DONNAN POTENTIALS FROM STRIATED MUSCLE LIQUID CRYSTALS

A-Band and I-Band Measurements

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ABSTRACT A-band and I-band potentials are measured selectively in crayfish skinned long-tonic muscle fibers. The microelectrode tip diameters used in this study are shown to be sufficiently small to permit the discrete placement of an electrode into either an A-band or I-band. Random and directed impalements into mechanically skinned fibers with microelectrodes yields reproducible trimodal distributions of potentials where the modalities represent the A-band (−1.80 mV), the I-band (−0.76 mV), and the Z-line vicinity (−3.63 mV). In conjunction with Donnan equilibrium theory, fixed charge concentrations are calculated from the measured potentials for the A-band (25.9 mmol e^-/l), I-band (10.9 mmol e^-/l), and Z-line vicinity (52.3 mmol e^-/l). When skinned fibers are treated with Triton X-100, the mean potentials (and charge concentrations) decrease: A-band to −1.71 mV (24.6 mmol e^-/l), I-band to −0.71 mV (10.2 mmol e^-/l), and the Z-line vicinity to −3.40 mV (49.0 mmol e^-/l). In the A-band this represents a loss of 1.3 mmol e^-/l while in the I-band 0.7 mmol e^-/l are lost; both decreases are attributed to the removal of internal membranous structures. In the rigor condition the A-band increases to −2.18 mV (33.1 mmol e^-/l) and the I-band increases to −0.88 mV (13.3 mmol e^-/l). Relative to the relaxed condition, this represents an increase of 8.5 mmol e^-/l and 3.1 mmol e^-/l in the A-band and I-band, respectively. The evidence shows that it is practical to measure A-band and I-band potentials selectively. Further, it is demonstrated that similar measurements can be obtained from agar, another polyelectrolyte gel system (see Appendix).

INTRODUCTION

Potentials have been measured from skinned and glycerinated muscle (Aldoroty and April, 1981, 1982; Bartels and Elliott, 1981 *a,b,* 1982; Dewey et al., 1982; Stephenson et al., 1981; Bartels et al., 1980; Hinke, 1980; Scordalis et al., 1975; Pemrick and Edwards, 1974; Collins and Edwards, 1971; Weiss et al., 1967; Naylor and Merrillees, 1964; Chichibu, 1961). Further, recent brief reports have suggested that it is possible to measure potentials selectively and reproducibly from the A-band and I-band regions of skinned fibers (Aldoroty and April, 1981, 1982; Bartels and Elliott, 1981*a,b,* 1982).

Experiments that selectively measure the potentials generated by the A-band and I-band regions from long-tonic fibers of the crayfish will be reported. First, to test the hypothesis that it is possible to measure A-band and I-band potentials selectively, random microlectrode impalements were made into skinned muscle fibers. The resultant trimodal distribution of potentials is explained by the morphology of the muscle fiber. Second, to test the hypothesis that the internal membranous structures (e.g., sarcoplasmic reticulum) contribute to the measured potentials, skinned fibers were treated with a nonionic detergent, Triton X-100. Electron microscopy demonstrates that upon treatment with the nonionic detergent the internal membranous structures are removed from the fiber. Since the internal membranes represent a source of negative static electrical charge, the removal of that charge should make the measured potentials less negative relative to a preparation in which the internal membranous structures remain.

These experiments demonstrate the feasibility, reproducibility, and precision with which selective measurements of A-band and I-band potentials can be made; they are prerequisite to investigating the source and behavior of these potentials. For example, the source of this potential was first proposed to be due to a Donnan equilibrium (Collins and Edwards, 1971). Earlier, Overbeek (1956) addressed this type of problem theoretically; a Donnan equilibrium can result from a charged structural component suspended in an aqueous ionic solution (e.g., a gel or resin). The contractile apparatus of straited muscle fulfills the above requirements (Hawkins and April, 1981, 1983; April, 1975 *a,b,* 1978; Elliott and Rome, 1969). The

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charged structural components of the system are the myosin filaments (thick filaments) and the actin filaments (thin filaments). These charged structures are surrounded by an aqueous ionic solution.

In the crayfish walking-leg muscles, the myosin filaments are ~ 4.5 μm long and 18 nm in diameter (April and Wong, 1976; April, 1969). The isoelectric point of crayfish myosin filaments is approximately pH 4.4 (April, 1978). Therefore, at physiological pH and under the experimental conditions, the myosin filaments carry a net negative charge. The actin filaments interdigitate the hexagonally packed myosin filaments, producing a mixed lattice with an approximate 6:1 ratio of actin filaments to myosin filaments. The actin filaments are 3.6 μm long and 8 nm in diameter (April, 1969).

The skinned long-tonic striated muscle fiber of crayfish is an ideal system in which to investigate A-band and I-band potentials. In this muscle the sarcomere is long (cf. vertebrate muscle). Within the physiological range, the sarcomere length can be varied from where the actin filaments completely overlap the myosin filaments (sarcomere length = 7.4 μm) to where the length of myosin filament overlapped by the actin filaments becomes nominally zero (sarcomere length = 11.9 μm). The length of the A-band, defined by the length of a myosin filament, is 4.5 μm, and the width of a Z-line is 0.2 μm; both are invariant (April, 1969). The I-band length increases from 1.3 to 3.6 μm as the sarcomere length increases from full overlap to zero overlap. The A-bands and I-bands are sufficiently large to be viewed with a light microscope. Further, given that the tip diameter of a microelectrode can be made to 0.5 μm or less, it should be possible to place a microelectrode selectively into an A-band or I-band. A preliminary report of measurements of A-band and I-band potentials has appeared (Aldoroty and April, 1981).

METHODS AND MATERIALS

All experiments were performed on long-tonic fibers from the carpodite extensor muscle of crayfish (*Orconectes*). The dissections followed previously described methods (Reuben et al., 1971; April et al., 1971; Girardier et al., 1963). After obtaining single muscle fibers 100 to 200 μm in diameter, the fibers were transferred from crustacean saline (van Harreveld, 1936) to a crustacean relaxing solution (Table I). The cell membranes were removed completely with a fine needle (Reuben et al., 1971). The sarcomere length was adjusted to 9.7 μm using a micromanipulator. Sarcomere length was monitored using light diffraction (He-Ne laser, 0.3 mW, λ = 0.6328 μm, Spectra-Physics Inc., Mountain View, CA) (April et al., 1971). To remove internal membranous structures, mechanically skinned fibers were immersed for 30 min in crustacean relaxing solution containing either 1.0 g/dl Brij 58 (polyoxyethylene [20] cetyl ether) (Orentlicher et al., 1974) or 1.0 ml/dl Triton X-100 (alkylaryl polyether alcohol). Over the next 30 min the preparations were washed with three 25 ml vol of crustacean relaxing solution. Skinned fiber controls were not treated with any detergent but otherwise were handled identically to the detergent-treated fibers.

The physiologic conditions necessary to maintain the relaxed and rigor conditions in long-tonic crayfish striated muscle fibers are known (Kawai and Brandt, 1976). The relaxed condition was ensured by the composition of the relaxing solution, which contained an excess of calcium-free

TABLE I
PREPARATION OF RELAXING AND RIGOR SOLUTIONS, pH 7.4

Component	Concentrations	
	Relax	Rigor
	mmol/l	
Potassium propionate	173	170
Tris	5	5
$MgCl_2$	1.12	0
Na_2H_2ATP	2	0
H_4EGTA	5	0
H_4EDTA	0	10

Tris, Tris(hydroxymethyl)aminomethane; H_4EGTA, ethylene glycol-bis-(β-amino-ethyl ether) *N,N'*-tetraacetic acid; H_4EDTA, ethylene diamine-*N,N,N',N'*-tetraacetic acid.
The reference was Kawai and Brandt (1976).

Mg-ATP, and by frequent changes of relaxing solution. The rigor condition was induced by changing the bathing medium from relaxing solution to low-rigor solution (Table I) with multiple washings. The establishment of the rigor condition was confirmed by measuring the decrease of fiber diameter relative to the relaxed condition (April and Schreder, 1979).

Transmission Electron Microscopy

Fibers for morphological study were dissected free and stretched to a sarcomere length of 9.7 μm. Before fixation the experimental fibers were detergent treated with either 1.0 g/dl Brij 58 or 1.0 ml/dl Triton X-100. After detergent treatment the fibers were immediately fixed at room temperature. Aldehyde fixation was accomplished in two 30-min periods. First the fibers were bathed in 0.3 ml/dl gluteraldehyde and 0.3 g/dl paraformaldehyde in relaxing solution. Then the concentration of the fixatives in the relaxing solution was increased to 3.0 ml/dl gluteraldehyde and 3.0 g/dl paraformaldehyde. Post-aldehyde fixation was accomplished with 1.0 g/dl OsO_4 at pH 7.4 for 1 h. The specimens were dehydrated in graded ethanol solutions (25, 50, 75, and 95 ml/dl) followed by three 10-min changes of absolute ethanol. They were prepared for Epon embedding by three 10-min changes of propylene oxide followed by graded Epon/propylene oxided solutions (1:2 vol/vol and 2:1 vol/vol) for 8 h each. After three changes of 100% Epon over 24 h, the blocks were polymerized at 60°C for 72 h. Silver-gold sections were cut with a microtome (M-2; Du Pont de Nemours & Co. Inc., Sorvall Instruments Div., Newtown, CT) and stained with aqueous uranyl acetate and lead citrate. Sections were examined by transmission electron microscopy (EM100 Phillips, EM300 Phillips, or 200CX, JEOL USA, Electron Optics Div., Peabody, MA).

Measurement of Microelectrode Tip Diameters

Single-fiber glass capillaries (1 mm o.d. × 0.5 mm i.d.), W-P Instruments Co., New Haven, CT were pulled at various degrees of heating (Alexander and Nastuk, 1963). The capillary glass was similar to that described by Sato (1977) and Tasaki et al. (1968). The electrodes were filled with 3 mol/l KCl and electrode resistance was measured under the experimental conditions by passing current through the electrode such that 1 mV is equivalent to 1 MΩ. Then the microelectrodes were backfilled with distilled water followed by acetone and dried overnight in a desiccator at room temperature. Each electrode was mounted on a specially designed aluminum block using an electrically conductive adhesive. The entire mount was coated with gold/palladium in a sputter coater (Technics, Inc., Springfield, VA; Hummer I). The long axis of the electrode was

placed parallel to the surface of the gold/palladium cathode. To minimize the charging effect of the electrically nonconductive glass, while using a conductive coating of minimum thickness, the coating was accomplished in three 2-min periods. After each coating, the electrode was rotated 120° about the long axis. The electrodes were examined by scanning electron microscopy (JEOL, USA, JSM-25).

A typical micrograph of an electrode is shown in Fig. 1 *a*. The results of a series of microelectrode resistance measurements versus microelectrode tip diameter measurements are plotted in Fig. 1 *b*. This plot was used to determine the approximate microelectrode tip diameter by measuring the electrode resistance under the experimental conditions. The electrode tips used in this study had an outer tip diameter of 0.3–0.4 μm, which is clearly small enough to permit selective placement into an A-band or I-band. The contractile-filament spacing is one order of magnitude less than the microelectrode tip diameter. Therefore, the electrode must either average ion concentrations or average the electrostatic potentials due to the individual myosin filaments and actin filaments (Elliott and Bartels, 1982; Naylor, 1982).

Measurement of Potentials

Measurements of potentials were made using 3 mol/l KCl-filled microelectrodes. Single-fiber glass capillaries (1 mm o.d. × 0.5 mm i.d., WP Instrument Co.) were pulled to tip diameters of 0.3 to 0.4 μm (Alexander and Nastuk, 1963). An Ag/AgCl electrode provided continuity between the fluid phase within the glass microelectrode and the positive terminal

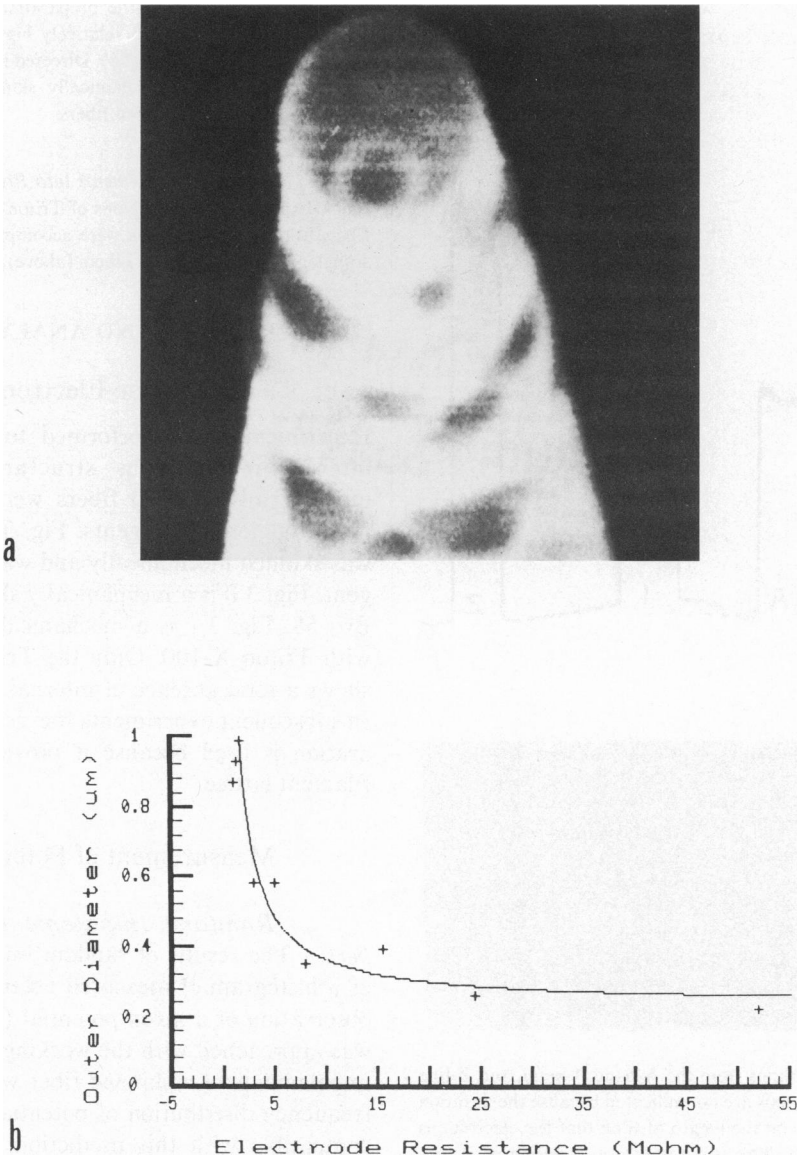

FIGURE 1 (*a*) Micrograph of a microelectrode tip. Scanning electron micrographs such as this were used to calibrate the resistance of microelectrodes against the measured outer tip diameter. The small circle within the tip, six o'clock, is probably the glass monofilament of the capillary. The magnification is 57,400. (*b*) Microelectrode tip diameter versus resistance. The curve represents a regression fit to the experimental points. Using the equations of Amatniek (1958) or Firth and De Felice (1971) the resistance of a microelectrode is inversely proportional to the tip diameter. Hence, the data was linearized as 1/resistance vs. diameter (slope = 1.26, *y*-intercept = 0.23 μm, $r = 0.9714$). The *y*-intercept, the diameter at which the effective capillary bore is lost, is twice the tip wall thickness. Therefore, (inner diameter) = (outer diameter) − (*y*-intercept).

of the preamplifier (NF1; Bioelectronics). The reference electrode, which was in the bath external to the fiber, consisted of a 3.0 g/dl agar-salt bridge filled with 3 mol/l KCl. Continuity with the common terminal of the preamplifier was provided by an Ag/AgCl electrode. All measurements were recorded by a chart recorder (Gould 2200 series with a Gould medium gain DC preamplifier, 13-461510; Gould Inc., Cleveland, OH). Electrode resistance was monitored by passing current through the electrode such that 1 mV was equivalent to 1 MΩ. All electrodes had resistances of 10–15 MΩ. The entire apparatus was protected from random radio frequency by appropriate screening and grounding.

The skinned fiber preparation was mounted on the stage of an inverted microscope (M2, Nikon Inc., Instrum. Div., Garden City, NY). The sarcomere length of the fiber was maintained at 9.7 μm and monitored. No experiment was carried beyond 6 h, even though it was shown that the preparations survived overnight at 4°C. Typical experiment tracings are shown in Fig. 2 *a*.

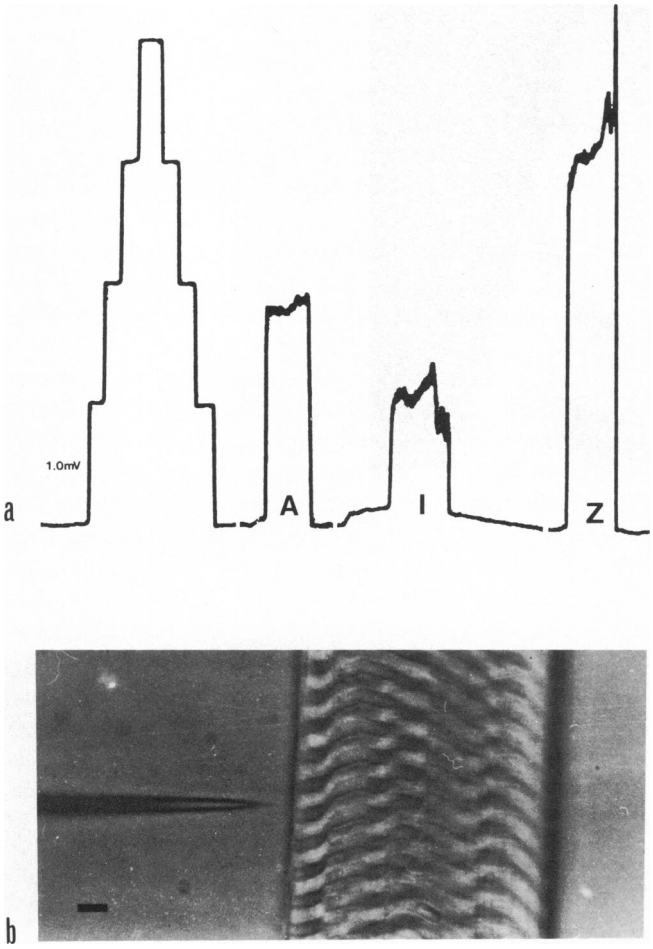

FIGURE 2 (*a*) Typical recordings from the A-band, I-band, and Z-line vicinity. The units on the time axis are not indicated because the duration of the trace is dependent only on the length of time that the electrode is allowed to remain in the fiber. This was tested over a period of 5 min, during which the potential did not vary. Immediately upon impaling the fiber, the upstroke rises to the plateau. Upon removing the electrode from the fiber, the potential returns to baseline immediately. (*b*) Micrograph of a microelectrode adjacent to an A-band. The A-bands are anisotropic. Even when the polarizers are slightly uncrossed to allow a view of the microelectrode, the A-bands appear bright. The marker bar represents 10 μm. The experimenter views the preparation at a magnification of 600.

Random Impalements Into Relaxed Fibers. Impalements without regard to location, along relaxed skinned muscle fibers that had not been treated with detergent, were accomplished by viewing the preparation with nonpolarized light at a magnification of 150. At this relatively low magnification it was impossible to visually determine whether the electrode tip had been placed in an A-band or I-band. However, the magnification was sufficient to allow consistent impalements into a fiber. Impalements were never made in the same place. After each impalement, the microelectrode was advanced in one direction only along the long axis of the fiber. When an end of the fiber was reached, the experiment was terminated.

Directed Impalements into Relaxed Fibers. Impalements into the A-band and I-band regions of relaxed skinned muscle fibers were accomplished by viewing the preparation with crossed polarizers at a magnification of 600. This relatively high magnification delineates the A-bands and I-bands (Fig. 2 *b*). Directed impalements were made in both non–detergent-treated mechanically skinned fibers and in detergent-treated mechanically skinned fibers.

Directed Impalements into Rigor Fibers. Impalements into the A-band and I-band regions of Triton X-100-treated skinned muscle fibers in the rigor condition were accomplished as described in Directed Impalements into Relaxed Fibers (above).

RESULTS AND ANALYSIS

Transmission Electron Microscopy

Experiments were performed to assess the presence of internal membranous structures. Prior to fixation, mechanically skinned fibers were treated with two different nonionic detergents. Fig. 3 *a* is a control; the fiber was skinned mechanically and was not treated with detergent. Fig. 3 *b* is a mechanically skinned fiber treated with Brij 58. Fig. 3 *c* is a mechanically skinned fiber treated with Triton X-100. Only the Triton X-100-treated fiber shows a total absence of internal membranous structures. In subsequent experiments the Triton X-100-treated preparation is used because it provides a purer contractile-filament lattice.

Measurement of Potentials

Random Impalements into Relaxed Fibers. The results of random impalements are presented as a histogram of measured potential vs. the frequency of observation of a given potential (Fig. 4). The experiment was approached with the working hypothesis that random impalements of a skinned fiber would result in a bimodal frequency distribution of potentials. The data in Fig. 4 is compatible with this prediction. The trimodality of the histogram implies that there is a third region from which potentials can be measured. The results of the directed impalement experiments that follow indicate that these modalities of increasingly negative mean potentials correlate with the I-band, the A-band, and the Z-line vicinity, respectively.

Directed Impalements into Relaxed Fibers. The results of directed impalements into Triton X-100-treated and non–Triton X-100-treated skinned relaxed fibers are demonstrated by histograms in Fig. 5 *a* and *b* and summarized in Table II. By employing crossed polarizers and a magnification of 600, it was possible to label each potential measurement as being the result of either an A-band or I-band impalement. The frequency distributions of the measured A-band and I-band potentials were plotted separately. Plotting the data in this manner yields a discrete A-band distribution and a bimodal I-band distribution. The application of a *t* test (Ostle and Mensing, 1975), comparing the less negative modality of the I-band data with the A-band data at any given condition, demonstrates that the A-band and I-band distributions are discrete populations ($P > 0.9999$).

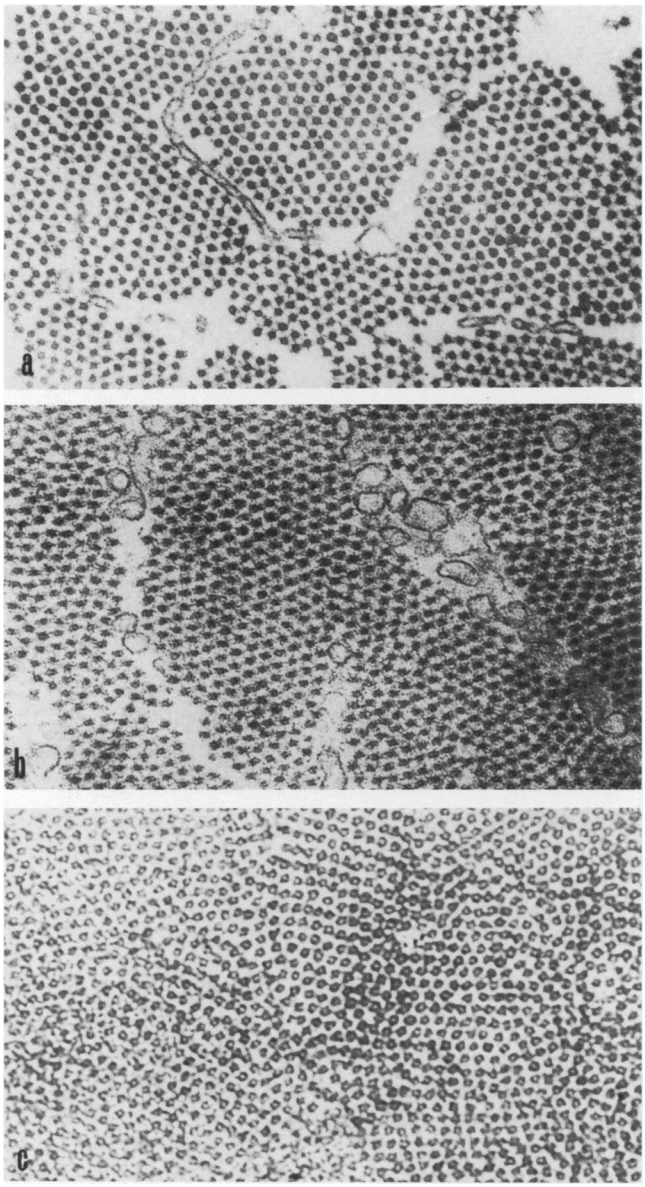

FIGURE 3 Electron micrographs of cross sections of crayfish striated muscle A-bands. The micrographs are at a magnification of 87,200. Fibers were mechanically skinned prior to detergent treatment. (*a*) Control skinned fiber. This was not treated with detergent but otherwise was handled identically to the detergent-treated fibers shown in *b* and *c*. The presence of membranes and the swelling that occurs between the myofibrils is obvious. (*b*) Brij58-treated skinned fiber. The intermyofibrillar swelling remains while the membranous structures assume a vesicular form. (*c*) Triton X-100-treated skinned fiber. There is a total absence of any internal membranes, which allows coalescence of the myofibrils.

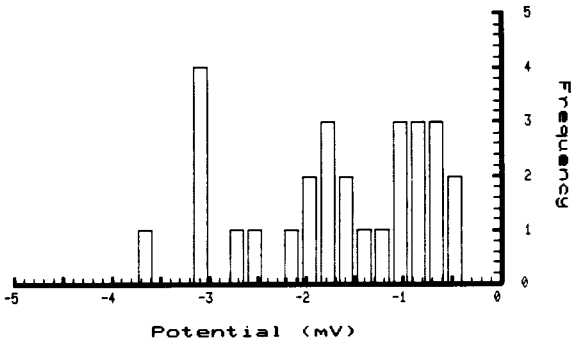

FIGURE 4 Histogram of potentials obtained from random impalements. The trimodal distribution demonstrates that there are three distinct regions along the muscle fiber from which potentials can be measured. It is reasonable to expect that these modalities correspond to structural aspects of the sarcomere.

The bimodality of the I-band data is more complex, because the samplings of the more negative I-band distribution are rare and *t* tests were not applied. However, these measurements probably represent a discrete distribution because the mean of the measurements falls outside the seventh standard deviation of the I-band distributions. The hypothesis is that the distribution at less negative potentials represents measurements from the I-band proper, while the relatively few potentials that segregate to more negative values represent an occasional impalement in the vicinity of a Z-line (Fig. 5). This interpretation of the I-band data is based upon the findings that (*a*) the highly negative I-band potentials are rarely obtained during the course of an experiment; (*b*) the impalements that produce these potentials correspond visually to the centers of I-bands; and (*c*) the density of protein in a Z-line is high and the resultant greater structural charge concentration would produce higher potentials than would the actin filaments in the I-band proper. Since internal membranous structures can be ruled out as a source of these potentials, it is reasonable that the bimodality of the data from these directed impalement experiments is explained by potentials detected from the I-band and the Z-line vicinity. Therefore, the trimodal distribution observed in the random impalement experiments represents the sum of the bimodal I-band data and the unimodal A-band data.

The distributions in non–detergent-treated fibers can be compared with those in detergent-treated fibers. A *t* test

shows that the distributions of measured A-band potentials from non–detergent-treated and detergent-treated A-bands appear to represent different populations (P = 0.8738). The equivalent A-band and I-band protein charge concentrations, calculated from Donnan equilibrium theory for the non–detergent-treated fiber and the Triton X-100-treated fiber, are shown in Table II. The differences of 1.3 and 0.7 mmol e^-/l for the A-band and I-band, respectively, can be attributed to the removal of membranous structures by Triton X-100.

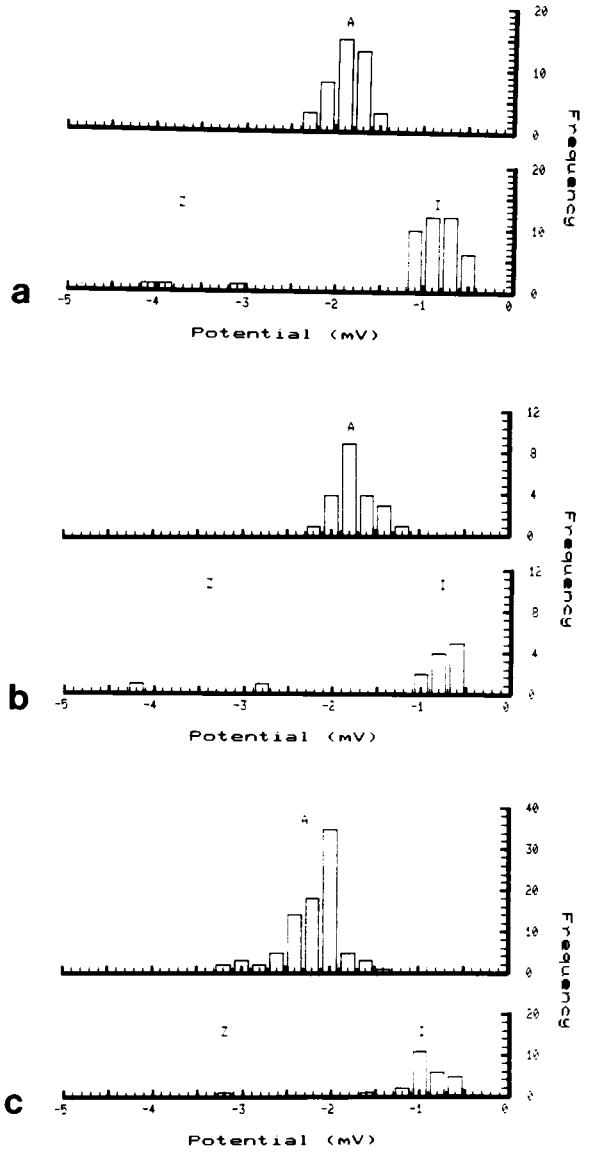

FIGURE 5 Histograms of potentials obtained from directed impalements. As impalements were made into a fiber, the region that the electrode entered was determined visually. The data were segregated based upon the visual determination and plotted as histograms. (A) marks the mean of the measured potentials from the A-band. (I) marks the mean of the measured potentials from the I-band. (Z) marks the mean of the measured potentials from the Z-line vicinity. (*a*) The results from relaxed fibers that were only mechanically skinned. (*b*) The results from relaxed fibers that were treated with Triton X-100 after they were mechanically skinned. (*c*) The results from fibers in the rigor condition that otherwise were treated identically to those in *b*.

Directed Impalements into Rigor Fibers. The results of directed impalements into the A-bands and I-bands of skinned Triton X-100-treated fibers in the rigor condition are demonstrated by histograms (Fig. 5 *c*) and are summarized in Table II. The application of a t test (Ostle and Mensing, 1975), which compares the A-band data in the relaxed condition to the A-band data in the rigor condition, demonstrates that these are discrete populations ($P > 0.9999$). Again applying Donnan equilibrium theory, the equivalent protein charge concentrations for the A-band and I-band are calculated (Table II). In the rigor condition, there is an increase of 8.5 mmol e^-/l in the A-band and an increase of 3.1 mmol e^-/l in the I-band, relative to the relaxed condition.

Calculation of Fixed-Charge Concentration

The basis for calculating the equivalent structural charge concentration and the applicable equations have been presented in detail elsewhere (Benedek and Villars, 1979; Naylor, 1977; Overbeek, 1956). Briefly, if one assumes that all the mobile ions are univalent then the fixed charge concentration due to proteins, Γ, can be calculated from the assumption of bulk neutrality:

$$C^+(\text{external}) = C^-(\text{external}) \tag{1a}$$

$$C^+(\text{internal}) = C^-(\text{internal}) + \Gamma, \tag{1b}$$

where C^+ and C^- represent the concentrations of the cations and anions in the internal and external phases. Further, the Nernst potential may be written as

$$\frac{C^+(\text{internal})}{C^+(\text{external})} = \frac{C^-(\text{external})}{C^-(\text{internal})} = \exp\left(\frac{-\psi_D e}{kT}\right). \tag{2}$$

Combining the above equations yields

$$\Gamma = C^+(\text{external})\left[\exp\left(\frac{-\psi_D e}{kT}\right) - \exp\left(\frac{\psi_D e}{kT}\right)\right]. \tag{3}$$

Standard deviations for the equivalent structural charge concentrations (Table II) were calculated from compounding of error theory (Wilson, 1952).

DISCUSSION

One working hypothesis tested the premise that it is possible to measure potentials selectively from the A-band and I-band regions of skinned crayfish striated muscle, and therefore random impalements (i.e., without respect to location in the sarcomere) would generate a bimodal distribution where the modalities represent samplings from the A-band and I-band. The results confirmed this hypothesis and implied that there was a third region within the sarcomere that could be sampled (Fig. 4). Based upon the results of experiments in which the placement of the

TABLE II
EXPERIMENTAL RESULTS AND STATISTICS

	Treatment								
	No detergent			Triton X-100					
	Relax			Relax			Rigor		
	A-band	I-band	Z-line	A-band	I-band	Z-line	A-band	I-band	Z-line
Measured potential (*mV*)	−1.80	−0.76	−3.63	−1.71	−0.71	−3.40	−2.18	−0.88	−3.20
Standard deviation (*mV*)	0.20	0.18	0.57	0.24	0.16	0.99	0.33	0.22	—
Number of samplings	42	40	3	22	11	2	88	25	1
Protein charge concentration (*mmol e^-/l*)	25.9	10.9	52.3	24.6	10.2	49.0	33.1	13.3	46.1
Standard deviation (*mmol e^-/l*)	2.9	2.6	8.2	3.4	2.3	14.2	5.0	3.3	—

microelectrode was visually determined, these modalities were assigned to the A-band, the I-band, and the Z-line vicinity (Figs. 1 and 5). Another working hypothesis tested the premise that the charge on internal membranous structures (e.g., the sarcoplasmic reticulum) contributes to the measured potentials. Electron microscopy demonstrated that internal membranous structures are removed by a nonionic detergent, Triton X-100 (Fig. 3). Experiments on these detergent-treated fibers confirmed this hypothesis as the measured potentials in the A-band and the I-band decreased (Table II). The source of this detergent-labile charge is most likely attributable to lipids and lipid-soluble proteins that constitute the internal membranes of the muscle cell (Chiu et al., 1980; Helenius and Simons, 1975; Dowben and Koehler, 1961).

In the last series of experiments presented, potentials were measured from Triton X-100-treated fibers in the rigor condition. The results indicate that, relative to the relaxed condition, there is an increase of 8.5 mmol e^-/l in the A-band. Also noted is that upon induction of rigor the I-band potential becomes more negative. These results could be caused by two separate effects. First, there could

TABLE III
SUMMARY OF THE AVAILABLE EXPERIMENTAL DATA: RELAXED CONDITION, pH 7.0 – 7.5

Preparation	% Myosin rod overlapped by actin filaments	A-band	I-band	Z-line	A-band	I-band	Z-line	Investigator
		mV			*mmol e^-/l*			
Rat semitendinosous (glycerinated)	—	−2.5	−2.6	—	36	36	—	Bartels and Elliott (1982)
Rat semitendinosous (saponin skinned)	—	−5.0	−5.0	—	70	70	—	Bartels and Elliott (1982)
Rat semitendinosous (saponin skinned and Brij treated)	—	−2.5	−2.4	—	36	36	—	Bartels and Elliott (1982)
Rabbit psoas (glycerinated)	—	−2.3	−2.2	—	33	32	—	Bartels and Elliott (1981a)
Rabbit psoas (glycerinated)	56	(−6.7)		—	—	—	—	Pemrick and Edwards (1974)
Rabbit skeletal (glycerinated)	—	(−7.5)[i]		—	—	—	—	Scordalis et al. (1975)
Barnacle (mechanically skinned)	—	−8.8	−8.7	—	125	124	—	Bartels and Elliott (1981b)
Dog ventricular (glycerinated)	—	(−46)[e]		—	—	—	—	Weiss et al. (1967)
Crayfish (mechanically skinned)	44	−1.8	−0.8	−3.6	26	11	52	Aldoroty and April (1982)
Crayfish (mechanically skinned and Triton treated)	44	−1.7	−0.7	−3.4	24	10	49	Aldoroty and April (1982)

Missing values indicate that the data was not reported.
Values that are between columns surrounded by parentheses indicate that no distinction between A-band or I-band was made.
"e" indicates that the value was estimated from the available data.
"i" indicates that the value was interpolated from their Fig. 4.

be a number increase in the negative charge on the structural components (i.e., the myosin filaments and actin filaments). Second, there could be a decrease in the volume that the structural components occupy. Either or both effects would increase the equivalent structural charge concentration. There is evidence that upon induction of rigor there is a decrease of the fiber-lattice volume relative to the relaxed condition (April and Schreder, 1979; Shapiro et al., 1979). The resolution of this question represents another investigation in which fiber volume is varied independently of the ionic conditions.

The work of other investigators is summarized for the relaxed condition in Table III and for the rigor condition in Table IV. The drawing of any quantitative conclusions based upon a comparison of this study with those in the literature is plagued by differences in species, relative sarcomere lengths, and ionic strengths. We note that most of the experiments summarized in Tables III and IV were performed at approximately one-half the ionic concentration of our experiments. The measured potential varies approximately inversely with the external ion concentration. Regardless of the differences in experimental method, this study and the work on psoas and semitendinosus muscle (Bartels and Elliott, 1982, 1981*a*) concur qualitatively in that the induction of rigor increases structural charge concentration. This is in contrast to the barnacle work of Bartels and Elliott (1981*b*), and the much earlier rabbit psoas muscle work of Scordalis et al. (1975) and

TABLE IV
SUMMARY OF THE AVAILABLE EXPERIMENTAL DATA: RIGOR CONDITION, pH 7.0 – 7.5

Preparation	% Myosin rod overlapped by actin filaments	A-band	I-band	Z-line	A-band	I-band	Z-line	Investigator
		mV			*mmol e⁻/l*			
Rat semitendinosous (glycerinated)	—	−5.3	−2.7	—	36	36	—	Bartels and Elliott (1982)
Rat semitendinosous (saponin skinned)	—	−5.2	−2.7	—	59	30	—	Bartels and Elliott (1982)
Rat semitendinosous (saponin skinned and Brij treated)	—	−5.4	−2.4	—	59	30	—	Bartels and Elliott (1982)
Rabbit psoas (glycerinated)	—	−4.7	−2.6	—	53	29	—	Bartels and Elliott (1981a)
Rabbit psoas (glycerinated)	—	(−11.9)		—	(137)		—	Elliott et al. (1978)
Rabbit psoas (glycerinated)	56	(−12.2)		—	—		—	Pemrick & Edwards (1974)
Rabbit skeletal (glycerinated)	—	(−3.3)[i]		—	—		—	Scordalis et al. (1975)
Barnacle (mechanically skinned)	—	−10.4	−6.2	—	119	70	—	Bartels and Elliott (1981b)
Frog ventricular (glycerinated)	—	(−7)		—	(56)		—	Collins and Edwards (1971)
Toad ventricular (glycerinated)	—	(−33)		—	—		—	Naylor et al. (1964)
Dog ventricular (glycerinated)	—	(−46)		—	—		—	Weiss et al. (1967)
Dog ventricular (glycerinated and mechanically skinned)	—	(−28)		—	—		—	Weiss et al. (1967)
Cat papillary (pulvarized)	—	(−24)		—	—		—	Weiss et al. (1967)
Crayfish (glycerinated)	—	(−18)[e]		—	—		—	Chichibu (1961)
Crayfish (mechanically skinned and Triton treated)	44	−2.2	−0.9	−3.2	33	13	46	Aldoroty and April (1982)

Missing values indicate that the data was not reported.
Values that are between columns surrounded by parentheses indicate that no distinction between A-band or I-band was made.
'e' indicates that the value was estimated from the available data.
'i' indicates that the value was interpolated from their Fig. 4.

Pemrick and Edwards (1974), in which, upon induction of rigor, they measured less negative potentials relative to the relaxed condition (Tables III and IV).

The data presented in this paper establish that in crayfish walking-leg long-tonic muscle fibers it is possible to obtain reproducible, precise, and selective measurements of potentials from the A-band, the I-band, and the Z-line vicinity. Under the reported experimental conditions, these regions are represented by distributions of potentials that are statistically distinct from each other. Further, internal membranous components contribute to the negativity of the measured potentials and therefore must be removed or accounted for if one is to consider using such measurements as a probe of the charge on the protein portion of the contractile apparatus.

APPENDIX

Measurements of Potential from Agar Gels

In the manner in which Collins and Edwards (1971) measured potentials from boiled egg white as positive controls for their glycerinated muscle experiments, we used agar (Fisher Scientific Co. Pittsburgh, PA) to validate the techniques used in this study. Agar is an extract of *Gelidium* species and other forms of red algae. It is known to be composed of 3,6-anhydro-L-galactose and D-galactopyranose with esterifield sulfate residues (Furia, 1978). It is probably a complex range of polysaccharides that can be separated into a gelling neutral agarose and a nongelling sulfated agaropectin (Windholz, 1983). As such, an agar gel is a charged structural component which, when placed in an ionic solution, establishes a Donnan equilibrium.

Agar gels were prepared in various concentrations in either 0.173 mol/l potassium chloride or 0.173 mol/l potassium propionate; both contained 5 mmol/l Tris buffer, pH 7.0. Agar, dissolved by heating and stirring until the solution cleared, was drawn into glass capillaries (1 mm i. d.) and allowed to set. The agar gels were extruded from the glass capillaries. Thereafter, the cylindrical agar gels were handled as described for the skinned muscle fibers. The gels were immersed in either 0.173 mol/l potassium chloride or 0.173 mol/l potassium propionate, both contained 5 mmol/l Tris buffer, pH 7.0, and both were impaled with microelectrodes. The resultant electrochemical potentials were recorded.

Since no work can be obtained from a system that is in equilibrium, a Donnan potential only can be measured using a salt bridge. This adds a necessary element of irreversibility to the system. However, each salt bridge unavoidably introduces a liquid junction potential (Overbeek, 1956). Experimentally, Kushmerick and Podolsky (1969) concluded that since the diffusivity of K^+ is reduced by a factor of 1.8 in the muscle cell relative to the external aqueous bathing medium, the ionic mobilities inside a muscle cell are not influenced significantly by chemical interaction. This result reduces the possibility of diffusion potentials based upon ionic mobility. Further, measurements of ionic activities support the applicability of the Nernst equation and Donnan equilibrium theory, and show that the activity coefficients of K^+ and probably of Cl^- are close to unity (Hinke and Gayton, 1971). Both of these reports are discussed by Elliott and Bartels (1982).

In the first set of experiments only the concentration of the agar was varied. The results are summarized in Table AI and plotted in Fig. 6. Potentials become more negative as the concentration of the polyelectrolyte increases. Based upon the potential measured from an agar gel of 5 g/dl, the line shown is calculated using Donnan equilibrium theory (calculation is outlined in this paper).

In other experiments, all performed on 4 g/dl agar gels, the resistance of the electrode (tip diameter) was varied; the concentration of the KCl contained in the electrode was varied; and the external and internal salt solutions were changed from 0.173 mol/l potassium chloride to 0.173 mol/l potassium propionate. The results of these experiments are summarized in Table *A* II. An analysis of variance (Ostle and Mensing, 1975) demonstrates that there is no significant difference between the distributions of measured potentials ($P = 0.9959$). When a t test (Ostle and Mensing, 1975) individually compares each perturbation with the control conditions, the result is similar ($0.8351 \leq P \leq 0.9993$).

TABLE AI
RESULTS OF POTENTIALS MEASURED AS A FUNCTION OF AGAR CONCENTRATION

Gel*		Bathing Solution*	Electrode		$\langle\psi_D\rangle$	SD	n
[Agar]	[KCl]	[KCl]	[KCl]	R			
g/dl		*mol/l*		*MΩ*	*mV*		
1	0.173	0.173	3.0	13	−1.0	0.4	18
2	0.173	0.173	3.0	14	−2.5	0.4	25
3	0.173	0.173	3.0	13	−4.4	1.0	21
4	0.173	0.173	3.0	13	−7.2	1.0	11
5	0.173	0.173	3.0	13	−8.3	3.3	18

Brackets indicate concentration.
R indicates resistance; $\langle\psi_D\rangle$, mean measured potential; SD, standard deviation; and n, number of measurements.
*Contains 5 mmol/l Tris(hydroxymethyl)aminomethane, pH 7.0.

Experiments on agar gels, surrogates for skinned striated muscle fibers, both provide a positive control for the experimental method (i.e, potentials are recorded from a different polyelectrolyte gel system) and avoid the inherent technical difficulties of skinned muscle fiber preparations. These experiments demonstrate that electrochemical potentials measured as a function of agar concentration are described by Donnan equilibrium theory. Further, it is shown that the microelectrode diffusion potentials must be insignificant because diffusion potentials must vary with the perturbations imposed upon the system. The measured potentials are not significantly different when the salt concentration in the electrode is varied (1–3 mol/l), when the tip diameter of the electrode (resistance) is varied, or when potassium chloride is exchanged with potassium propionate, either in the bathing medium or within the gel. The absence of significant variation in the measured potentials under conditions that would change the magnitude of artifactual diffusion potentials (functions

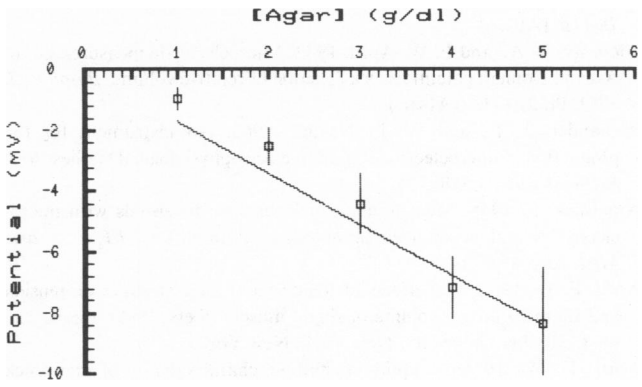

FIGURE 6 Potentials measured as a function of agar concentration. Bars represent standard deviations. The curve was calculated from Donnan theory (details of such calculations are presented in subsequent papers). When agar concentration increases, the measured potential increases (agar is a polyelectrolyte gel). This result agrees qualitatively with the predictions of Donnan equilibrium theory.

TABLE AII
RESULTS OF POTENTIALS MEASURED UNDER A VARIETY OF SALT CONDITIONS

Gel*			Bathing solution*		Electrode		$\langle\psi_D\rangle$	SD	n
[Agar]	[KCl]	[KProp]	[KCl]	[KProp]	[KCl]	R			
g/dl			*mol/l*			*MΩ*	*mV*		
4‡	0.173‡	—	0.173‡	—	3.0‡	13‡	−7.2	1.0	11
4	0.173	—	0.173	—	3.0	*30*	−6.8	0.4	8
4	0.173	—	0.173	—	*1.0*	16	−6.8	0.7	17
4	0.173	—	0.173	—	*2.0*	*24*	−6.9	0.8	16
4	0.173	—	—	*0.173*	3.0	13	−7.0	0.8	16
4	—	*0.173*	—	*0.173*	3.0	14	−5.7	0.7	16

Brackets indicate concentration.
Italics indicate conditions that were perturbed.
R indicates resistance; $\langle\psi_D\rangle$, mean measured potential; SD, standard deviation; and n, number of measurement.
*Contains 5 mmol/l Tris(hydroxymethyl)aminomathane, pH 7.0.
‡Indicates the control conditions.

of ionic mobilities and transference numbers) demonstrates that artifactual diffusion potentials do not significantly contribute to these electrochemical measurements.

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