilled, washed, and exposed for varying intervals to 0.5 percent pronase made up in a Ca^{++}- and Mg^{++}-free balanced salt solution at *p*H 6.8 to 7.0. The ovaries were placed in the enzyme solution at 37.5°C and were agitated every 10 minutes. As dispersal of the ovary occurred, samples of fluid containing cellular materials were taken and examined microscopically. Cell viability was assessed by testing the dispersed material with nigrosin (*5*).

When the ovaries were taken from immature animals at ages close to the onset of vaginal opening, the elements initially found in the fluid were single whole follicles. These follicles were the outermost follicles of the ovary. After release, each follicle dispersed; the outer cell layers were liberated first. Concurrent with the dispersal of the liberated follicles from the periphery, follicles from the interior of the ovary were released. Disaggregation of the follicle took place only after release from the ovary.

The treatment of ovaries from animals in the follicular phase of the estrus cycle yielded follicles in all stages of maturation (Figs. 1–3). It was possible to obtain intact follicles with large antra but these were more susceptible to dispersal or to rupture than were the less mature follicles with small antra or those at stages prior to antrum formation.

Corpora lutea, when present, were released first, then follicles from the interior. Suspensions of luteal cells were obtained by subjecting the liberated corpora lutea to the enzyme.

When enzymatic degradation of the ovary was attempted with other proteolytic enzymes (trypsin, chymotrypsin, pancreatin, collagenase, elastase) suspensions of cells appeared without the step by step breakdown that took place with pronase.

After dispersal the cellular elements were collected by either mild centrifugation or on Millipore filters, washed with saline, and resuspended in a balanced salt solution containing 0.5 percent nigrosin. In most instances only 10 to 15 percent of the free cells allowed the dye to permeate, and no dye penetration occurred in the whole follicles or corpora lutea.

Any of the tissues isolated may be suitable for study. Embryonic cells isolated by pronase have been used to establish cell cultures (*6*). Other data indicate that the ovarian elements obtained by pronase digestion can be grown in culture and may retain their function.

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Conduction of the Action Potential in the Frog Ventricle

Abstract. *Cardiac muscle fibers are made up of individual cells joined end to end; it has been suggested that the action potential must be conducted from cell to cell by some specialized mechanism. The alternative is that the electrical resistance is low at the junctions between the cells, so that the fiber behaves like a cable. The shape and time course of the foot of the action potential and the conduction velocity in the frog ventricle fit the predictions made from the cable theory.*

The conduction of the action potential in nerve and skeletal muscle is understood from core conductor theory; that is, the tissue is regarded as a long cable, with the cell membrane separating solutions of relatively low resistance on the inside and outside of the fiber. When one region of the fiber is excited, a current flows from the excited membrane through the intracellular solution and outward through the resting membrane. This local current flow depolarizes the resting membrane and leads to excitation (*1*). Many cardiac electrophysiologists have assumed that the same principles apply to conduction in the heart. A difficulty arises because the fibers of cardiac muscle are made up of individual cells, separated one from the other at the intercalated discs (*2*). The interposed membrane might add a large resistance to longitudinal current flow and prevent the flow of local currents.

Some studies suggest that the membranes at the intercalated discs are barriers to current flow (*3*, *4*). One possibility is that transmission across the discs is achieved by the release of a chemical (*5*); most of the evidence for specialized transmission mechanisms comes from experiments on the frog ventricle. On the other hand, the discs must have quite a low resistance for electrical transmission to occur (*6*).

If the fibers in the frog ventricle do behave like a cable, the initial depolarization at the earliest part of the rising phase (the foot) of the action potential would be generated by local current flow from excited regions of the fiber. The foot of the action potential should be exponential, and the time constant, t_c, of the foot of the action potential will be given by:

$$\frac{2\lambda^2}{v\,[v\tau + (v^2\tau^2 + 4\lambda^2)^{1/2}]} = t_c \qquad (1)$$

where λ is the space constant, τ is the time constant of the membrane, and v is the conduction velocity (*7*). Our experiments were conducted to see whether the time course of the foot of the action potential in the frog ventricle could be predicted by Eq. 1. To do this we had to know the time constant of the foot of the action potential, the space constant, the time constant of the membrane, and the conduction velocity.

The foot of the action potential of the isolated frog ventricle in Ringer solution was recorded by using glass capillary microelectrodes filled with 3*M* KCl, a capacity-compensated preamplifier, and an oscilloscope. The ventricles were stimulated electrically with 2 msec square waves applied between a cathode in the tissue and an anode in the Ringer solution. The sample record in Fig. 1*A* shows that the rapid upstroke of the action potential is preceded by a slower depolarization—the foot of the action potential. In Fig. 2, the semilogarithmic plot of the time course of the foot of another action potential shows that the depolarization is exponential. The time constant t_c was determined from plots such as that shown in Fig. 2. Conduction velocity (v) was estimated by assuming that the action potential was conducted along a direct line between the stimulating and recording electrodes. In some of the experiments conduction velocity was slowed by cooling the tissue.

The membrane time constant, τ, was

Table 1. Measured values of conduction velocity and the time constant of the foot of the action potential compared with calculated values for the time constant.

v measured (cm/sec)	t_c measured (msec)	t_c calculated (msec)
11.6	2.0	1.8
14.4	1.6	1.3
11.1	2.5	1.9
11.1	2.7	1.9
7.3	4.5	3.4
6.0	4.2	4.5

Table 2. Electrical constants of frog muscle fibers at rest. (Specific resistance of sarcoplasm, R_i, and of membrane, R_m; specific capacitance of membrane, C_m.)

R_i (Ω cm)	R_m (Ω cm^2)	C_m (μF cm^{-2})
	Ventricle	
460	2600	1.2
	Toe muscle	
260	4000	4.5

measured by following the changing voltage across the membrane when a hyperpolarizing current pulse was passed through an intracellular microelectrode. The voltage change across the microelectrode itself was compensated by using a bridge circuit (*8*). The mean from eleven measurements was 3.2 msec (range 2.7 to 3.7).

We assumed a space constant (λ) of 3.5×10^{-2}cm, the value found by Trautwein *et al.* for strips of frog auricle (*9*), in which current appears to spread as predicted by simple cable theory.

The time constant (t_c) for the foot of the action potential is calculated by

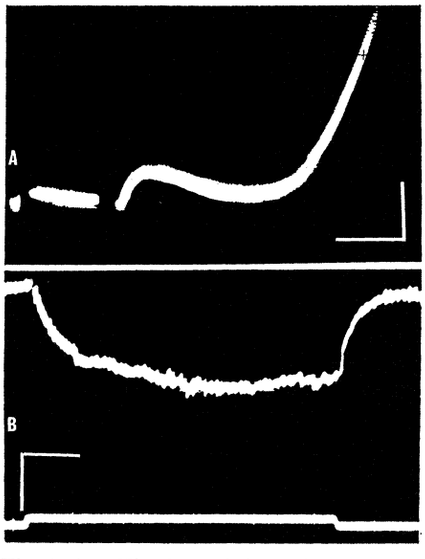

Fig. 1. (*A*) The foot of the action potential recorded from a frog ventricle. Calibrations: 5 msec and 10 mv. (*B*) Upper trace: The effect of a current passed through the microelectrode on the membrane potential of a fiber in the frog ventricle. Lower trace: current. Calibrations: 5 msec, 20 mv, and 5×10^{-9} amp.

Eq. 1; in Table 1 calculated and measured values are compared. The agreement is reasonably good and supports the idea that the foot of the action potential is generated by local current flow in a cable-like structure.

From the data we can also calculate the important electrical constants for fibers in the frog ventricle, using the well-known relationships from cable theory. Equation 1 solved for λ gives

$$\lambda = (v^2 t_c^2 + v^2 t_c \tau)^{1/2} \qquad (2)$$

From the measured values of v, t_c (line 1, Table 1), and τ, λ is found to be 3.75×10^{-2} cm. Furthermore,

$$\lambda = (r_m / r_i)^{1/2} \qquad (3)$$

where r_m is the resistance of the membrane and r_i is the resistance of the sarcoplasm for a unit of length of the fiber. These resistances can be estimated because our measurements gave values for another quantity which is a function of r_m and r_i:

$$\Delta V / I = \tfrac{1}{2} (r_m r_i)^{1/2} \qquad (4)$$

where ΔV is the change in membrane potential produced by passing a current, I, through the membrane in experiments like that illustrated in Fig. 1*B*. From eleven measurements the average $\Delta V/I$ was 11 megohms (range 5.2 to 29.3 megohms). Our result is close to the 12.4 megohms obtained by a two-electrode method (*4*).

Equations 3 and 4 were solved for r_m and r_i. The results can be compared with other tissues by calculating the specific resistances:

$$R_m = 2\pi r r_m, \text{ and } R_i = \pi r^2 r_i \qquad (5)$$

where R_m is the specific resistance of the membrane and R_i is the specific resistance of the sarcoplasm; r is the radius of the fiber, 5×10^{-4} cm. The specific capacitance of the membrane (C_m) is calculated from

$$\tau = C_m R_m \qquad (6)$$

The calculated constants for the ventricle are shown in Table 2, where they are compared with values for the toe muscle of the frog (*10*). The notable point is how similar the constants for cardiac muscle are to those for skeletal muscle.

We believe that the evidence supports the idea that conduction between the cells making up the frog ventricle occurs by local current flow. This means that the membranes at the junctions between ventricular cells have a comparatively low electrical resistance. One possibility which might account for the low electrical resistance is that the intercalated discs are "leaky" in regions where there

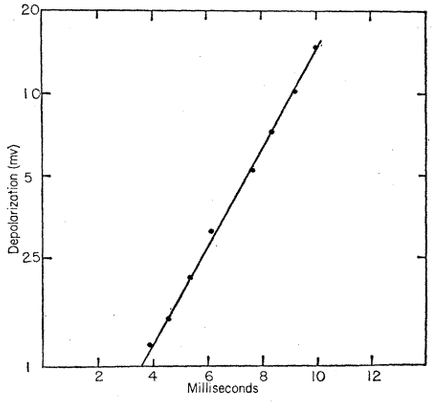

Fig. 2. The change in membrane potential at the foot of the action potential of the frog ventricle as a function of time.

are desmosomes or at the regions of membrane fusion (*11*). Although simple core conductor theory seems to give an acceptable fit for our data from the frog ventricle, we recognize that the theory is a simplification because of the branching of the fibers in cardiac muscle. In many experimental situations more complicated models may be needed to accurately fit the data (*6, 12*).

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2 July 1964