Effect of Diameter on Membrane Capacity and Conductance of Sheep Cardiac Purkinje Fibers

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ABSTRACT Membrane electrical properties were measured in sheep cardiac Purkinje fibers, having diameters ranging from 50 to 300 μm. Both membrane capacitance and conductance per unit area of apparent fiber surface varied fourfold over this range. Membrane time constant, and capacitance per unit apparent surface area calculated from the foot of the action potential were independent of fiber diameter, having average values of 18.8 ± 0.7 ms, and 3.4 ± 0.25 μF/cm², respectively (mean ± SEM). The conduction velocity and time constant of the foot of the action potential also appeared independent of diameter, having values of 3.0 ± 0.1 m/s and 0.10 ± 0.007 ms. These findings are consistent with earlier suggestions that in addition to membrane on the surface of the fiber, there exists a large fraction of membrane in continuity with the extracellular space but not directly on the surface of the fiber. Combining the electrical and morphological information, it was possible to predict a passive length constant for the internal membranes of about 100 μm and a time constant for charging these membranes in a passive 100-μm fiber of 1.7 ms.

In 1952 Weidmann reported that the membrane capacity of cardiac Purkinje fibers was 10–15 μF/cm². While this was far larger than the 1 μF/cm² found for squid axon membrane and most other cell membranes (Curtis and Cole, 1938), it did not differ markedly from the 5–8 μF/cm² found for frog skeletal muscle (Fatt and Katz, 1951). It was subsequently shown for skeletal muscle by Falk and Fatt (1964) and for cardiac Purkinje fibers by Fozzard (1966) that only about 3 μF/cm² could be attributed to the surface of the fiber, and the rest was associated with less accessible membrane. Sommer and Johnson (1968) suggested that this less accessible membrane in Purkinje fibers was found largely in intercellular clefts, associated with the external boundaries of the cells comprising the fiber bundle. Mobley and Page (1972) made quan-
titative measurements of this membrane, finding that both the fiber surface membrane and the interfiber cell boundaries were folded, resulting in an even larger membrane area than would be calculated from knowledge of the overall dimensions of the cells.

Assuming that the cells within a fiber are of a uniform size, a larger fiber would be composed of more cells. In this case more fiber membrane would be distant from the fiber surface, resulting in a larger membrane capacitance per unit surface area when calculated using only the apparent fiber surface area. Cable analyses in fairly large Purkinje fibers reported by Fozzard and Schoenberg (1972) revealed average membrane capacitance values of 17–19 μF/cm², with some values as high as 36 μF/cm². The present study adds additional measurements of the cable properties of Purkinje fibers to those already reported, and examines the relation of these parameters to fiber diameter. The prediction of variation with diameter of membrane capacity and conductance per unit apparent surface area was substantiated.

METHODS

Hearts were obtained from sheep less than 2 min after exsanguination and transported to the laboratory in chilled Tyrode solution. Free-running Purkinje fibers were excised from the left ventricle. All experiments were carried out in Tyrode solution containing NaCl, 137 mM; KCl, 5.4 mM; CaCl₂, 1.8 mM; MgCl₂, 1.05 mM; NaHCO₃, 13.5 mM; NaH₂PO₄, 2.4 mM; glucose, 11 mM. The solution was gassed with 95% O₂ and 5% CO₂, resulting in a pO₂ = 7.2–7.4. Temperature was regulated within 1°C, and kept at 35–37°C. Glass micropipettes, filled with 3 M KCl, had DC resistances of 5–10 MΩ. Cable analysis was performed as previously described (Fozzard, 1966), with one pipette placed at the end of the fiber column to pass small 300-ms hyperpolarizing currents and one or two other pipettes placed at various distances along the fiber to record the voltage response. Transmembrane potential was measured as the difference between the intracellular pipette and one placed outside the fiber. Resting potential was measured by withdrawal of a voltage-recording pipette during quiescence. Conduction velocity was calculated between two widely separated pipettes. Apparent capacity filled by the foot of the action potential was calculated according to the method of Tasaki and Hagiwara (1957). Diameter was usually measured using a calibrated eyepiece in a dissecting microscope (Carl Zeiss, Inc., New York) with indirect lighting. This was confirmed in some cases by frozen section and staining with Safranin O. Errors in diameter by the visual method did not exceed 20%. Current was measured by an operational amplifier (Tektronix, Inc., Beaverton, Ore., type O) that kept the tissue bath at virtual ground.

Twenty-eight new analyses of cable properties were made and added to 21 previously reported by Fozzard (1966) and Fozzard and Schoenberg (1972). It should be noted that measurement of fiber diameter was not as accurate as many of the electrical measurements. As described above, comparison of the optical method with frozen section showed differences as large as 20%. In addition, the diameter of the
fiber was not perfectly uniform along its length. Furthermore, the experiments were done over a range of 6 yr and the original set of cable analyses were being done in Bern, Switzerland. Because of this there could have been small differences in microelectrode techniques which might account for some of the scatter in the data.

RESULTS

Fiber measurements and calculations may be found in Table I. Since we were interested in comparing different populations of fibers and also determining whether various intercepts were statistically different from zero, we have expressed our computed values as "mean ± 95% confidence limits" rather than the more standard "mean ± SEM." Since all of our populations were fairly large (N > 20), the 95% confidence limits were all approximately twice the standard error of the mean (Natrela, 1963).

Membrane Capacitance

When total membrane capacitance per unit area was calculated with reference to the apparent surface area of the Purkinje fiber (calculated from measurements of diameter as described in the Methods section), it was found to increase markedly with diameter. Fig. 1 shows all the data points along with the least squares linear regression line of capacitance on diameter. The correlation coefficient of the data, r, was 0.716 (N = 48). The intercept of the regression line was 4.1 ± 4.1 μF/cm² (mean ± 95% confidence limits). The slope was 890 ± 256 μF/cm². The capacitance per unit area filled by the foot of the action potential was relatively independent of diameter. Its value was 3.4 ± 0.5 μF/cm² (N = 32).

Membrane Conductance

The membrane conductance per unit apparent surface area, G_m, was also found to increase markedly with diameter (Fig. 2). The correlation coefficient, r, was 0.707 (N = 44). The zero diameter intercept of the linear regression line was 0.18 ± 0.28 mmho/cm² (±95% confidence limits). The slope was 53.5 ± 16.5 mmho/cm².

Internal Conductivity

The average value of internal conductivity, G_i, was 8.9 ± 2.0 mmho/cm (mean ± 95% confidence limits, N = 38). Although there was large scatter in the data, there was some negative correlation (r = -0.47) between internal conductivity and diameter. The correlation was due mainly to the large values obtained for G_i in the smallest fibers (fibers 50–70 μm in diameter; see Fig. 3). In the series reported by Weidmann (1952), similarly large values of G_i were obtained for fibers of 50–70 μm.
### TABLE I
ELECTRICAL MEASUREMENTS IN PURKINJE FIBERS

| Fiber | Diameter | Vd/Io | λ | r | Rm | Cm | R1 | r1 | θ | Cj | Vm | APD |
|-------|----------|-------|---|---|----|----|----|----|---|----|-----|-----|
| FL 1  | 50       | 300   | 2.6| 24| 2,300| 10.5| 46 | 0.09| 3.1| 3.5| 250  |
| FL 2  | 52       | 457   | 2.2| 23| 1,850| 12.4| 53 | 0.09| 3.0| 2.9| 240  |
| FL 3  | 66       | 400   | 2.0| 20| 1,500| 13.3| 57 | 0.12| 3.6| 1.85|     |
| FL 4  | 80       | 430   | 1.5| 17.3| 1,130| 15  | 100| 0.10| 2.9| 2.3| 210  |
| FL 5  | 120      | 460   | 1.3| 18 | 1,800| 10  | 315| 0.22| 1.3| 2.6|     |
| FL 6  | 100      | 130   | 2.3| 23.5| 1,700| 13.8| 157| 0.10| 2.1| 3.5| 240  |
| FL 7  | 80       | 760   | 1.8| 25 | 1,720| 14.5| 85 |     |     |     | 240  |
| FS 1  | 80       |       |    |    |     |     |    |     |     |     | 7.5   |
| FS 2  | 100      |       |    |    |     |     |    |     |     |     | 13.9  |
| FS 3  | 110      |       |    |    |     |     |    |     |     |     | 8.4   |
| FS 4  | 80       |       |    |    |     |     |    |     |     |     | 7.7   |
| SL 1  | 150      | 120   | 2.0| 19.5| 1,220| 16  | 115| 0.07| 3.7| 3.3|     |
| SL 2  | 200      | 154   | 2.2| 23.3| 2,180| 10.7| 220| 0.09| 3.0| 3.6|     |
| SL 3  | 200      | 84    | 2.33| 7 | 708  | 30  | 200| 0.11| 2.85| 2.9|     |
| SL 4  | 125      | 97    | 2.1| 22.7| 1,550| 13.8| 116| 0.09| 2.84| 3.7|     |
| SS 1  | 200      | 164   |    |    |     |     |    |     |     |     |     |
| SS 2  | 150      | 214   |    |    |     |     |    |     |     |     |     |
| SS 3  | 150      | 110   |    |    |     |     |    |     |     |     |     |
| SS 7  | 100      | 240   |    |    |     |     |    |     |     |     |     |
| SS 8  | 80       | 520   |    |    |     |     |    |     |     |     |     |
| SS 9  | 88       | 300   |    |    |     |     |    |     |     |     |     |
| DF 1  | 160      | 185   | 1.7| 23.8| 1,926| 12.3| 285| 0.08| 2.84| 2.1|     |
| DF 2  | 138      | 133   | 1.12| 17| 1,064| 16  | 291| 0.09| 3.1 | 1.4 |     |
| DF 3  | 110      | 144   | 1.2 | 9  | 845  | 10.6| 174| 0.08| 3.2 | 1.85|     |
| DF 4  | 140      | 87    |    |    |     |     |    |     |     |     | 3.5  |
| DF 5  | 120      | 69    | 2.8 | 14| 1,411| 10  | 108| 0.04| 3.8 | 4.3 | -77 |
| DF 6  | 110      | 108   | 3.2 | 16| 1,234| 13  | 33 | 0.09| 4.2 | 5.2 | -80 |
| DF 7  | 110      | 125   | 2.26| 18| 1,235| 14.4| 67 | 0.11| 3.2 | 3.4 | -76 |
| DF 8  | 120      | 90    | 1.32| 14| 879  | 15.9| 502| 0.04| 2.9 | 3.0 | -78 |
| DF 9  | 160      | 120   | 1.72| 16| 1,037| 16  | 95 | 0.20| 2.4 | 3.7 | -79 |
| DF 10 | 165      | 107   | 1.76| 18| 1,239| 14.2| 169| 0.11| 2.9 | 2.7 | -81 |
| DF 11 | 220      | 100   | 1.2 | 15.5| 666  | 23  | 252| 0.08| 2.1 | 5.9 | -69 |
| DF 12 | 174      | 70    | 2.86| 20| 1,115| 18  | 60 | 0.15| 3.2 | 4.6 | -75 |
| DF 13 | 192      | 140   | 1.32| 15| 1,320| 15.3| 367| 0.08| 3.8 | 1.1 | -77 |
| DF 14 | 165      | 75    | 1.2 | 11| 446  | 24  | 137| 0.08| 3.25| 3.8 | -72 |
| DF 15 | 110      | 150   | 2.0 | 18| 1,056| 17  | 74 | 0.08| 2.8 | 6.1 | -75 |
| DF 16 | 136      | 160   | 2.06| 17| 1,620| 14.5| 197| 1.8 |     | -75 |
| DF 17 | 220      | 53    | 2.7 | 17| 934  | 18  | 76 | 0.11| 3.4 | 5.9 | -350 |
| DF 18 | 120      | 88    | 3.5 | 30| 2,312| 13  | 114| 0.04| 3.44| 5.0 | -410 |
| DF 19 | 260      | 54    | 1.34| 11| 590  | 18.6| 212| 0.06| 4.6 | 2.44| -86 |
| DF 20 | 192      | 94    | 1.7 | 12.5| 980  | 12.5| 160| 0.09| 3.0 | 3.6 | -71 |
| DF 21 | 192      | 112   | 1.2 | 18.7| 808  | 23  | 269| 0.15| 1.6 | 4.6 | -80 |
| DF 22 | 160      | 89    | 1.34| 20| 800  | 25  | 154| 0.16| 1.9 | 5.15|     |
| DF 23 | 128      | 212   | 2.0 | 19| 1,700| 11.2| 137| 0.07| 4.3 | 1.8 | -81 |
| F 1   | 300      | 35    | 1.65| 16| 545  | 29.4| 150|     |     |     |     |
| F 2   | 250      | 43    | 1.55| 18| 501  | 36  | 124|     |     |     |     |
| F 3   | 250      | 53    | 2.0 | 24| 850  | 29  | 130|     |     |     |     |
| F 4   | 300      | 38.5  | 1.4 | 18| 507  | 35.4| 194|     |     |     |     |
| F 5   | 200      | 61    | 1.3 | 16| 520  | 30  | 150|     |     |     |     |

Legend to Table I on facing page.
**Input Resistance**

Input resistance decreased markedly with diameter (Fig. 4). For fibers less than 100-μm diameter, the input resistance averaged 465 kΩ \((N = 7)\), and for fibers 250 μm or greater in diameter, the input resistance averaged 55 kΩ \((N = 8)\). The input resistance is related to the membrane conductance and internal conductivity according to the relationship, input resistance \(= (2/\pi)(DG_mG_i)^{-1/2}\). The line drawn in Fig. 4 was calculated using the re-

| Table I Legend. |
|------------------|
| Fibers identified as FL and FS are long cable and short segment studies previously reported by Fozzard (1966). SL and SS indicate long and short fibers, respectively, already reported by Fozzard and Schoenberg (1972). The series labelled DF and F are newly reported data. Diameter is in microns; \(V_d/I_o\) is input resistance in kilohms; \(\lambda\) is cable length constant in millimeters; \(\tau\) is membrane time constant in milliseconds; \(R_m\) is resistance of 1 cm\(^3\) of apparent surface membrane in ohms cm\(^2\); \(C_m\) is capacitance of 1 cm\(^3\) of apparent surface membrane in microfarads per square centimeter; \(R_i\) is specific core resistivity in ohmcentimeters; \(\tau_f\) is the time constant of the foot of the action potential in milliseconds; \(\theta\) is conduction velocity in meters per second; \(C_f\) is capacitance per unit apparent surface area calculated from the foot of the propagated action potential in microfarads per square centimeter; \(V_m\) is resting membrane potential in millivolts; APD is duration of the action potential in milliseconds measured at 90% repolarization. |
Figure 2. Membrane conductance per unit apparent surface area versus diameter. Solid line is least squares regression line.

Figure 3. Internal conductivity versus diameter. Solid curve is $G_i = 7.9 \text{ mmho/cm}$, a value 1 SEM less than the mean. This value was used in the calculations of Figs. 4 and 6 since it seemed to give a better fit to the data of Fig. 4. Note the apparent increase in $G_i$ for the smallest fibers (see text).
Figure 4. Input resistance versus diameter. Solid line is drawn according to the relationship, input resistance = \((2/\pi) (D G_m G_i)^{-1/2}\), where \(G_i = 7.9\ \text{mmho/cm}\) and \(G_m\) varies with diameter according to the regression line of Fig. 2.

Regression line of Fig. 2 for the variation of membrane conductance with diameter and using \(G_i = 7.9\ \text{mmho/cm}\). A value for \(G_i\) 1 SEM less than the mean was chosen for the calculations because it gave a slightly better fit to the data. This seemed reasonable since it appeared as if a few large values of \(G_i\) might have raised the calculated value of the mean.

**Length Constant**

No variation of length constant with diameter could be demonstrated \((r = 0.32, N = 38)\). The expected change, using the regression line of Fig. 2 and \(G_i = 7.9\ \text{mmho/cm}\), is less than 10% over the diameter range of 100–300 \(\mu\text{m}\). If the regression line of Fig. 2 actually went through the origin, no change of length constant with diameter would be expected. The average value of the length constant was \(1.9 \pm 0.2\ \text{mm}\).

**Time Constants**

The time constant for the foot of the action potential, \(\tau_f\), was independent of diameter \((r = 0.04, N = 31)\) and had an average value of \(0.10 \pm 0.014\ \text{ms}\). The membrane time constant was also independent of diameter \((r = 0.255, N = 34)\), having an average value of \(18.8 \pm 1.4\ \text{ms}\).
Conduction Velocity and Other Parameters

Surprisingly, the conduction velocity, \( \theta \), appeared to be independent of diameter \( (r = 0.04, N = 33) \). Its value was 3.0 ± 0.2 m/s. There was no relationship between fiber diameter and resting membrane potential \((-77 ± 2 \text{ mV}, \ r = 0.02, N = 17) \) or fiber diameter and action potential duration \((310 ± 53 \text{ ms}, r = 0.26, N = 24) \).

Calculations

Membrane Capacitance and Conductance

Capacitance per unit area is usually calculated on the basis of a smooth envelope of membrane at the surface of the fiber. Weidmann (1952) suggested that the apparent average membrane capacitance of 12 \( \mu \text{F/cm}^2 \) was unusually large because the estimation of membrane area was in error. Quantitative measurements of membrane area have been made by Mobley and Page (1972) in cardiac Purkinje fibers of about 100-\( \mu \text{m} \) diameter. They found that the amount of membrane was about 12 times the amount calculated by assuming a smooth surface envelope. The additional membrane was found in boundaries between cells within the fiber and in a redundancy or folding of both the surface membrane and these “internal” membranes. From their studies it seemed possible that the actual membrane capacitance of Purkinje fibers might be 1 \( \mu \text{F/cm}^2 \). They also suggested that calculation of membrane parameters based upon apparent surface area might depend on fiber diameter, since the ratio of internal membrane to surface membrane would increase with fibers of larger diameter. This would be the case only if the current injected at the surface were capable of spreading down the cell interspaces to interior cells. The present study clearly suggests that this is the case.

An extremely simplified representation of the Purkinje fiber morphologically described by Mobley and Page (1972) is shown in Fig. 5a. Each of the Purkinje cells is drawn as an equilateral triangle with 50-\( \mu \text{m} \) sides. This was chosen so that each cell of a 100-\( \mu \text{m} \) fiber would have an apparent cell surface to volume ratio three times the apparent surface to volume ratio of the whole fiber, as was reported by Mobley and Page (1972). Also, sheep Purkinje cells are known to have a diameter of 40–50 \( \mu \text{m} \) (Mobley and Page, 1972).

Mobley and Page found that both the internal and external membranes were extensively folded. The internal membrane had a folding factor, \( \phi_i \), of 1.9 and the external membrane had a folding factor, \( \phi_e \), of 1.35. These measurements were made from electron micrographs of transverse sections. Using their assumption that the folding factors in the longitudinal plane were similar, so that the surface area increment due to folding is proportional to \( \phi^3 \), the specific membrane capacitance, \( C'_m \), and conductance, \( G'_m \), can be calculated. From Figs. 1 and 2, a 100-\( \mu \text{m} \) fiber has a capacitance per unit apparent
Schoenberg, Dominguez, and Fozzard  Diameter and Cable Properties  449

Figure 5. Simplified representation of a Purkinje fiber. Fig. 5 a shows a 100-μm fiber composed of six triangular Purkinje cells. Salient features include folding of internal and external membranes; apparent surface area to volume ratio of fiber equaling one-third that of cell (apparent surface area implies neglecting folding as in Fig. 5 b); constant separation between neighboring cells. Fig. 5 b schematically shows individual Purkinje cell and how 200- and 300-μm fibers may be represented as composed of individual cells.

Surface area of approximately 13 μF/cm² and a conductance of 0.71 mmho/cm². Therefore, for Fig. 5 a,

\[(6 \times \phi^2 \times 50 \times 10^{-4} + 12 \times \phi^2 \times 50 \times 10^{-4}) C_m' = 13 \times 6 \times 50 \times 10^{-4},\]

or \(C_m' = 1.45 \mu F/cm^2\). Similarly, the specific membrane conductance is calculated as 0.08 mmho/cm².

Types of Models

Frog skeletal muscle because of the presence of transverse tubules also has membrane in addition to that on the external surface of the fibers. Hodgkin and Nakajima (1972 a, b) have demonstrated that membrane capacitance and conductance per unit surface area are larger for larger fibers. A model assuming a constant volume of transverse tubules per unit volume of the cell had been developed by Adrian et al. (1969) and Hodgkin and Nakajima (1972 a, b) found that this model predicted the observed variation in membrane capacitance and conductance. In addition, the model accounted for
the measured capacity filled by the foot of the action potential and could be used to calculate the time constant for voltage clamping of the transverse tubular system. It would be helpful if an equivalent model could be derived for Purkinje fibers.

A very simple model for Purkinje fibers of different diameter is represented in Fig. 5a and 5b. The model incorporates the concept that larger fibers are composed simply of larger numbers of individual Purkinje cells as suggested by electronmicrographs. An interesting difference between a model of this type and that of Adrian et al. (1969) is that in the former the real surface to volume ratio of the fiber is similar to the real surface to volume ratio of each cell (ignoring the cell interspaces and the difference in folding factor between membrane in the interior and that near the cell surface). As a result, the extrapolated zero diameter intercept of the capacitance or conductance per unit apparent surface area versus diameter plot need not necessarily be different from zero, as is the case in the model of Adrian et al., 1969. It is interesting to note that the intercepts of the regression lines in Figs. 1 and 2 are not significantly different from zero whereas those of Hodgkin and Nakajima (1972 b) are. However, these findings could just as easily be explained by assuming greater scatter in our data.

Another important difference between the model of Adrian et al. (1969) and that represented by Fig. 5a is that the former has a greater fraction of the internal membrane near the surface. As a result, if the parameters of the Adrian et al. model are chosen for the Purkinje fiber to give the correct value for the average amount of internal membrane per unit volume, the model predicts too great a value for the capacitance filled by the foot of the action potential. The model represented by Fig. 5a gives a much better prediction, as shown in the next section.

**Capacitance Filled by the Foot of the Action Potential**

Hodgkin and Nakajima (1972 b) showed that the capacitance filled by the foot of the action potential in frog skeletal muscle is equal to the capacitance of the surface membrane plus an additional contribution of the T-tubules. This additional contribution is equal to $Y_{Tf} \tau_f$, where $\tau_f$ is the exponential time constant of the foot of the action potential, and $Y_{Tf}$ is the ratio of tubular current to surface voltage for an exponential voltage at the surface. The capacitance filled by the foot of the action potential in a Purkinje fiber may be calculated in a similar manner, substituting $Y_{cf}$ for $Y_{Tf}$, using the current into the clefts in place of the tubular current.

For a cleft, $Y_{cf}$ may be found by substituting $V_j = V_o e^{ei\omega}$ in Eq. 4 a of the Appendix. As suggested by Hodgkin and Nakajima (1972 b), Eqs. 4 a and 2 a may then be readily solved in a manner identical to the solution for the steady
Schoenberg, Dominguez, and Fozzard  Diameter and Cable Properties

state, yielding the voltage distribution

$$V = V_f \frac{\cosh (x/\lambda_{sf})}{\cosh (\alpha/\lambda_{sf})},$$

where $V_f$ is the voltage at the mouth of the cleft, and the current

$$I_{sf} = \frac{V_f \bar{G}_L}{\lambda_{sf}} \frac{\sinh (\alpha/\lambda_{sf})}{\cosh (\alpha/\lambda_{sf})},$$

where $\lambda_{sf}$, the "length constant" for an exponentially rising voltage, is $\bar{G}_L / (\bar{C}_m + \bar{G}_m/\tau_f)^{1/2}$. We then have

$$Y_{sf} = \frac{\bar{G}_L}{\lambda_{sf}} \tanh (\alpha/\lambda_{sf}).$$

$\bar{G}_L$ is the conductivity per unit depth of the fluid in the lumen of the cleft for a cleft of 1-cm length (mho centimeter). $\bar{C}_m$ and $\bar{G}_m$ are the capacitance and conductance per unit depth, again for a slit 1 cm in length. The appropriate units for $\bar{C}_m$ and $\bar{G}_m$ are F/cm and mho/cm, respectively.

For a single cleft of the fiber represented by Fig. 5a, we have

$$\bar{G}_L = \frac{G'_L d}{\phi_i} \times 1 \text{ cm},$$

$$\bar{C}_m = 2G'_m \phi_i \times 1 \text{ cm},$$

$$\bar{C}_m = 2G'_m \phi_i \times 1 \text{ cm},$$

where $G'_L$ is assumed equal to the conductivity of Tyrode solution, $2 \times 10^{-2}$ mho/cm and $d$ is the width of the cleft. Sommer and Johnson (1968), from their electronmicrographic studies, estimated the distance between cells ($d$) in sheep Purkinje fibers to be relatively constant and approximately $3 \times 10^{-6}$ cm. Using $G'_m = 0.08$ mmho/cm$^2$, and $C'_m = 1.45 \mu F/cm^2$ and $\phi_i = 1.9$ from the previous section, we have $\bar{G}_L = 3.1 \times 10^{-8}$ mho cm, $\bar{C}_m = 0.3$ mmho/cm and $\bar{C}_m = 5.5 \mu F/cm$. $\tau_f$ equals 0.1 ms (see Results). This yields an "exponential" length constant, $\lambda_{sf} = 7.5 \times 10^{-4}$ cm. For comparison, the DC length constant, $\lambda_e = (\bar{G}_L/\bar{G}_m)^{1/2}$, which is used in the next section, is $10^{-4}$ cm.

It is now easy to calculate the expected capacitance filled by the foot of an action potential for the fiber model of Fig. 5a. The surface capacitance in 1 cm of fiber length is

$$6 \times 50 \times 10^{-4} C'_m \phi_i^2 = 7.92 \times 10^{-2} \mu F,$$
taking \( C'_m = 1.45 \mu F/cm^2 \), \( \phi_0 = 1.35 \), and taking the side of each triangular "cell," \( a \), to be \( 50 \times 10^{-4} \) cm.

For each of the six clefts, \( Y_{c\ell} \), for 1 cm of membrane, equals \( \frac{\bar{\sigma}_L}{\lambda_{c\ell}} \) since \( a/\lambda_{c\ell} \) is much greater than 1. Therefore, the total capacitance of 1-cm real length of cleft filled by the foot of an action potential with a time constant, \( \tau_f = 0.1 \) ms is

\[
(6 \frac{\bar{\sigma}_L}{\lambda_{c\ell}}) \tau_f = 2.48 \times 10^{-2} \mu F.
\]

This is the capacitance contribution of the clefts for 1-cm length of membrane. However, because of longitudinal folding of the membranes, 1 cm of fiber has an amount of membrane equal to \( 1 \times \phi \). Because \( \lambda_{c\ell} \) is only 7.5 \( \mu m \), compared with a cell diameter of 100 \( \mu m \), it is probably more reasonable to use the folding factor of membrane near the surface \( \phi_0 = 1.35 \) rather than the internal membrane folding factor, \( \phi_i = 1.9 \). The total contribution of the clefts for 1 cm of fiber is therefore \( 2.48 \times 10^{-2} \times 1.35 = 3.35 \times 10^{-2} \mu F \). Adding this to the capacitance of the surface membrane and normalizing by the apparent surface area (\( 3 \times 10^{-2} \) cm\(^2\)) yields an expected capacitance filled by the foot of the action potential per unit apparent area of \( 3.76 \mu F/cm^2 \) for the model of Fig. 5 a. The average value measured in our experiments (see Results) was \( 3.4 \pm 0.5 \mu F/cm^2 \). The extremely close agreement is probably somewhat fortuitous considering the crudeness of the model. However, it is of interest that the model appears to give reasonable values. Since the length constant of the clefts for the foot of the action potential is only the order of 7-8 \( \mu m \), the capacitance per unit apparent area filled by foot would not be expected to vary much for diameters between 50-300 \( \mu m \).

**DISCUSSION**

*Radial Uniformity*

Radial nonuniformity of potential along intercellular clefts during passage of DC currents would be expected to affect measurements of membrane capacitance and conductance in larger fibers. This is because a potential drop could occur in the cleft between the center of the fiber bundle and the outside, if a significant resistance to ion movement was present. Existence of standing gradients would be an important source of error in efforts to "voltage clamp" cardiac Purkinje fibers. These standing gradients have been calculated by Sommer and Johnson (1968) for membranes of various membrane resistances. Ignoring membrane folding, they predicted negligible gradients if the specific membrane resistances were greater than 10,000 \( \Omega cm^2 \).

A more complete description of the voltage clamp of a cleft is given in the Appendix. From Eq. 6 a (also Sommer and Johnson, 1968), the ratio of the steady-state voltage in the center of a fiber to that on the surface is equal to \( [\cosh (a/\lambda_0)]^{-1} \). From calculations described in the previous section the DC
length constant, \( \lambda = (\bar{G}_L/\bar{G}_m)^{1/2} \), for a cleft width of \( 3 \times 10^{-6} \) cm is approximately 100 \( \mu \)m. For a 100-\( \mu \)m fiber (\( a = 50 \mu \)m), the steady-state voltage in the center of the fiber after a voltage step is then 89\% that at the surface. The time constant with which this voltage is established, which is identical with the time constant for current decay, may be obtained from Eq. 9 a and is equal to 1.7 ms. This is similar to the average time constant of 2.1 ms measured by Fozzard (1966) for current decay in four voltage clamped fibers.

It thus appears that the very simple model represented by Fig. 5 a makes reasonable predictions for the behavior of the 100-\( \mu \)m passive Purkinje fiber. It appears that in response to a surface depolarization, substantial amounts of internal membrane are brought to a voltage not too different from the surface voltage with a time-course of only a few milliseconds in the passive fiber. How much or how quickly charge spreads to the internal membrane in an active Purkinje fiber is a topic much open to discussion. It cannot be answered fully without some knowledge of the time and voltage dependence of the active currents.

**Conduction**

The time constant of the foot of the action potential, \( \tau_f \), did not vary with diameter (\( r = 0.04, N = 31 \)). This is compatible with the idea that the sites generating ionic current are proportional to the effective capacitance of a fiber for fibers of different diameter. This follows from Eq. 3 of Hodgkin and Huxley (1952) where \( K = 4\theta C_m/DG_i \approx 1/\tau_f \), remains independent of diameter if \( C_m \) scales with the ionic conductances.

Surprisingly, conduction velocity, \( \theta \), also showed no variation with diameter (\( r = 0.04, N = 33 \)), although this might have been due to the large scatter in the data. Fig. 6 shows the data along with two alternative curves for

![Figure 6](image-url)
\[ \theta = \left( \frac{DG_i}{4\tau_iG_i} \right)^{1/2}. \]

Since the capacitance filled by the foot of the action potential seems to be that of membrane very near the surface of the fiber, \( G_i \) was taken as constant and equal to 3.4 \( \mu \text{F/cm}^2 \) while \( \tau_i \) was taken as 0.1 ms. The solid curve assumes \( G_i \) is constant (\( G_i = 7.9 \text{ mmho/cm} \)) so that \( \theta = \sqrt{D} \); the dashed curve assumes \( \theta \) is constant which implies \( G_i \propto D^{-1} \). We found it impossible, on the basis of the data, to distinguish between these two possibilities. In an ideal core conductor, \( G_i \) is constant and \( \theta \) varies as the square root of diameter. However, one case in which \( \theta \) would be independent of diameter is if in the larger fibers internal currents were confined mainly to the peripheral cells. Compatible with this, Fig. 3 appears to show an increase in \( G_i \) for smaller fibers. Whether this is real, or possibly an artifact due to differences in cross-sectional shape between large and small fibers (perhaps elliptical versus spherical), is not clear. Although the possibility that in larger fibers the internal currents are not distributed equally must be regarded as very tentative, one wonders whether portions of the Purkinje system where diameters are even larger (near the bundle of His, for example) have similar conduction velocities and whether this might not have some functional role in coordination of ventricular contraction. Clearly a larger range of fiber diameter needs to be studied with more data at the extremes. It may be, as suggested by Sommer and Johnson (1968), that sheep Purkinje fibers much larger than 100 \( \mu \text{m} \) cannot be treated as simple cables.

**Excitation**

It has been shown (Schoenberg and Fozzard, 1971; Fozzard and Schoenberg, 1972) that many properties relating to excitation can be understood in terms of the liminal length concept of Rushton (Rushton, 1937) which states that a minimum or "liminal" length of membrane has to be raised above threshold before an action potential can be propagated. Recently, Noble (1972) suggested a model of an excitable system in which a simple analytic expression for the liminal length could be obtained. He assumed that the current-voltage relation of membrane below threshold could be expressed by the linear relation \( i = g_sV \) and above threshold by the linear relation \( i = g_AV \) where \( g_s \) was negative. Solving for the steady-state voltage distribution of such a system and assuming that an action potential is generated when the active depolarizing currents are large enough to balance the passive repolarizing currents, the length of membrane above threshold necessary for activation is equal to \( (\pi/2)\lambda_m(-g_s/g_A)^{1/2} \) where \( \lambda_m \) is the longitudinal cable DC length constant.

Fozzard and Schoenberg (1972) found that compared to the Hodgkin-Huxley squid axon, Purkinje fibers have a much smaller ratio of liminal length to DC length constant. It may be that the explanation for this difference lies in the different geometries. Charge from the current electrode would be expected to spread through the internal membrane near the current elec-
trode more rapidly than charge would flow across internal membrane more distal. If the internal membrane were excitable, ionic currents from this membrane would tend to cause a greater concentration of depolarizing currents near the current electrode, possibly more rapidly than distal internal membrane could contribute to the passive repolarizing currents. This would lead to a relatively shorter liminal length than if all the membrane were on the surface and equally accessible to charge as in the case of the squid axon. Again, however, these conclusions must remain tentative until more is known about the properties of the internal membranes in the active Purkinje fiber.

In conclusion, it seems reasonably certain that internal membrane of the passive Purkinje fiber is accessible to charge injected at the surface and that for a 100-μm fiber the charging time constant is on the order of 1-2 ms. The DC length constant for the clefts appears to be the order of 100 μm and the length constant for the action potential about 10 μm, but both of these numbers are very sensitive to specific values chosen for geometric variables which may be in error, such as the exact separation between adjacent cell membranes and the precise degree of folding of internal membrane.

**APPENDIX**

**Current and Voltage Distribution down a Cleft**

Fig. 7 shows a single cleft with the important variables demonstrated. The cleft is of depth 2a, with x representing distance from the center of the cleft, x = 0. i(x, t) is

\[
\begin{align*}
  x &= a \\
  x &= 0 \\
  x &= -a
\end{align*}
\]

\[G_m, \quad i_m, \quad i, \quad \bar{G}_L, \quad C_m, \quad d\]

**Figure 7.** Transverse section of a cleft, showing important parameters and variables. a, one-half depth of cleft (centimeters). \(\bar{G}_L\), conductivity per unit depth of fluid in cleft (mho centimeter). d, separation of cleft walls (centimeters). \(i_m\), outward membrane wall current density (amperes per centimeter). \(i\), outward lumen current (amperes). Schematic shows parallel R-C properties of lumen wall. \(\bar{G}_m\) = membrane conductance per unit depth (mho per centimeter). \(C_m\) = membrane capacitance per unit depth (farad per centimeter).
the outward current in the lumen of the cleft, while \( i_m(x, t) \) is the outward membrane current passing through the walls of the cleft. The walls have a conductance and a capacitance per unit depth of \( G_w \) and \( C_w \), respectively. The conductivity of the fluid in the lumen of the cleft per unit depth is \( G_L \). \( V(x, t) \) is the voltage distribution along the cleft measured as internal voltage minus lumen voltage.

From Kirchhoff's current law

\[ i_m = \frac{\partial i}{\partial x}. \quad (1a) \]

From Ohm's law

\[ i = G_L \frac{\partial V}{\partial x}. \quad (2a) \]

From the current voltage relationship across the cleft membrane

\[ i_m = G_m V + C_m \frac{\partial V}{\partial t}. \quad (3a) \]

Finding \( \frac{\partial i}{\partial x} \) from Eq. 2a and equating 1a and 3a yields

\[ G_L \frac{\partial^2 V}{\partial x^2} = G_m V + C_m \frac{\partial V}{\partial t}. \quad (4a) \]

If the cleft is voltage clamped at time zero such that the ends \( x = \pm a \) are kept at \( V = V_0 \), the voltage distribution as a function of time may be found by applying Danckwert's method to the corresponding solutions of the diffusion equation. (See Carslaw and Jaeger, 1959, pp. 33, 100.)

The complete solution is

\[
V = 1 - \sum_{n=0}^{\infty} \frac{4(-1)^n}{\pi(2n + 1)} \left[ 1 - \frac{1}{(2n + 1)^2 \pi^2 \lambda_c^2 + 1} \right] \cos \left( \frac{(2n + 1)\pi x}{2a} \right) \\
- \sum_{n=0}^{\infty} \frac{4(-1)^n}{\pi(2n + 1)} \left[ 1 - \frac{1}{(2n + 1)^2 \pi^2 \lambda_c^2 + 1} \right] \cos \left( \frac{(2n + 1)\pi x}{2a} \right) \times \exp \left\{ -\left[ \frac{(2n + 1)^2 \pi^2 \lambda_c^2}{4a^2} + 1 \right] \left[ \frac{t}{\tau_m} \right] \right\},
\]

where \( \lambda_c = (G_L/G_m)^{1/2} \) and \( \tau_m = C_m/G_m \). The steady-state part of solution may be expressed in an alternate form by setting \( \partial V/\partial t = 0 \) in Eq. 4a and solving for the steady-state voltage, \( V_{ss} \), yielding

\[ V_{ss} = V_0 \frac{\cosh \left( x/\lambda_c \right)}{\cosh \left( a/\lambda_c \right)}. \quad (6a) \]

The total outward current through the mouth of the cleft, \( I_o \), during the voltage
clamp may be found by evaluating Eq. 2 at \( x = a \) or \( x = -a \). The result is

\[
I_e = \frac{V_o \hat{G}_L}{\lambda_e} \sinh \left( \frac{a}{\lambda_e} \right) + \frac{2V_o \hat{G}_L}{a} \sum_{n=0}^{\infty} \left[ \frac{1}{\frac{(2n+1)^2 \pi^2 \lambda_e^2}{4a^2} + 1} \right] \left\{ \frac{t}{\tau_m} \right\}.
\]

(7a)

making use of the fact that \((-1)^n \sin [(2n+1)\pi/2] = 1\) for all \(n\).

If \(a/\lambda_e < 1\), for all but the shortest times Eq. 7 may be approximated by the first term of the infinite sum. This yields

\[
I_e = \frac{V_o \hat{G}_L}{\lambda_e} \tanh \left( \frac{a}{\lambda_e} \right) + \frac{2V_o \hat{G}_L}{a} \left[ \frac{\pi^2 \lambda_e^2}{4a^2} \right] \exp \left\{ -\left[ \frac{\pi^2 \lambda_e^2}{4a^2} \right] \left[ \frac{t}{\tau_m} \right] \right\}.
\]

(8a)

It is seen that the current following a voltage clamp soon decays as a single exponential with a time constant, \(\tau_e\), equal to

\[
\tau_e = \frac{\tau_m}{1 + \frac{\pi^2}{4} (\lambda_e/a)^2}.
\]

(9a)

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Note Added in Proof While this paper was in press, a paper by D. C. Hellam and J. W. Studt (1974. J. Physiol. (Lond.). 243:637–694) reported the results of an electronmicroscopic and electrophysiologic study designed to study the variability in geometrical and electrical properties of Purkinje fibers. It is clear from their results that much of the scatter in the present paper is likely related to remaining differences in structure even among fibers of the same diameter. Another interesting result reported by Hellam and Studt is that in their hands the intercellular cleft distance was quite variable, averaging about 4 × 10^{-6} cm. This is in contrast to the work of Sommer and Johnson (1968) who reported a reasonably constant value of 3 × 10^{-6} cm for the cleft width.

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