Cable Parameters, Sodium, Potassium, Chloride, and Water Content, and Potassium Efflux in Isolated External Intercostal Muscle of Normal Volunteers and Patients with Myotonia Congenita

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ABSTRACT In isolated fiber bundles of external intercostal muscle from each of 13 normal volunteers and each of 6 patients with myotonia congenita, some or all of the following were measured: concentrations of Na^+, K^+, and Cl^-, extracellular volume, water content, K^+ efflux, fiber size, fiber cable parameters, and fiber resting potentials.

Muscle from patients with myotonia congenita differed significantly ($0.001 < P < 0.025$) with respect to the following mean values (myotonia congenita vs. normal): the membrane resistance was greater (5729 vs. 2619 $\Omega \cdot cm^2$), the internal resistivity was less (75.0 vs. 123.2 $\Omega \cdot cm$), the water content was less (788.2 vs. 808.2 ml/kg wet weight), and the mean resting potential was greater (68 vs. 61 mv).

No significant differences were found with respect to the following variables: K^+ content (73.5 vs. 66.7 mEq/kg wet weight) and the calculated intracellular K^+ concentration (215 vs. 191 mEq/liter fiber water), fiber capacitance (5.90 vs. 5.15 $\mu f/cm^2$), Na^+ content (97.7 vs. 94.1 mEq/kg wet weight), Cl^- content (79.0 vs. 74.7 mEq/kg wet weight), mannitol extracellular volume (45.1 vs. 46.6 cc/100 g wet weight), and K^+ efflux (23.2 vs. 21.5 moles $\times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$).

These abnormalities of skeletal muscle in human myotonia congenita are like those of skeletal muscle in goats with hereditary myotonia. We tentatively conclude that a decreased Cl^- permeability accounts for some of the abnormal electrical properties of skeletal muscle in myotonia congenita.

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INTRODUCTION

We reported abnormalities of cable parameters, ion content, and Cl^- permeability in external intercostal muscle isolated from goats with hereditary myotonia (1, 2). On extending these observations to man, we realized that cable parameters in isolated human external intercostal muscle have been reported for only seven fibers from two persons (3); ion content after several hours in physiological salt solution has been reported in only one other investigation (4); and no studies of the kinetics of exchange of K^+ have been reported for any isolated human muscle.

We now report findings in isolated external intercostal muscle from patients with myotonia congenita and from normal volunteers. We have compared our human results with similar data we obtained in isolated external intercostal muscle from the goat (1, 2).

METHODS

Normal volunteers. Our normals are 13 males aged 21–33 yr with no evidence of neuropathy or myopathy. One (R. G.) had diabetes mellitus; another (T. P.) had probable pulmonary sarcoidosis (minimal pulmonary fibrosis on chest X-ray and scalene node biopsy positive for noncaseating granuloma). At the time of muscle biopsy, T. P. had no symptoms and his chest X-ray was clearer (without therapy) than 1 yr earlier. No volunteer had taken any medication for at least 5 days before the external intercostal muscle biopsy except for the patient with diabetes, who received insulin up to and including the day before biopsy. The remaining 11 subjects were healthy and the results of laboratory studies (chest X-ray, electrocardiogram, hemoglobin, hematocrit, white blood cell count and differential, urinalysis, serum urea nitrogen, fasting blood sugar, Wasserman, serum creatinine, serum ions [Na^+, K^+, Cl^-], serum Ca and Mg, total serum proteins, serum alkaline phosphatase, serum glutamic oxalacetic transaminase, and creatinine phosphokinase) were normal.

Patients with myotonia congenita. The presence of myotonia was clinically obvious and was confirmed by electromyography in each of the six patients studied. Standard histopathological and histochemical examinations of a portion of each biopsy showed no diagnostic abnormalities. The patients shared the following clinical features: (*a*) myotonia was the principal complaint, there was no associated weakness or easy fatigability, and cooling was not necessary to elicit myotonic signs; (*b*) legs, arms, hands, extraocular muscles, tongue, etc., were all myotonic; (*c*) the lower extremities appeared more affected by the myotonia than upper extremities; (*d*) motor difficulties dated back to early childhood, or, at the latest, grade school; (*e*) each had a history of having fallen more than once, because of myotonia; (*f*) each had well developed musculature without atrophy in any muscle group; (*g*) there was no evidence of other organ system involvement or of other concurrent disease (in the male subjects, there was no characteristic balding nor testicular atrophy); (*h*) percussion myotonia was easily elicited from most muscles; (*i*) classical "warm-up," i.e., myotonia, decreasing with activity, was present. The results of laboratory studies in each of the patients (chest X-ray, electrocardiogram, hemoglobin, hematocrit, white blood count and differential, urinalysis, serum urea, nitrogen, fasting blood sugar, Wasserman, serum creatinine, serum ions [Na^+, K^+, Cl^-], serum Ca and Mg, total serum proteins, serum alkaline phosphatase, serum glutamic oxalacetic transaminase, and creatinine phosphokinase) were normal.

Their ages ranged from 25 (L. H.) to 42 yr (L. T.) and they were male except for P. N. and L. H. Patients P. N., E. K., and L. H. were siblings from a family with 10 family members known to be myotonic. Patient R. L. had one myotonic sibling but no other family history. Patients R. D. and L. T. were the only affected members of their respective families. No patients had taken any medication for 5 days before biopsy.

Biopsy techniques. Biopsies of the anterior border of external intercostal muscle (2.5–5 cm in length) were obtained under local anesthesia from the right eighth intercostal space. Preoperative medications were pentobarbital (from 100 to 200 mg), and morphine (from 10 to 12 mg). Intercostal nerves in the seventh, eighth, and ninth interspaces were blocked in the posterior axillary line by 2% lidocaine with epinephrine or 2% mepivacaine with epinephrine. In addition, the local anesthetic solution was infiltrated subcutaneously in a rectangular track around the interspace of the biopsy at least 6 cm from the line of subsequent incision.

The biopsy was placed in a thermos bottle containing previously gassed (95% O_2, 5% CO_2) physiological salt solution at a temperature of from 35 to 38°C, for transportation to the laboratory. Each biopsy was dissected, as previously described (1), so that one or more preparations were used for electrophysiological studies and two to seven preparations for measurement of ion content, ion flux, or extracellular space. Each preparation was dissected so that intact fibers ran from tendon to tendon. Preparations were less than 1 mm thick, varied in mass from 8 to 140 mg, contained from about 100 to 1000 fibers, and were about 1.5–2.5 cm long.

Electrophysiological methods. Tetrodotoxin, 1.3×10^{-6} M, was present in the physiological salt solution during all of the electrical measurements of myotonic preparations and during measurement of two of the normal (M. R. and L. C.) preparations. Electrophysiological equipment and recording methods used were as reported previously (2). Temperature of the preparations was 38 ±1.5°C.

The measured variables were: (*a*) I_o, the square wave hyperpolarizing current pulse (amperes $\times 10^{-8}$) passed through the current electrode; (*b*) t_{84}, the fiber time constant (milliseconds), the time required for the electrotonic potential at 0.05 mm to reach 84% of its steady-state value (this measurement approximates the product of the membrane resistance and the sum of the surface membrane and tubular membrane capacities [see equation 7, reference 5]); (*c*) V_x, the steady-state electrotonic potential (millivolts), recorded at distance x (millimeters) from the current electrode. Distances between the current and voltage electrodes were measured by ocular micrometer at a magnification of 25–50. The nearer electrode, for measuring $V_{0.05}$, was always at a distance too small for accurate estimation and therefore was assumed to be 0.05 mm from the current electrode.

The space constant, λ, (millimeters) was calculated from:

$$\lambda = (x - 0.05)/\ln(V_{0.05}/V_x) \quad (1)$$

where $V_{0.05}$ and V_x are steady-state potentials (millivolts) at 0.05 mm and x distances, respectively, from the current electrode. The input resistance of the fiber, R_{in}, in megohms (MΩ) was calculated from:

$$R_{in} = 0.1(V_{0.05}/I_o)\cdot e^{0.05/\lambda}. \quad (2)$$

From the parameters R_{in}, λ, and t_{84} we calculated r_i, the fiber resistance per unit length (megohms per centimeter), r_m, the membrane resistance of a unit length (MΩ·cm), and c_t, the fiber capacitance per unit length (microfarads per centimeter), as follows:

$$r_i = 20R_{in}/\lambda \quad (3)$$

$$r_m = 0.2R_{in}\cdot\lambda \quad (4)$$

$$c_t = (t_{84}/r_m)\cdot 10^{-3}. \quad (5)$$

The parameters r_i, r_m, and c_t were averaged for each preparation, and P, the mean corrected perimeter (microns), and A, the mean corrected cross-sectional area (μ^2), (histologically determined, see below), of the same preparation were used to calculate the R_m, the mean membrane resistance for unit area of fiber surface ($\Omega\cdot cm^2$), R_i, the mean myoplasmic resistivity (Ω·cm), and C_t, the mean fiber capacitance per unit of fiber surface ($\mu f/cm^2$), from the following:

$$R_m = r_m\cdot P\cdot 10^2 \quad (6)$$

$$R_i = r_i\cdot A\cdot 10^{-2} \quad (7)$$

$$C_t = [c_t/P]\cdot 10^4. \quad (8)$$

Resting potentials were recorded, to the nearest millivolt, upon each withdrawal of the voltage electrode when making cable parameter measurements. Resting potentials were obtained for these fibers by averaging the two observations in each fiber and then giving each average potential equal weight in calculating the mean. When resting potential was measured in fibers, other than those being used for cable parameter measurements, resting potential was determined once in each fiber and each determination given equal weight in calculating the mean.

Cable parameters were measured near the center of each fiber, three- to five-space constants from the tendonous insertions. The length of each preparation was adjusted, by eye, to about 10% greater than resting.

Ion content and washout techniques. The washout procedure was performed as previously described (1). When fat-free dry weight (FFDW)[1] was determined, the procedure of Nichols, Hazelwood, and Barnes (6) was followed. The temperature of the loading solution and of the washout solution was 38 ±1.5°C. All preparations were loaded with both

[1] *Abbreviations used in this paper:* ECV, extracellular volume; FFDW, fat-free dry weight.

^{42}K[2] and ^{36}Cl.[3] Loading times, constant for each experiment, were 100 min in ^{36}Cl followed by 20 min in ^{42}K and ^{36}Cl.

Concentrations of Na^+ and K^+ in tissue elution fluids were determined with a Perkin-Elmer, Model 290, Atomic Absorption Spectrometer.[4] Chloride in tissue elution fluids was determined with a Buchler-Cotlove chloridometer.[5] ^{42}K was counted with a Nuclear-Chicago NaI scintillation spectrometer,[6] and ^{14}C was counted with a Nuclear-Chicago low-background planchet counting system,[6] in Geiger mode. When ^{42}K and ^{36}Cl were present simultaneously, ^{42}K was counted immediately upon completion of the experiment with the scintillation spectrometer set to exclude energies below 0.8 MeV.

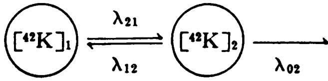
The ^{42}K used was of high specific activity, manufactured by an (n,γ) reaction from ^{41}K. This procedure also produces ^{35}S, which was therefore a contaminant in some washout experiments on biopsies from normal volunteers. Although the ^{35}S did not influence the counting of ^{42}K (since energies below 0.8 Mev were excluded), its presence made the control ^{36}Cl data invalid. ^{42}K of high specific activity with undetectable ^{35}S can be made and was used in the majority of our experiments.

Method of analyzing K^+ washout curves. The K^+ washout curves appear to be the sum of two exponentials. The form of the washout curve can therefore be described as:

$$\text{Fraction } ^{42}\text{K in muscle} = Ae^{-at} + Be^{-bt} \tag{9}$$

where A represents the fraction of total ^{42}K in the rapidly moving component, and a represents the rate constant governing A's movement. Likewise, B represents the fraction of total ^{42}K in the slower exchanging component and b represents its rate constant. It has been customary (7) to assume that the first term (Ae^{-at}) is small at long times (since a is large) and therefore contributes little to the slope of the terminal portion of the washout curve. Consequently, it has further been assumed that the rate constant derived from the slope of the terminal portion of the washout curve is the rate constant of efflux of K^+ from the muscle cells.

We have deviated from this analysis by assuming that the tissue can be represented by two compartments, an intracellular phase and an extracellular phase, which exchange with one another and with the nonradioactive physiological salt solution in each washout test tube according to first-order reactions. This can be depicted by the following diagram:



where $[^{42}K]_1$ represents the intracellular compartment, $[^{42}K]_2$ the extracellular compartment, and λ_{21}, λ_{12}, and λ_{02}, the first-order rate constants for efflux, influx, and extracellular exchange with the bathing solution of each washout test tube, respectively. This compartmental model can then be represented by the following:

$$\frac{d[^{42}K]_1}{dt} = \lambda_{12}[^{42}K]_2 - \lambda_{21}[^{42}K]_1 \tag{10}$$

and

$$\frac{d[^{42}K]_2}{dt} = \lambda_{21}[^{42}K]_1 - (\lambda_{12} + \lambda_{02})[^{42}K]_2. \tag{11}$$

Estimates of λ_{21}, λ_{12}, and λ_{02} can be derived by fitting the model to the K^+ washout curves by standard techniques of compartmental analysis. For our work we used an EAI, TR-20 analog computer[7] for initial estimates of rate constants and then a digital program, SAAM 23 (developed by M. Berman of the National Institutes of Health), for final fits. The digital fits gave fractional standard deviations of 2–10% for the final values of the rate constants.

Given equations 10 and 11 and the experimental data points, a unique set of values for λ_{12}, λ_{21}, and λ_{02} can be determined if one can provide the initial conditions (i.e., the magnitude of the intracellular compartment at zero time) or can fix the ratio of any two of the three rate constants. Since initial condition estimates require extracellular volume estimates, subject to error, we chose to assume that the tissue was in steady state with respect to K^+ content, over the time studied. This allows the ratio of $\lambda_{12}/\lambda_{21}$ to be fixed at the ratio of intracellular to extracellular K^+ concentration. Since human external intercostal preparation is much like goat and since goat external intercostal has an unchanging K^+ content for as long as 24 hr out of the animal,[8] this assumption seems reasonable.

In the Results we will present analysis by customary and by compartmental methods. Potassium efflux was calculated from

$$\text{Efflux} = (A/P)k[K]_i \cdot 10^2 \tag{12}$$

where efflux is in moles $\times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$; A/P (microns) is the mean ratio of area to perimeter, corrected for shrinkage, for the preparation studied; k (sec^{-1}) is the mean model efflux rate constant (λ_{21}) or the apparent rate constant (b of equation 9) for the preparations studied; and $[K]_i$ (mEq/liter) is the mean internal K^+ concentration for the entire group.

Extracellular volume and cell geometry. Extracellular volume was determined by equilibration of preparations of external intercostal muscle with ^{14}C-labeled mannitol[9] by methods previously reported (1). Cell dimensions were measured from projected cross sections (magnification 1000) of formalin-fixed, paraffin-embedded preparations (1). It is generally recognized that during conventional histological preparation a shrinkage occurs. A correction factor (1.42 for perimeter and 2.02 for area), calculated from Creese (8), has been applied to the measured perimeter and area in calculating cable parameters and ion flux data (Tables I, II, V, and VI); it was applied to data reported by Bryant (2) but not to data reported by Lipicky and Bryant (1).

Physiological salt solution. The physiological salt solution, also used for isolated goat external intercostal muscle (1, 2), had the following composition (millimolar): NaCl 148, KCl 4.5, $CaCl_2$ 2.0, $MgCl_2$ 1.0, NaH_2PO_4 0.44, $NaHCO_3$ 12, and glucose 5.5; on equilibration with 95% O_2 and 5% CO_2 at 38°C the pH was 7.1.

Tetrodotoxin[10] was Crystalline 3 × (manufactured by Sankyo Company Limited, Tokyo, Japan). A stock solution (1×10^{-4} g/ml) was prepared from the original ampule material and stored at 4°C no longer than 3 months before use.

Presentation of data. Our data are presented as mean ±SEM. The symbol "n" represents the number of observations (i.e., the number of fibers in any one preparation that had cable measurements, or the number of preparations from any one biopsy that had ion measurements), and "N" repre-

[2] Obtained from Nuclear Science, Irvine, Calif.

[3] Obtained from Oak Ridge National Laboratories, Oak Ridge, Tenn.

[4] Perkin-Elmer Corp., Norwalk, Conn.

[5] Buchler Instruments Inc., Fort Lee, N. J.

[6] Nuclear-Chicago Corp., Des Plaines, Ill.

[7] Electronic Associates, Inc., West Long Branch, N. J.

[8] Lipicky, R. J., S. H. Bryant, and J. H. Salmon. Unpublished observations.

[9] Obtained from Amersham/Searle Corp., Des Plaines, Illinois.

[10] Calbiochem, Los Angeles, Calif.

TABLE I

Mean Cable Parameters (38°C) of Muscle Fibers from Normal Volunteers

Volunteer	P	A	n	All fibers measured RP	R_i	R_m	C_t	n	Selected fibers RP	R_i	R_m	C_t
	μ	μ^2		*mv*	*Ω·cm*	*Ω·cm²*	*μf/cm²*		*mv*	*Ω·cm*	*Ω·cm²*	*μf/cm²*
J. H.	174	2123	25	(55–85)	135	1371	4.5	15	(70–85)	111	1927	4.3
L. C.	171	1672	10	(52–81)	142	1980	9.8	5	(72–81)	120	2509	8.5
C. C.	225	3720	17	(55–97)	165	1913	3.9	9	(70–97)	129	1868	4.1
J. C.	243	3808	9	(60–85)	166	3128	4.4	8	(70–86)	167	2927	4.6
M. R.	166	1999	12	(59–90)	118	1601	7.9	9	(73–90)	85	1611	8.6
R. G.	151	1621	15	(41–109)	157	2612	5.5	4	(70–88)	153	3717	3.1
P. M.	184	2452	10	(60–90)	200	1804	4.6	4	(70–90)	145	2437	3.3
B. J.	204	2593	8	(25–71)	150	1068	6.0	—	—	—	—	—
D. S.	203	2884	5	(30–70)	106	1944	4.3	—	—	—	—	—
T. P.	199	2739	19	(43–86)	127	2999	4.7	10	(70–86)	131	2975	4.6
S. G.	165	1822	5	(63–73)	90	1503	6.6	4	(70–73)	85	1627	6.7
T. J.	184	2452	3	(70–77)	94	3743	4.6	3	(70–77)	94	3743	4.6
D. W.	189	2458	5	(42–75)	161	2933	4.5	3	(70–75)	135	3471	4.2
Group mean of this series			N = 13	68.1 ±2.0	139.3 ±8.8	2200 ±223	5.48 ±0.47	N = 11	75.6 ±1.1	123.2 ±8.2	2619 ±243	5.15 ±0.58
Isolated external intercostal normal human, 37°C (3)									81.0 ±2.0	125	4070 ±259	4.8 ±0.5
Isolated external intercostal normal goat, 38°C (2)									74.4 ±0.8	112 ±4	1897 ±86	4.1 ±0.2

The mean values (or ranges) of each variable measured in each volunteer are arranged horizontally, and are identified by the initials of the volunteer. The means at the bases of the vertical columns were obtained by giving each preparation (i.e., each volunteer) equal weight, irrespective of the number of fibers, n, measured in any individual preparation. The middle section of the table ("All fibers measured") shows results of every cable parameter measurement, the right section ("Selected fibers") shows only the cable parameters of fibers with resting potentials greater than 70 mv at each electrode position. All measurements were made at 38 ±1.5°C. Group means are expressed ±SEM.

Fiber dimensions (P = perimeter, A = area) are corrected for shrinkage attributed to histological procedures. The ranges of resting potential (RP) are shown for each preparation in parentheses. Specific cable parameters (R_i = myoplasmic resistivity, R_m = membrane resistance, and C_t = fiber capacitance) were calculated according to equations 6, 7, and 8.

TABLE II

Mean Cable Parameters (38°C) of Muscle Fibers from Patients with Myotonia Congenita

Volunteer	P	A	n	All fibers measured RP	R_i	R_m	C_t	n	Selected fibers RP	R_i	R_m	C_t
	μ	μ^2		*mv*	*Ω·cm*	*Ω·cm²*	*μf/cm²*		*mv*	*Ω·cm*	*Ω·cm²*	*μf/cm²*
R. D.	155	1807	5	(15–70)	62	8457	5.5					
R. L.	184	2466	11	(51–83)	39	5219	6.1	10	(70–83)	31	5195	5.8
L. T.	162	1947	14	(60–86)	116	4075	6.6	10	(70–86)	85	4754	4.8
P. N.	131	1311	14	(31–83)	123	11,219	7.1	6	(70–83)	116	7151	6.7
E. K.	154	1673	13	(40–67)	214	5963	10.2					
L. H.	148	1349	14	(60–82)	77	5225	7.9	10	(70–82)	68	5815	6.3
Group mean of this series			N = 6	65.2 ±4.0	105.1 ±25.4	6693 ±1085	7.23 ±0.68	N = 4	75.0 ±0.3	75.0 ±17.7	5729 ±522	5.90 ±0.41
Isolated external intercostal myotonic goat, 38°C (2)									76.0 ±0.6	102.5 ±3.4	5589 ±250	4.4 ±0.2

TABLE III

Mean Water and Ion Content of Isolated (38°C) Muscle Fiber Bundles from Normal Volunteers

Volunteer	n	Mean wet weight	Mean H_2O content	Mean ion content		
				Na^+	K^+	Cl^-
		mg	*ml/kg wet wt*		*mEq/kg wet wt*	
J. H.	5	17.1	809.6	84.7	68.2	91.9
L. C.	3	56.5	785.0	98.5	50.2	74.5
C. C.	5	86.3	855.2	79.3	58.1	87.4
J. C.	3	111.2	787.8	74.2	48.2	80.6
M. R.	3	44.9	818.4	84.1	74.6	58.6
R. G.	6	36.0	793.8	96.6	60.7	75.0
P. M.	4	40.1	818.2	76.1	81.5	63.5
B. J.	5	24.6	789.2	114.0	71.7	73.0
D. S.	5	16.6	801.9	113.1	73.1	80.1
T. P.	5	42.7	823.9	93.8	68.1	69.8
T. J.	9	57.7	812.0	117.9	71.1	69.2
D. W.	7	32.4	803.5	96.5	74.9	73.1
Group mean of this series	N = 12		808.2 ±5.6	94.1 ±4.3	66.7 ±3.0	74.7 ±2.7
Isolated external intercostal, normal human, 38°C (4)			752 ±23	117 ±11.7	62 ±7.3	
Isolated external intercostal, normal goat, 38°C (1)			789.7 ±4.8	91.6 ±3.4	68.9 ±1.6	70.0 ±2.2

The muscle bundles had been in physiological salt solution at 38 ±1.5°C for 8–10 hr after biopsy. From each biopsy, three to six (n) bundles were studied; the mean values of these bundles are reported for each volunteer. The means of these means are listed.

sents the number of subjects (the mean for each subject given equal weight) represented in the group mean. Means of other investigators' results, if not in their data, were calculated directly from their tables and are presented as mean ±SEM. Student's t test was used for testing the significance of differences between means.

RESULTS

Cable parameters. The current-voltage relationships of fibers studied were linear to at least 15 mv in the hyperpolarizing direction. For each measurement, the injected current was adjusted so that the hyperpolarized electrotonic potential at the near electrode was about 10 mv.

Table I summarizes the cable parameters of 143 fibers in 13 preparations from 13 normal volunteers ("All fibers measured"). Because the method for determining cable parameters necessitates the use of surface fibers, which are more likely to be depolarized than interior fibers, some selection, of results, is usually felt to be necessary in order to approximate the mean electrical constants of a preparation. Arbitrarily selecting results, by requiring that each fiber measured had resting potentials of 70 mv or more at each measurement point ("Selected fibers"), eliminates 2 of the 13 preparations, leaving 74 measurements in 11 preparations from 11 subjects.

Table II summarizes the cable parameters of 71 fibers in 6 preparations from 6 patients with myotonia congenita ("All fibers measured"). Selecting fibers with a resting potential of 70 mv or more, as above, ("Selected fibers") leaves 36 measurements in 4 preparations from 4 patients.

The most striking cable finding in muscle from patients with myotonia congenita was a two to three times greater-than-normal resting membrane resistance. In preparations from patients with myotonia congenita, the range of R_m (4075 to 11,219 $\Omega \cdot cm^2$), ("All fibers measured," Table II) shows no overlap with the maximum range of R_m (1068 to 3743 $\Omega \cdot cm^2$) in preparations from normal volunteers ("All fibers measured," Table I).

Considering the mean cable parameters of the ("Selected fibers") in the myotonia congenita group, Table II, R_m (5729 ±522 $\Omega \cdot cm^2$) was greater ($P < 0.001$) and R_i (75.0 ±17.7 $\Omega \cdot cm^2$) was less ($0.025 > P > 0.01$) than those of the normal volunteers (2619 ±243 $\Omega \cdot cm^2$ and 123.2 ±8.2 $\Omega \cdot cm$ for R_m and R_i, respectively). The fiber capacitance of the ("Selected fibers," Table II) did not differ ($P > 0.4$) from those of the normal volunteers.

Resting potentials. 3 preparations used for cable parameter measurements, a total of 263 fibers (46, 121, and 96 measurements), from the normal volunteers were

TABLE IV

Mean Water and Ion Contents of Isolated (38°C) Muscle Fiber Bundles from Patients with Myotonia Congenita

Volunteer	n	Mean wet weight	Mean H_2O content	Mean ion content Na^+	K^+	Cl^-
		mg	*ml/kg wet wt*	*mEq/kg wet wt*		
R. D.	6	18.1	808.8	94.9	71.8	73.2
R. L.	7	25.2	762.1	85.5	64.7	74.2
L. T.	11	22.3	771.8	101.2	67.9	67.3
P. N.	6	15.7	775.6	103.7	78.4	67.7
E. K.	6	32.9	781.3	98.8	75.9	63.1
L. H.	3	27.7	829.6	101.9	82.4	128.4
Group mean this series	N = 6		788.2 ±10.5	97.7 ±2.73	73.5 ±2.7	79.0 ±10.0
Isolated external intercostal myotonic goat (1)			789.7 ±0.48	101.0 ±3.9	79.2 ±2.0	68.2 ±2.1

systematically surveyed for distribution of resting potentials. Fibers were penetrated from the surface to a depth of up to five penetrations. Nearly all of the lowest potentials (20–40 mv) were in the first layer penetrated, which had mean resting potentials from 20 to 22 mv lower than the third or deeper layers ($P < 0.005$). This suggests that the dissection procedure injures the superficial fibers, possible by pulling on the fine connective tissue strands and so tearing the sarcolemma of the top fibers (see Fig. 3, reference 9). The distribution of resting potentials was skewed toward lower potentials. The mean was 61.2 ±1.0 mv, the mode was 72.5 mv, and the median was 60 mv. It is our impression that these preparations (T. J., S. G., and D. W.) had a larger number of fibers with low resting potentials than did others in this series of biopsies and may therefore represent a biased sampling.

5 preparations, a total of 377 fibers (41, 56, 103, 113, and 64 measurements) from patients with myotonia congenita were systematically surveyed for distribution of resting potentials. As in the normal population, there was a left skewing and the fibers with the lowest potentials were surface fibers. The mean was 68.1 ±1.1 mv, the mode was 85 mv, and the median was 70 mv.

Sodium, potassium, chloride, and water content. Some preparations were not subjected to washout procedure, but were in an isolated state for the same periods of time as the washout muscles. There was no significant difference between the ion content of those preparations which had been subjected to washout procedures and preparations that had not ($P > 0.1$). There was also no correlation between the size of the preparations and their ion content.

The ion content, wet weight (presented to show the average size of our preparations), and water content in biopsies from our normal volunteers are summarized in Table III. The means for 60 determinations in 12 volunteers (the mean for each volunteer was given equal weight in determining the group mean) resemble those obtained in isolated external intercostal muscle from the goat (1) and that determined by others from man (4).

The means for 39 measurements in 6 patients with myotonia congenita (the mean for each volunteer was given equal weight in determining the group mean) are summarized in Table IV. The K^+ content milliequivalent per kilogram wet weight) of the myotonic muscle (73.5 ±2.7) was not significantly greater than normal (66.7 ±3.0) and the H_2O content (milliliter per kilogram wet weight) of myotonic muscle (788.2 ±10.5) probably less ($P < 0.05$) than normal (808 ±5.6).

Fat content and extracellular volume. The over-all mean fat content in 28 determinations was 3.24 ±0.65% wet weight, 86% of the preparations having less than 6% fat content. There was no difference between preparations from patients with myotonia congenita and those from normal volunteers.

The mean mannitol extracellular volume (ECV) measured in seven preparations from five normal volunteers, was 46.6 ±3.6 cc/100 g wet weight, and that measured in five preparations from three patients with myotonia congenita was 45.0 ±5.0 cc/100 g wet weight. Eight preparations from five other patients with nondystrophic muscle disease (other parameters not reported because these patients did not have myotonia congenita) had a mannitol ECV of 41.3 ±2.1 cc/100 g wet weight. The mean ECV's of these three groups of subjects were not different ($P > 0.5$) from one another. We have chosen to combine the ECV measurements (20 preparations measured from 8 patients and 5 normal volunteers) into one estimate of 45.7 ±2.1 cc/100 g wet weight. We consider this mean to be the

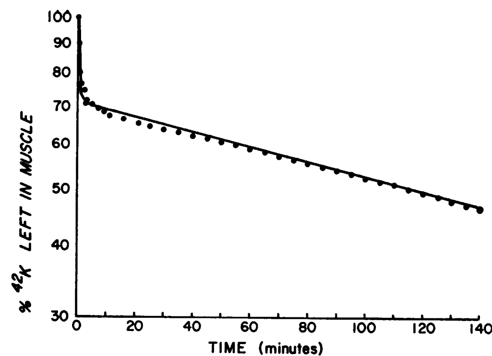


FIGURE 1 The time course of decline of ^{42}K in one preparation from one normal volunteer. The points are the experimental curve. The continuous line is the best-fit solution of the two-compartment open model for this washout experiment. The agreement between experimental and calculated curves is typical of all our washout experiments.

best estimate of the mannitol ECV in our isolated external intercostal muscle preparations and used this value in calculations where an ECV is required.

These ECV determinations in human muscle are different from those we reported (30.5 ±2.0 cc/100 g wet weight) for isolated goat external intercostal muscle (1). To substantiate this difference we repeated the measurement of the mannitol ECV in isolated goat external intercostal muscle. We found an ECV of 33.4 ±2.0 cc/100 g wet weight in seven preparations from five goats. These determinations, occurring over 4 yr apart, were indistinguishable ($0.1 > P > 0.05$). We conclude that measurement of the mannitol ECV is reproducible and therefore that the human preparations have a larger mannitol ECV than do those of goat preparations.

To evaluate the effects of injury, which may make fibers permeable to mannitol, five preparations from goat external intercostal muscle were deliberately pinched in several places with forceps or were partially transected with scissors, or both, before being equilibrated with mannitol-^{14}C. In these "grossly injured" goat preparations the mannitol ECV was 50.0 ±3.92 cc/100 g wet weight.

Intracellular ion concentrations. From the group mean ion and water contents (Tables III and IV) and the over-all mean mannitol ECV (45.7 cc/100 g wet weight) we calculated (see Discussion) intracellular concentrations of 59, 184, and 7 mEq/liter fiber water for Na^+, K^+, and Cl^-, respectively, for normal human preparations; 73, 215, and 20 mEq/liter fiber water for Na^+, K^+, and Cl^-, respectively, for the myotonia congenita preparations. These can be compared to concentrations of 90.9, 133.5, and 49.7 mEq/liter fiber water for Na^+, K^+, and Cl^-, respectively, for normal goat preparations; 107, 160, and 42 mEq/liter fiber water for Na^+, K^+, and Cl^-, respectively, for myotonic goat preparations (1).

Potassium efflux. The time course of decline of ^{42}K (washout curve) from an isolated bundle of external

TABLE V

Mean Potassium Efflux of Isolated (38°C) Muscle Fiber Bundles from Normal Volunteers

Volunteer	n	A/P	Apparent rate constant of efflux	Apparent potassium efflux	Model rate constant of efflux	Model potassium efflux
		μ	min^{-1}	$moles \times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$	min^{-1}	$moles \times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$
J. H.	2	11.7	0.00592	21.2	0.01097	39.3
J. C.	3	14.9	0.00415	18.9	0.00623	28.4
R. G.	3	10.4	0.00621	19.8	0.00770	24.5
P. M.	3	12.6	0.00439	16.9	0.00542	20.9
B. J.	3	12.6	0.00485	18.7	0.00547	21.1
D. S.	2	14.0	0.00274	11.7	0.00412	17.7
T. P.	2	13.4	0.00433	17.8	0.00618	25.4
T. J.	3	13.8	0.00257	10.9	0.00309	13.1
D. W.	3	12.8	0.00354	13.9	0.00446	17.5
Group mean this series		N = 9	0.00430 ±0.00042	16.6 ±1.2	0.00596 ±0.00077	23.1 ±2.5
Isolated external intercostal, normal goat, 38°C (1)				8.3 ±1.2		

Potassium efflux was calculated according to equation 12. A/P is corrected for histological shrinkage. The "apparent rate constants of efflux" were obtained directly from the washout curves and are equivalent to b of equation 10. The "model rate constants of efflux" are λ_{21} of our two-compartment open model.

TABLE VI
Mean Potassium Efflux of Isolated (38°C) Muscle Fiber Bundles from Patients with Myotonia Congenita

Volunteer	n	A/P	Apparent rate constant of efflux	Apparent potassium efflux	Model rate constant of efflux	Model potassium efflux
		μ	min^{-1}	$moles \times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$	min^{-1}	$moles \times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$
R. L.	3	13.4	0.00480	23.1	0.00563	27.1
L. T.	3	12.0	0.00622	26.8	0.00788	34.0
P. N.	3	10.0	0.00577	20.8	0.00714	24.2
E. K.	2	10.9	0.00317	12.4	0.00400	15.7
L. H.	3	9.1	0.00360	11.8	0.00456	14.9
Group mean of this series	N = 5	11.08 ±0.75	0.00471 ±0.00059	19.0 ±3.0	0.00584 ±0.00072	23.2 ±3.6
Isolated external intercostal, myotonic goat, 38°C (1)				11.7 ±0.4		

intercostal muscle of one volunteer is shown in Fig. 1. The points are washout data and the continuous line was obtained by solving the two-compartment open model. The agreement between the calculated and experimental curves is good.

The results of analysis of 24 washout curves in nine normal volunteers are summarized in Table V and those of 14 washout curves from patients with myotonia congenita are summarized in Table VI. The "apparent rate constant" and "apparent K^+ efflux" are values derived from graphical analysis, assuming that equation 9 (see Methods) holds. The "model rate constant" and "model K^+ efflux" are values obtained from the two-compartment open model. The "model rate constant" is greater than the "apparent rate constant" in each volunteer or patient studied. The "model potassium efflux" was 22–39% greater than the "apparent potassium efflux" in each volunteer or patient studies. There was no correlation between the size of the preparations and their potassium efflux.

The mean "model potassium efflux" (the mean for each subject was given equal weight in determining the group mean) of preparations from the normal volunteers was 23.1 ±2.5 moles $\times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$ and was indistinguishable ($P > 0.5$) from the mean "model potassium efflux" of preparations from the patients with myotonia congenita (23.2 ±3.6 moles $\times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$). The same conclusion is reached if the "apparent potassium effluxes" are compared. The "apparent potassium efflux" of external intercostal muscle preparations from normal goats and from goats with hereditary myotonia (8.3 and 11.7 moles $\times 10^{-12}$ $cm^{-2} \cdot sec^{-1}$, respectively), were also not different from one another (1) but each is less than that in its respective human counterpart.

DISCUSSION

The diagnosis of myotonia congenita was made on the basis of the clinical criteria outlined in Methods and not upon the results of our investigation. It turned out that the patients we selected on the basis of common clinical features were also relatively similar with regard to cable and ionic parameters of isolated muscle, and with respect to these parameters were similar to goats with hereditary myotonia. We do not, at the present time, wish to become involved in controversy as to whether myotonia congenita (Thomsen's disease), myotonia dystrophica (Steinert's disease) and paramyotonia are separate entities (see references 10 and 11 for details).

Suitability of biopsies of human external intercostal muscle as isolated preparations. Human external intercostal muscle has a complex orientation of fibers and of tendons within an interspace and, at least in the location from which our biopsies were obtained, the sample is equivalent to taking a muscle biopsy from the belly of some larger muscle. Although it is possible to make satisfactory preparations of human external intercostal muscle, the fine dissection is more tedious and takes two to three times longer than is our experience in the goat. From one normal volunteer we were unable to get usable preparations.

The variation noted in the ion contents (Tables III and IV) is probably related to these difficulties of dissection. Variation among subjects could occur from the fact that sometimes whole biopsies (as judged from the

ease of finding surface fibers that were suitable for cable-parameter studies) seemed to be in poorer condition than others. Likewise, within any one biopsy, some preparations were easier to dissect than others and selection of preparations on the basis of ability to twitch (to external stimulation) after long time periods was not possible once a preparation was committed to the ion procedure.

External intercostal muscle is the only easily accessible human muscle which can be removed from tendon to tendon. We, as others (3, 4, 12–14), have found that it can provide useful, reproducible electrophysiological, ion flux, and ion content data.

Cable model. The cable model (illustrated as part of a distributed network) that serves as a framework for interpretation of our data, is shown in Fig. 2. The basis for arrangement of the elements was shown by Falk and Fatt in frog sartorius muscle (15). Data from frog muscle show that the elements r_e ($\Omega \cdot$cm) and c_e (microfarads per centimeter) are associated with the interior of the cell (related in some way to the transverse tubules or other components of the sarcoplasmic reticulum), whereas the components r_m ($\Omega \cdot$cm) and c_m (μf/cm) are associated with the surface membrane of the muscle cell (16). The ratios of r_e to r_m and c_e to c_m are 0.1 and 2, respectively (17). The validity of this model has not been tested for mammalian muscle, and in toad muscle r_e may be too small to be detected (18). Assuming the validity of this model for mammalian skeletal muscle, our measurements based on the use of steady-state potentials do not measure r_e and give a capacitance c_t derived from t_{84} (see equation 5) that approximates the sum of c_m and c_e (5). Therefore, as was done previously (2), the capacitance per cm^2 has been labeled C_t (approximate total fiber capacitance) rather than C_m (surface membrane capacitance), in anticipation of extending the Falk and Fatt model to mammalian skeletal muscle.

Cable parameters, R_m and C_t. For the sake of comparison, the mean parameters t_{84} and R_{in} from our ("Selected fibers") of normal volunteers (Table I) are 12.8 $\pm$1.3 msec and 0.43 $\pm$0.04 MΩ, respectively. McComas, Mrozek, Gardner-Medwin, and Stanton (19), from recordings *in situ* in biceps and quadriceps muscle of normal volunteers, reported a t_{84} of 6.33 $\pm$0.4 msec and an R_{in} of 0.27 $\pm$0.02 MΩ. Elmqvist, Johns, and Thesleff (3), in isolated external intercostal muscle of normal subjects reported a t_{84} of 18.9 $\pm$1.2 msec and an R_{in} of 0.48 $\pm$0.05 MΩ. Our mean values are between those of these two previous reports.

To calculate a value for R_m and C_t from t_{84} and R_{in}, without benefit of the measured space constant (λ), would require an assumption of R_i and fiber diameter. In such a calculation R_m would vary as the cube of the diameter and therefore be subject to large errors.

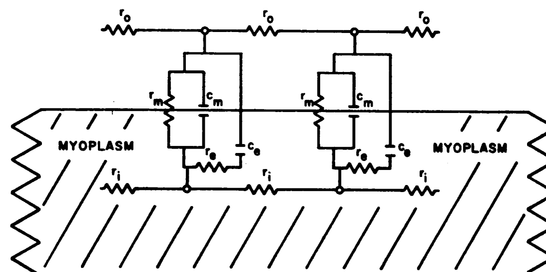


FIGURE 2 Equivalent circuit diagram of the cable model postulated by Falk and Fatt (14). Symbols: extracellular resistance (r_o), myoplasmic resistance (r_i), membrane resistance (r_m), membrane capacitance (c_m), a resistance associated with elements of the cell other than the surface membrane (r_e), and a capacitance associated with elements of the cell other than the surface membrane (c_e). r_o was assumed to be so small with respect to r_i that it can be neglected in calculation. The parameters (in lower case) indicate that the dimensions of the cell have not been taken into account. Knowing the dimensions of the cell and applying equations 6–8 allow expression of these parameters in a form that allows meaningful comparison between different preparations that have varying cell size.

McComas et al. did not measure R_i, or diameter. If values for R_m were to be calculated from their data (assuming our R_i and fiber size), R_m would be less than that which we are reporting for muscle from normal volunteers. Our mean cable parameters (Table I, "Selected fibers" or All fibers measured") for normal volunteers differ from those reported by Elmqvist et al. (3), in that our R_m is smaller ($P < 0.01$).

It is clear that isolated human external intercostal has a significantly higher R_m than goat external intercostal (see Table I) or other mammalian skeletal muscle that has been tested (i.e., 1400 $\Omega \cdot cm^2$, cat tenuissimus (20); 370–540 $\Omega \cdot cm^2$, rat soleus and rat extensor digitorum longus (21, 22]). Because potassium permeability (from flux data, see below) appears to be greater in human intercostal than in goat intercostal, we tentatively conclude that the chloride permeability is lower in normal human muscle than in normal goat muscle (K^+ and Cl^- being assumed to be the only major ions accounting for the resting transmembrane conductance).

The mean capacitance (5.15 $\mu f/cm^2$) reported here for normal human external intercostal muscle is not significantly different from other values reported for this same muscle from normal man or the normal goat, 4.8 and 4.1 $\mu f/cm^2$, respectively (see Table I). As in the goat (2), if attention is restricted to the ("Selected fibers," Table II), the C_t of myotonia congenita fibers is not different from normal. However, the unselected fibers ("All fibers measured") do show a greater C_t in myotonic muscle ($P = 0.05$) than in normal muscle. An explanation for this difference between ("All fibers measured" and "Selected fibers") is not immediately apparent.

Cable parameter R_i. Assuming handbook equivalent conductances, corrected to 38°C, and the internal concentrations of Na^+, K^+, and Cl^- calculated for muscle from normal volunteers (see Results), R_i would be 40 Ω·cm. The measured value ("Selected fibers," Table I) was 123.2 Ω·cm. The mean R_i (75 Ω·cm) of the ("Selected fibers," Table II) from the myotonia congenita patient population, significantly less than normal, approaches the calculated R_i (33 Ω·cm).

A disparity between measured myoplasmic resistivity, and values of myoplasmic resistivity calculated from ionic concentrations, has been noted before (23, p. 638). Recently, measurement of the apparent diffusivities of potassium, sodium, and other substances inside single frog muscle fibers has been reported (24). The apparent diffusivities of most of the substances were about half their diffusivities in aqueous solution. The investigators concluded that the reduced diffusivity was due to physical factors rather than chemical interactions in the interior of the cell.

Accepting that some sort of physical barriers interfere with the diffusivity of ions inside muscle fibers leads us to speculate that there would be differences in internal current paths (which may or may not be apparent as electronmicroscopic differences in ultrastructure) in skeletal muscle from patients with myotonia congenita.

We note ("All fibers measured," Table II), that patients R. D., R. L., and L. H. have low values for R_i and that patients L. T., P. N., and E. K. have normal to high values for R_i. This raises the possibility that R_i differentiates the dominant from the recessive (25, 26) forms of myotonia congenita.

Resting potentials and their influence on cable parameters. The mean resting potentials reported for isolated, normal, human, external intercostal muscle fibers are 81 ±1.7 (3), 72.6 ±0.8 (4), 78.8 ±2.5 (13), and 79.8 ±0.6 (14) mv. Our means of 75.6 ±1.1 and 75 ±0.3 mv, for ("Selected fibers"), normal and myotonic preparations, respectively, are within this range. Thus, the cable parameters reported for normal and myotonic ("Selected fibers") can be taken as representative of typical isolated human external intercostal fibers. Data from ("All fibers measured") are also reported in order to show that the greater than normal R_m of the myotonic fibers was present whether or not the fibers were selected for resting potentials greater than 70 mv.

Considering either individual subjects or group means there are some differences in mean cable parameters between ("All fibers measured" and "Selected fibers"). We do not have enough data to satisfactorily test the hypothesis that cable parameters depend upon resting potential and, at the present time, we are unable to ascribe biological significance to differences between ("All fibers measured" and "Selected fibers").

Our results suggest that myotonic fibers have higher resting potentials than do normal fibers since the mean, median, and mode of resting potentials of myotonic preparations was higher than that of the normal preparations. This is consistent with findings in the goat (2) and in human myotonia congenita (27). Moreover, the higher-than-normal intracellular potassium concentration of myotonic fibers (see Results) and their lower chloride permeability (see below) is consistent with a higher resting potential in myotonic fibers.

Extracellular volume and intracellular ion concentrations. Calculation of ion efflux, permeability, conductance, and ion equilibrium potentials depends upon knowing the intracellular concentration of the ion under consideration. The sodium, potassium, and chloride measured in the elution fluids comes from at least two sources: (*a*) the ion content of the physiological salt solution that has equilibrated with the extracellular volume of the preparations, and (*b*) the ion content of the myoplasm (intracellular content in solution) or the ion content of structures that either bind or concentrate ions.

The values for intracellular ion concentrations depend on the extracellular volume determination. The amount of ions present in the elution fluids that came from intracellular (or bound) sources is obtained by the difference between that actually measured (ion contents, Tables III and IV) and that estimated to have come from the ECV. Likewise, the H_2O content actually measured (Tables III and IV) is divided between intracellular and extracellular phases by the differences between the total content and the ECVestimate(assuming that the ECV has a specific gravity of one). As can be seen from our results in frog sartorius (see below), it is possible to end up with a negative intracellular ion concentration.

Mannitol, sucrose, and inulin are commonly used for the measurement of extracellular volume, both in intact animals and in isolated tissues. In cardiac muscle, each of these substances is excluded from the intracellular space that is important to electrophysiological function (28). In isolated frog sartorius muscles, mannitol gives values of extracellular volume in close agreement with those predicted from physiochemical considerations (29). Yet variation in the magnitude of measured extracellular volume occurs (30).

In order to explore the accuracy of the mannitol extracellular volume in determining intracellular ion concentrations, we tested its ability to predict the intracellular chloride concentration in frog sartorius muscles and compared mannitol estimates to those of other substances. The intracellular chloride concentration of frog sartorius muscles is estimated to be between 3.1 and 3.8 mEq/liter fiber water (31) from measurements independent of measurement of extracellular

volume. We found intracellular concentrations of chloride (mEq/liter fiber water) to be 7.2 ± 5.4 ($n = 28$), -27.7 ± 6.4 ($n = 22$), -9.8 ± 5.8 ($n = 42$), and 11.4 ± 1.5 ($n = 15$) from measurements of ECV with mannitol, sucrose, inulin, and ^{131}I-labeled–human serum albumin, respectively. Although mannitol, albumin, or inulin give reasonable intracellular Cl^- concentrations, we chose mannitol for use in muscle from man because the measurements were more conveniently made and because it gave a mean intracellular Cl^- concentration closest to the above 3.1 to 3.8 mEq/liter fiber water.

The larger mean ECV (cc/100 g wet weight) of human external intercostal muscle preparations (45.7) compared to the goat (30.5–33.4) is consistent with the fact that human muscle required more dissection and had a more open and loose appearance than did goat preparations. Also consistent with the larger ECV of human preparations is the greater water content (cubic centimeter per kilogram wet weight) in human preparations (810) than in the goat (790). Isolated external intercostal muscle from both goat and man have considerably larger ECV's than are reported for other mammalian muscles *in situ* (32), which range from 13.5 to 17 cc/100 g wet weight. This difference is probably related to the fact that the muscles *in situ* have not been exposed to dissection and teasing.

In goat preparations we demonstrated that the mannitol ECV could go up from control values of 30.5–50 cc/100 g wet weight. However, it is unlikely that human preparations were injured to the degree that the deliberately mutilated goat preparations were injured. Although injury may have elevated the mean mannitol ECV by some unknown amount, we think that the contribution of injury was small and therefore that our isolated human external intercostal preparations have a true ECV near that which we are reporting. Similar conclusions were reached by Creese et al. (4) with isolated human external intercostal muscle.

Potassium permeability. Given the potassium efflux (moles $\times 10^{-12}$ cm$^{-2}\cdot$sec^{-1}), the internal potassium concentration (moles $\times 10^{-6}$ cm^{-3}), and the resting potential mode (millivolts), the permeability of the muscle membranes (cm$\cdot$sec^{-1}) can be calculated from the Goldman constant field equations (see reference 33). This relationship can be expressed as follows (34):

$$P_K = \frac{\text{Efflux}}{[K]_i \frac{EF/RT}{[1 - e^{-EF/RT}]}}, \quad (14)$$

where E is the potential difference across the membrane, its sign taken as negative, since, in the case of potassium efflux, the ion movement is opposed by the electric field; F/RT have their usual meaning; and $[K]_i$ is the internal potassium concentration in the muscle fibers. Calculation gives 6.40×10^{-7} cm$\cdot$sec^{-1} for P_K in the isolated external intercostal muscle (at 38°C) from normal volunteers. This can be compared to 6.2×10^{-7} and 3.65×10^{-7} cm$\cdot$sec^{-1} in frog sartorius (34), and external intercostal from normal goats (1), respectively. The P_K in human external intercostal is almost twice that in normal goat external intercostal.

Similar calculation for muscle from patients with myotonia congenita gives a P_K of 7.63×10^{-7} cm$\cdot$sec^{-1}. This can be compared with that of the myotonic goat which was 4.9×10^{-7} cm$\cdot$sec^{-1} (1). Clearly, human external intercostal muscle has a greater P_K than goat external intercostal muscle. Since the cable parameters, resting potentials, and ion contents indicate that human preparations are in the same condition, with respect to injury, as goat preparations, the greater P_K of human muscle is probably a feature of the muscle membrane. By deduction, the P_{Cl} in normal human skeletal muscle would be expected to be less than that of normal goat muscle.

Since the R_m of fibers from patients with myotonia congenita was greater than normal (i.e. total conductance or permeability less than normal) and since the P_K calculated from potassium efflux was greater than normal, we conclude that P_{Cl} must be abnormally low in myotonia congenita. In this way, as well as with respect to R_m, myotonia congenita muscle is like that of goats with hereditary myotonia (1).

Relationship of these findings to myotonia congenita. Measurement of the cable parameters was one of our primary goals. Based upon our experience with muscle from goats with hereditary myotonia (1, 2), that of Norris (35), and McComas and Mrozek (36) in human myotonia congenita, a means of avoiding the repetitive electrical activity associated with microelectrode penetration was necessary in order to make the cable measurements. Tetrodotoxin was selected for this purpose because of its selective blockade of the sodium mechanism involved in the action potential (37, 38). Tetrodotoxin did not alter the cable parameters of myotonic goat fibers (2) and presumably did not significantly alter the cable parameters we are currently reporting.

Because of the tetrodotoxin, and because the cable parameter measurements were complicated enough to occupy full time, we did not systematically study the excitability characteristics of our preparations. However, the myotonic preparations that were studied would probably have exhibited the expected repetitive activity since: (*a*) intra-operative electromyography showed typical "dive-bomber" responses in regions of external intercostal adjacent to the area removed for our studies, (*b*) during the fine dissection, preparations often exhibited a periodic rhythmic "beating," and (*c*) an occasional observation, before adding tetrodotoxin

(TTX), was made of repetitive activity upon microelectrode penetration.

From the high R_m we would predict that, as in myotonic goat muscle (39), there would be a decreased rheobase to electrical stimulation in fibers from patients with myotonia congenita. The high R_m may explain the myotonic response to mechanical stimulation, i.e., percussion myotonia, and may be related to the mechanism of Erb's reaction and the lack of accommodation described in myotonic muscle (40). In both isolated frog (41) and mammalian muscle (42) replacing external chloride with an impermeant anion, presumably decreasing chloride conductance, leads to repetitive activity. It is tempting to speculate that the decreased Cl^- shunt conductance, i.e., the decreased Cl^- permeability, is related to the lack of accommodation, but a satisfactory model has not been developed.

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REFERENCES

1. Lipicky, R. J., and S. H. Bryant. 1966. Sodium, potassium, and chloride fluxes in intercostal muscle from normal goats and goats with hereditary myotonia. *J. Gen. Physiol.* **50**: 89.
2. Bryant, S. H. 1969. Cable properties of external intercostal muscle fibres from myotonic and nonmyotonic goats. *J. Physiol. (London).* **204**: 539.
3. Elmqvist, D., T. R. Johns, and S. Thesleff. 1960. A study of some electrophysiological properties of human intercostal muscle. *J. Physiol. (London).* **154**: 602.
4. Creese, R., J. B. Dillon, J. Marshall, P. B. Sabawala, D. J. Schneider, D. B. Taylor, and D. E. Zinn. 1957. The effect of neuromuscular blocking agents on isolated human intercostal muscles. *J. Pharmacol. Exp. Ther.* **119**: 485.
5. Freygang, W. H., Jr., I. S. Rapoport, and L. D. Peachey. 1967. Some relations between changes in the linear electrical properties of striated muscle fibers and changes in ultrastructure. *J. Gen. Physiol.* **50**: 2437.
6. Nichols, B. L., C. F. Hazelwood, and D. J. Barnes. 1968. Percutaneous needle biopsy of quadriceps muscle: potassium analysis in normal children. *J. Pediat.* **72**: 840.
7. Huxley, A. F. 1960. Compartmental analysis. *In* Mineral Metabolism. C. L. Comar and F. Bronner, editors. Academic Press, Inc., New York. 1 (Pt. A, Appendix 2): 163.
8. Creese, R. 1968. Sodium fluxes in diaphragm muscle and the effects of insulin and serum proteins. *J. Physiol. (London).* **197**: 255.
9. Hakansson, C. H. 1956. Conduction velocity and amplitude of the action potential as related to circumference in the isolated fibre of frog muscle. *Acta Physiol. Scand.* **37**: 14.
10. Maas, O., and A. S. Paterson. 1950. Myotonia congenita, dystrophia myotonica and paramyotonia; reaffirmation of their identity. *Brain.* **73**: 318.
11. Caughey, J. E. 1968. Transitional forms between myotonia congenita, dystrophia myotonica and paramyotonia congenita. *N. Z. Med. J.* **67**: 347.
12. Dillon, J., J. Fields, T. Gumas, D. J. Jenden, and D. B. Taylor. 1955. An isolated human voluntary muscle preparation. *Proc. Soc. Exp. Biol. Med.* **90**: 409.
13. Hofmann, W. W., W. Alston, and G. Rowe. 1966. A study of individual neuro-muscular junctions in myotonia. *Electroencephalogr. Clin. Neurophysiol.* **21**: 521.
14. Ludin, H. P. 1969. Microelectrode study of normal human skeletal muscle. *Eur. Neurol.* **2**: 340.
15. Falk, G., and P. Fatt. 1964. Linear electrical properties of striated muscle fibres observed with intracellular electrodes. *Proc. Roy. Soc. Ser. B.* **160**: 69.
16. Gage, P. W., and R. S. Eisenberg. 1969. Capacitance of the surface and transverse tubular membrane of frog sartorius muscle fibers. *J. Gen. Physiol.* **53**: 265.
17. Falk, G. 1968. Predicted delays in the activation of the contractile system. *Biophys. J.* **8**: 608.
18. Pugsley, I. D. 1966. Contribution of the transverse tubular system to the membrane capacitance of striated muscle of the toad (*Bufo marinus*). *Aust. J. Exp. Biol. Med. Sci.* **44**: 9.
19. McComas, A. J., K. Mrozek, D. Gardner-Medwin, and W. H. Stanton. 1968. Electrical properties of muscle fibre membranes in man. *J. Neurol. Neurosurg. Psychiat.* **31**: 434.
20. Boyd, I. A., and A. R. Martin. 1959. Membrane constants of mammalian muscle fibres. *J. Physiol. (London).* **147**: 450.
21. Kiyohara, T., and M. Sato. 1967. Membrane constants of red and white muscle fibers in the rat. *Jap. J. Physiol.* **17**: 720.
22. Albuquerque, E. X., and S. Thesleff. 1968. A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscles of the rat. *Acta Physiol. Scand.* **73**: 471.
23. Fatt, P. 1964. An analysis of the transverse electrical impedance of striated muscle. *Proc. Roy. Soc. Ser. B.* **159**: 606.
24. Kushmerick, M. J., and R. J. Podolsky. 1969. Ionic mobility in muscle cells. *Science (Washington).* **166**: 1297.
25. Becker, P. E. 1961. Heterogeneity of myotonia. *Proc. Int. Congr. Hum. Genet. 2nd. 1961.* 79.
26. Kuhn, E., and V. Schneider. 1968. Dickdarmanomalien bei autosomal rezessiv vererbter myotonia congenita. *Schweiz. Med. Wochenschr.* **98**: 114.
27. Riecker, G., H. Dobbelstein, D. Rohl, and H. D. Bolte. 1964. Messungen des membranpotentials einzelner quergestreifter muskelzellen bei myotonia congenita (Thomsen). *Klin. Wochenschr.* **42**: 519.
28. Page, E. 1962. Cat heart muscle *in vitro* III. The extracellular space. *J. Gen. Physiol.* **46**: 201.
29. Ling, G. N., and M. H. Kromash. 1967. The extracellular space of voluntary muscle tissues. *J. Gen Physiol.* **50**: 677.
30. Bohr, D. F. 1964. Electrolytes and smooth muscle contraction. *Pharmacol. Rev.* **16**: 85.

31. Adrian, R. H. 1961. Internal chloride concentration and chloride efflux of frog muscle. *J. Physiol. (London).* **156**: 623.
32. Burr, L. H., and H. McLennan. 1960. The apparent extracellular space of mammalian skeletal muscle: a comparison of the inulin space in normal and dystrophic mouse tissues. *Can. J. Biochem. Physiol.* **38**: 829.
33. Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (London).* **108**: 37.
34. Katz, B. 1966. Nerve, Muscle and Synapse. McGraw-Hill Book Company, New York.
35. Norris, F. H., Jr. 1962. Unstable membrane potential in human myotonic muscle. *Electroencephalogr. Clin. Neurophysiol.* **14**: 197.
36. McComas, A. J., and K. Mrozek. 1968. The electrical properties of muscle fibre membranes in dystrophia myotonica and myotonia congenita. *J. Neurol. Neurosurg. Psychiat.* **31**: 441.
37. Moore, J. W., and T. Narahashi. 1967. Tetrodotoxin's highly selective blockage of an ionic channel. *Fed. Proc.* **26**: 1655.
38. Narahashi, T., T. Deguchi, N. Urakawa, and Y. Ohkubo. 1960. Stabilization and rectification of muscle fiber membrane by tetrodotoxin. *Amer. J. Physiol.* **198**: 934.
39. Herzog, W. H. 1962. Studies on the mechanism of repetitive firing in a muscle disease, myotonia congenita, and in the squid giant axon induced by a drug, aconitine. Ph.D. thesis, University of Cincinnati, Cincinnati, Ohio 45219.
40. Bryant, S. H., R. J. Lipicky, and W. H. Herzog. 1968. Variability of myotonic signs in myotonic goats. *Amer. J. Vet. Res.* **29**: 2371.
41. Falk G., and J. F. Landa. 1960. Effects of potassium on frog skeletal muscle in a chloride-deficient medium. *Amer. J. Physiol.* **198**: 1225.
42. Lüllmann, H. 1961. Das verhalten normaler und denervierter skeletmuskulatur in chloridfreiem medium (Methylsulfat-Tyrodelösung). *Arch. Exp. Pathol. Pharmakol.* **240**: 351.