Evidence for Non-Syncytial Nature of Cardiac Muscle from Impedance Measurements.* (25032)

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Until recently the spread of excitation in cardiac tissue was considered to be explicable in terms of protoplasmic continuity between cells. However, electron microscopy of cardiac muscle has shown that cellular membranes at the intercalated discs separate adjacent cells(1,2). If the myocardium is not a morphological syncytium, then some other basis for impulse transmission from cell to cell must be sought. Two possibilities are a) that the heart is a functional syncytium (*i.e.*, low resistance junctional membranes), or b) that some type of junctional transmission process is involved. In a previous study of frog heart with intracellular electrodes, ventricular cells were electrically isolated from their neighbors during perfusion with hypertonic solution(3). From these results it was concluded that the myocardium is not a functional syncytium. In the present study additional evidence is presented in support of this hypothesis. This evidence is based upon a comparison of the specific resistance of cardiac muscle with that of short-celled intestinal smooth muscle, and that of long-celled frog sartorius muscle. A syncytial structure would be expected to behave electrically like the long-celled tissue.

Methods. The D. C. resistances of 3 types of muscle were measured in the direction of fiber orientation: a) frog sartorius muscle, b) ganglion-free circular smooth muscle of cat intestine (*cf.* 4 for method of preparation), and c) ventricular muscle strips from dog and beef hearts. Relatively uninjured strips of ventricular trabeculae were excised from fresh beef hearts. Strips of parallel fibers were cut from papillary muscle of fresh dog heart. Each muscle strip was prepared and selected for uniformity of the transverse dimensions throughout the length of the strip. No samples thicker than 3 mm were used. The lengths varied from 15 to 30 mm, and weights from 15 to 150 mg. The muscle cross-sectional area (cm^2) was taken as average of one estimate obtained from direct caliper measurements of the transverse dimensions and one estimate from length and wet weight (uncorrected for specific gravity). D.C. resistances were measured with Wheatstone bridge technic using a cathode ray oscilloscope as the null-point detector. A Grass S-4 stimulator and stimulus isolation unit supplied rectangular pulses (3/sec., 30 msec. duration), and produced a voltage across the muscle of about 1 volt. A metal clip mounted on the micromanipulator served as one electrode and also supported one end of the muscle. A second electrode was immersed in reservoir filled with Tyrode's solution. The free end of the muscle was appropriately weighted and lowered into the reservoir in 4 steps. At each step, D.C. resistance and exposed length of muscle were measured. There was no evidence of polarization since polarity reversal did not alter the measured resistance. The resistance at each position was plotted against corresponding muscle length. From this linear plot, the slope or resistance per cm length of muscle was calculated. This slope was multiplied by the cross-sectional area to obtain the specific resistance in ohm-cm for a particular muscle strip. Resistance measurements were performed on each sample after soaking for about 2 hours in Tyrode's solution and again after soaking 2 hours in 10% Tyrode's made isotonic with sucrose. In some experiments the muscle was re-soaked in Tyrode's and a resistance value close to initial value was obtained. Tyrode's solution was used for mammalian tissues and frog Ringer's for frog sartorius. For mammalian muscles the 10% Tyrode's was made by mixing 9 volumes of 0.3 M sucrose with one volume of mammalian Tyrode's. The 10% frog Ringer's was made in a similar manner using 0.22 M sucrose. The volume of the soaking solution

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TABLE I. Mean Specific Resistances of Cardiac, Smooth, and Skeletal Muscles before and after Reduction of Interspace Ion Concentration.

Muscle	No. of samples	Specific resistance (ohm-cm)* Tyrode's	10% Tyrode's	Resistance ratio*	Interspace vol (ml/100 g muscle wt)	
Cardiac muscle						
Beef ventricular trabeculae	10	251 ± 17	1890 ± 116	7.6 ± .5	Cardiac	19† 30‡
Dog papillary strip	12	286 ± 13	1819 ± 85	6.4 ± .2		19§
Smooth muscle						
Cat intestine smooth muscle	20	118 ± 5	778 ± 42	6.7 ± .3	Smooth	28‖ 35¶
Skeletal muscle						
Frog sartorius	12	133 ± 11	367 ± 23	2.9 ± .2	Skeletal	35** 31†† 13‡‡

* Each value is mean ± stand. error of mean. Resistance ratio was calculated by dividing tissue resistance measured after soaking in 10% Tyrode's with tissue resistance measured after soaking in Tyrode's. † Sucrose space of cat ventricle *in situ*, calculated assuming 80% water content from data in(5). ‡ Cl or Na space of rabbit ventricle(6). § Histologically determined interspace of beef moderator band(7). ‖ Inulin space of frog stomach smooth muscle(8). ¶ Cl or Na space of rabbit small intestine(6). ** Sucrose, SO_4, and rapidly exchanging Na space of Ringer's soaked frog sartorius(9). †† Rapidly exchanging Na space of Ringer's soaked sartorius(10). ‡‡ Cl space with Donnan corrections(11).

was over 200 times the muscle volume and at least one replacement was made during the soaking period. All procedures were carried out at room temperature.

Results. The mean D.C. specific resistances of cardiac, (frog) skeletal, and intestinal smooth muscles were 270 ± 11, 133 ± 11, 118 ± 5, ohm-cm, respectively (Table I).‡ Ventricular muscle specific resistance was significantly higher ($p<.001$) than that of either skeletal or smooth muscle. Mean specific resistances after soaking for a minimum of 2 hr in 10% Tyrode's solution were 1838 ± 70, 367 ± 23, and 778 ± 42 ohm-cm, respectively for cardiac, skeletal and smooth muscles. Mean ratios of resistances in the 2 solutions for cardiac, skeletal and intestinal smooth muscles were 7.0 ± 0.3, 2.9 ± 0.2, and 6.7 ± 0.3, respectively. The resistance ratio of ventricular muscle was not significantly different from that of smooth muscle ($p>0.1$). However, the resistance ratio of skeletal muscle was significantly lower than that of either cardiac ($p<.001$) or smooth muscle ($p<0.01$). In other words the tissue resistances of cardiac and smooth muscles increased much more than did that of skeletal muscle when the interspace ion concentration was reduced 10-fold.

The large increase in ventricular muscle resistance after soaking in 10% Tyrode's is not attributable to a loss of intracellular potassium. Beef ventricular trabeculae immersed for 2 hours in 0.3 M sucrose lost 9 μeq potassium/g wet weight of muscle. This amount is less than 10% of the total muscle potassium and far from the loss necessary to account for the resistance increase found. The relatively small increase in sartorius muscle resistance after soaking in 10% Tyrode's could not be due to insufficient reduction of interspace ion concentration since duration of the soaking period employed was about 10 times the reported half-time for interspace sodium washout(12).

Discussion. The resistance increase following interspace ion depletion was found to be less in skeletal muscle than in cardiac or smooth muscles. The most probable explanation for this difference in resistance change is that a smaller proportion of the total current passed through the cells of cardiac or smooth muscles than those of skeletal muscle. Preferential current flow through the interspace in cardiac and smooth muscles could have been due to a large fraction of the total cross-sec-

‡ Specific resistances of beef and dog ventricular muscle were not significantly different and have been pooled for comparison with skeletal and smooth muscles.

tional area being interspace, or to a large resistance in current flow through the cells. If the whole tissue were interspace, all the current would of necessity pass through the interspace and the expected specific resistance would be that of Tyrode's (44 ohm-cm(4)). However, the reported values for the interspaces of cardiac, skeletal, and smooth muscles, although variable, are not sufficiently different to account for the differences in the resistance changes (Table I). Therefore, the current flow paths through the cells of cardiac and smooth muscles appear to be of high resistance. This high resistance is more likely due to the high resistance of cell membranes than to high myoplasmic resistance.

Other studies predict that the current flow path through smooth muscle cells should involve a succession of high resistance cellular membranes(13). Our results lead to the conclusion that this may also be true of cardiac muscle. This is consistent with the observation that ventricular muscle specific resistance is larger than that of skeletal muscle. It would appear that cardiac muscle cells are separated from each other by high resistance cellular membranes and do not form a low resistance functional syncytium.

Summary. The D.C. specific resistances of dog and beef ventricular muscle strips were compared with those of cat intestine circular smooth muscle, which is a non-syncytial tissue, and of frog sartorius muscle where individual cells extend almost the full length of muscle. Resistances were measured in the direction of fiber orientation before and after the interspace ion concentration was reduced by soaking in isotonic 10% Tyrode's-sucrose solution. Cardiac and smooth muscle resistances were much more sensitive to interspace ion depletion than was skeletal muscle resistance. It is suggested that the current flow path through the cells of cardiac muscle is of high resistance due to large number of high resistance cellular membranes involved, as in smooth muscle. It was concluded that cardiac muscle cells are separated from each other by high resistance membranes and do not form a functional syncytium.

1. Fawcett, D. W., Selby, C. C., *J. Biophys. Biochem. Cytol.*, 1958, v4, 63.

2. Moore, D. H., Ruska, H., *ibid.*, 1957, v3, 261.

3. Sperelakis, N., Hoshiko, T., Keller, R. F., Jr., Berne, R. M., *Am. J. Physiol.*, in press.

4. Prosser, C. L., Sperelakis, N., *ibid.*, 1956, v187, 536.

5. Robertson, W. Van B., Peyser, P., *ibid.*, 1956, v184, 171.

6. Manery, J. F., Bale, W. F., *ibid.*, 1941, v132, 215.

7. Truex, R. C., Copenhaver, W. M., *Am. J. Anat.*, 1947, v80, 173.

8. Bozler, E., Lavine, D., *Am. J. Physiol.*, 1958, v195, 45.

9. Johnson, J. A., *ibid.*, 1955, v181, 263.

10. Levi, H., Ussing, H. H., *Acta physiol. scandinav.*, 1948, v16, 232.

11. Boyle, P. J., Conway, E. J., Kane, F., O'Reilly, H. L., *J. Physiol.*, 1941, v99, 401.

12. Johnson, J. A., *Am. J. Physiol.*, 1957, v191, 487.

13. Sperelakis, N., Prosser, C. L., *ibid.*, 1959, v196, 850.

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Infectious Ribonucleic Acid Derived from Enteroviruses.* (25033)

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In recent years the ribonucleic acid (RNA)-containing fraction of a number of viruses has been shown to be infectious. The evidence supports the premise that the active ingredient of this infectious portion is RNA; infectivity is inactivated by crystalline ribonuclease (RNAase) but not by gamma globulin prepared from specific antiserum or by desoxyribonuclease (DNAase)(1). In earlier ex-

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