The Effect of Variations in the Intra- and Extracellular Ion Concentrations upon the Electrical Activity of Normal and Dystrophic Mouse Skeletal Muscle

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ABSTRACT The resting and action potentials of the m. gastrocnemii of normal and dystrophic mice have been measured *in vitro*, under various conditions of the ionic environment. The observed effects are consistent with the view that, when equilibrium is established between internal and external ionic concentrations, the resting potential is determined very largely, and perhaps entirely, by the gradient of K ions. Action potentials are associated with a greatly increased Na conductance in this as in other excitable tissues. No differences in electrical activity between normal and dystrophic muscle cells could be established.

INTRODUCTION

It is now widely accepted that the potential difference which exists at rest between the interior of a muscle cell and its external environment is largely that of a potassium concentration cell. The potential predicted by the Nernst equation agrees well with observed values at higher external K concentrations than are encountered physiologically: at lower K levels the predicted and measured potentials may deviate from one another, due to the contribution of the sodium and/or chloride ion ratio to the potential. Observations of this type were originally made for frog muscle by Ling and Gerard (1950), and have been extended by others to many tissues. The applicability of the concept to toad muscle has, however, been questioned by Shaw, Simon, Johnstone, and Holman (1956) and by Shaw, Simon, and Johnstone (1956).

Considerably less work of this nature has been carried out on mammalian muscle. Muscholl (1957), Creese, Scholes, and Whalen (1958), and Lüllmann (1958) have all studied rat diaphragm *in vitro*. Muscholl reported average values for the resting potential of 85.9 mv and 122 mv for the action potential when the tissue was bathed in 2.7 mM K; Creese *et al.* found resting potentials

of 70 to 80 mv in 5 mM K; and Lüllmann reported an average of 88 mv in 2.7 mM K.

Trautwein, Zink, and Kayser (1953) measured resting and action potentials in the muscles (mostly m. sartorius) of cats and guinea pigs *in situ*: their results average respectively 79.5 and 116 mv for cats and 84.5 and 121 mv for guinea pigs. Pillat, Kraupp, Giebisch, and Stormann (1958) perfused the legs of cats with media in which the K concentration was varied, and recorded potentials from the fibers of m. gracilis. They reported that at 3.8 mM K the average resting potential was 90 mv and that increases in the external K concentration reduced the potential approximately in accordance with the Nernst equation. Zierler (1959) reported an average value of 74 mv for the resting potential of rat muscle *in vitro* in 4.7 mM K; and Conrad and Glaser (1959) values of 71.2 and 70.2 mv for normal and dystrophic mouse muscles at *ca.* 25°C and in 5.5 mM K.

While these various reports are reasonably consistent, in that higher external K concentrations tend to yield lower resting potentials, there is little evidence to show whether or not the potential can be predicted accurately from a knowledge only of the internal/external K ratio. In the present study resting and action potentials have been recorded from mouse muscle cells *in vitro* under conditions in which the K and Na concentrations of the bathing medium have been changed, and after sufficient time had elapsed for new ionic equilibria to have become established. The results indicate that the resting potential *in vivo* is likely entirely to be attributed to the distribution of K. Comparison of normal and dystrophic muscles failed to reveal any differences.

METHODS

Normal and dystrophic mice of the Bar Harbor 129 strain were killed by cervical fracture. One leg was skinned and detached from the body by cutting well up on the thigh. In some experiments the sciatic nerve was dissected free in the thigh region and placed on electrodes for stimulation; in others the muscle was stimulated directly. Since no differences in the recorded action potentials evoked by these two methods were observed, the results have been combined in this paper.

The leg was arranged in a shallow dish and fixed with pins through the thigh muscles and foot so that m. gastrocnemius was uppermost. A saline medium warmed so that the temperature in the neighborhood of the muscle was kept at 36°C was allowed to flow through the dish at a rate of 15 ml/min. The superficial aspect of the muscle was covered by a layer of fluid about 1 mm deep, and 1½ hours was allowed to elapse before any measurements were made in order for new ionic equilibria to become established. Records of the resting and action potentials of the fibers lying within 200 μ of the surface were made with glass microelectrodes filled with 2.5 M KCl solution. Maximal square wave stimuli were applied at a rate of 1/sec.

The media used were variants upon one which contained, in millimoles/liter: Na 144, K 6, Ca 2, Mg 1, Cl 134, HCO_3 20, SO_4 1, glucose 10, and all were equilibrated

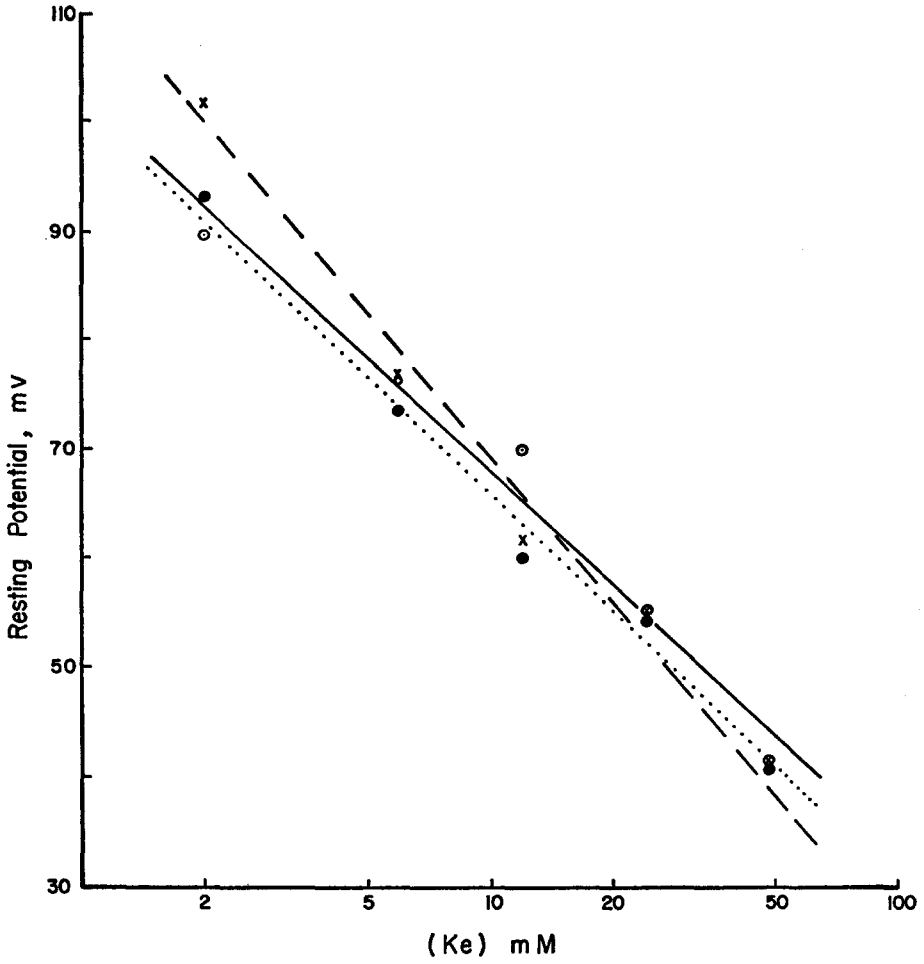

FIGURE 1. The variation of the resting potential of normal mouse skeletal muscle cells with alteration of the environmental potassium ion concentration (K_e).

o——o, measured values.

×---×, calculated from $E_r = \frac{RT}{F} \ln \frac{(K_i)}{(K_e)}$

●.....●, calculated from $E_r = \frac{RT}{F} \ln \frac{(K_i) + 0.005\ (Na_i)}{(K_e) + 0.005\ (Na_e)}$.

The values for K_i and Na_i are for the intracellular concentrations at the end of 2 hours' incubation in the various media.

with 95 per cent O_2–5 per cent CO_2. Changes in K concentration were balanced by alterations in Na to preserve isotonicity; Na removed was replaced by equivalent choline, but the medium with 288 mM Na was hypertonic.

Measurements of the concentrations of Na and K in the muscles were made with a

flame photometer on nitric acid digests, and are expressed in this paper as millimoles cation/liter of intracellular fluid. Values for the volumes of extracellular space were taken from Burr and McLennan (1960), and some figures for tissue cation contents are from Burr and McLennan (1961). The adequacy of oxygenation of the tissue was inferred from the observation that the cation contents were unchanged after 2 hours' incubation in the basic medium when compared to fresh material.

TABLE I

THE EFFECT OF CHANGES IN EXTERNAL K CONCENTRATION (K_e) ON MEASURED AND CALCULATED RESTING POTENTIALS, AND THE K AND NA CONTENTS AFTER 2 HOURS' INCUBATION, OF NORMAL MOUSE SKELETAL MUSCLE CELLS

(Number of determinations in parentheses. Experimental values ± standard error of the mean.)

K_e	Na_e	K_i	Na_i	Measured resting potentials	Calculated potentials from equation (1)	(2)
mM	mM	mM	mM	mv	mv	mv
2	148	99.2 ± 1.9 (10)	36.4 ± 2.4 (8)	89.5 ± 1.5 (58)	101.8	93.0
6	144	113.3 ± 1.1 (12)	22.5 ± 1.1 (11)	76.3 ± 0.6 (119)	76.5	73.5
12	138	126.3 ± 1.3 (7)	7.4 ± 1.8 (6)	69.9 ± 0.9 (37)	61.5	60.0
24	126	198.5 ± 2.0 (6)	~0	55.3 ± 1.3 (26)	55.1	54.3
48	102	233.5 ± 2.1 (6)	~0	41.5 ± 1.0 (30)	41.4	40.9

RESULTS

Resting Potentials

The effect upon the measured resting potentials of normal mouse muscle fibers of changes in the K concentration (K_e) of the bathing medium is shown in Fig. 1. The observed values fall about a line whose slope is −34.4 mv/tenfold increase in K_e. The dashed line in the figure is that of best fit for the potentials calculated from the Nernst equation:

$$E_r = \frac{RT}{F} \ln \frac{(K_i)}{(K_e)} \tag{1}$$

(slope −43.7 mv/tenfold change in K_e); the dotted line from:

$$E_r = \frac{RT}{F} \ln \frac{(K_i) + 0.005\ (Na_i)}{(K_e) + 0.005\ (Na_e)} \tag{2}$$

(slope −35.6 mv/tenfold change in K_e). The appropriate cation concentrations in the intracellular fluid of the muscles are shown in Table I, together with further details of the potential measurements. It should be emphasized

that changes in K_e are reflected by alterations in the internal concentrations of both Na and K, and that the values for Na_i and K_i used here are those found after 2 hours' incubation in the various media. All potential measurements were made in the period between 1½ and 2 hours of incubation.

The fit of the experimentally observed values by either equation (1) or (2) is reasonably good, the introduction of the small term for Na yielding a somewhat better result. This is confirmed by the observations set forth in Table II, where the resting potentials measured in solutions in which Na_e was varied are given. These results are well fitted by values calculated from equation (2). It is quite possible that the introduction of another term involving the chloride ion concentration ratio into equation (2) would further improve the agreement between observed and calculated values.

TABLE II

THE EFFECT OF CHANGES IN EXTERNAL NA CONCENTRATION ON RESTING POTENTIALS AND CATION CONTENTS OF NORMAL MUSCLES

(Number of determinations in parentheses. Experimental values ± S.E. of the mean.)

Na_e	K_e	Na_i	K_i	Measured resting potentials	Calculated potentials from equation (1)	(2)
mM	*mM*	*mM*	*mM*	*mv*	*mv*	*mv*
18	6	20.6 ± 0.7 (8)	114.0 ± 1.6 (8)	76.6 ± 1.2 (35)	76.8	76.4
144	6	22.5	113.5	76.3	76.5	73.5*
288	6	46.5 ± 0.7 (6)	106.5 ± 1.1 (6)	71.1 ± 1.0 (56)	75.0	69.3

* See Table I.

Measurements of resting potentials in muscle cells from dystrophic mice have also been made, and the results are shown in Table III. In no instance is there a significant difference from the normal in the reaction of these cells to changes in their environment.

Action Potentials

Table IV shows the effects of variations in K_e and Na_e upon the amplitude of observed action potentials in normal cells. Four solutions only were available, those with 2, 6, and 12 mM K and that with 288 mM Na. In solutions of higher K_e and in that with 18 mM Na the muscles were inexcitable, and occasional action potentials only could be recorded in 12 mM K.

As in many other tissues, the total amplitude of the action potential exceeds that of the resting potential, and the amplitude of this "overshoot" is little affected by changes in K_e. This is in accordance with the current conception

that excitation of muscle is associated with a large increase in inward movement of Na, and that the peak of the action potential tends towards the equilibrium potential for this ion. The experimentally observed overshoot potentials for these muscles can be approximately described, in the media

TABLE III

THE EFFECT OF CHANGES IN THEIR IONIC ENVIRONMENT ON THE RESTING POTENTIALS OF DYSTROPHIC MUSCLE CELLS

(Number of determinations in parentheses. Average values ± S.E. of the mean.)

K_e	Na_e	Measured resting potentials
mM	*mM*	*mv*
2	148	89.3 ± 1.2 (15)
6	144	74.0 ± 1.0 (40)
12	138	69.0 ± 1.4 (27)
6	18	82.5 ± 1.2 (20)
6	288	67.5 ± 0.9 (21)

TABLE IV

THE EFFECT OF CHANGES IN THEIR IONIC ENVIRONMENT ON THE ACTION POTENTIALS OF NORMAL MUSCLE CELLS

(Number of determinations in parentheses. Average values ± S.E. of the mean.)

K_e	Na_e	Average* resting potential	Measured action potentials	Average overshoot potential	Calculated potential from equation (3)
mM	*mM*	*mv*	*mv*	*mv*	*mv*
2	148	89.5	100.5 ± 1.8 (50)	11.0	9.9
6	144	76.3	87.3 ± 1.3 (80)	11.0	9.1
12	138	69.9	79.2 ± 1.6 (22)	9.3	13.8
6	288	71.1	90.6 ± 1.7 (36)	19.5	24.5

* From Tables I and II.

used, by the equation:

$$E_0 = \frac{RT}{F} \ln \frac{(K_e) + 1.5\,(Na_e)}{(K_i) + 1.5\,(Na_i)} \qquad (3)$$

indicating that an increase in Na movement of at least 300-fold (relative to K) over the resting state has occurred. Using equations (3.0) and (6.0) of Hodgkin and Katz (1949), it can be estimated that the membrane conductance has increased 85-fold at the peak of the action potential, assuming that the change has been produced entirely by increased Na movement.

No alterations in the action potentials recorded from dystrophic muscle cells could be discerned.

DISCUSSION

The results presented here show that, in mammalian muscle cells *in vitro*, the resting potential depends almost entirely upon the difference in K concentration between inside and outside, and that alteration of the external environment produces the expected effect when calculations based on new internal/external ratios are made. The fit between observed and calculated values is improved if a small term representing the contribution of Na distribution to the potential is added. However, if evidence from experiments on squid axons, disussed by Moore and Cole (1960), is applicable here, it seems likely that *in vivo* the resting potential is identical with the potassium equilibrium potential and that slightly lower values are measured *in vitro*. This suggestion is supported by the findings of Pillat *et al.* (1958), who perfused cats' legs with diluted blood and found complete agreement between the resting potential and the K content of the perfusing fluid. The present results are in accord with those obtained by others (see Introduction) on a variety of mammalian skeletal muscles.

The failure of Shaw and his coworkers (1956) to find a positive correlation between the ionic gradients after equilibration of toad muscles in various solutions and resting and action potentials is at variance with the present results, as with those of others. Lüllmann (1958) has also noted that, in rat diaphragm, resting potentials may be maintained unchanged for a number of hours, whereas the total tissue K declines and Na rises during the course of incubation. It is possible, however, that in his experiments measurement of total tissue K does not reflect the situation holding in the cells near the surface of the muscle from which the potential measurements were made. This situation has been discussed in another connection by Creese *et al.* (1958). Shaw *et al.* have also studied only the superficial layers of fibers for their electrical responses.

Conrad and Glaser (1959) have briefly reported on the effects of changes in the ionic environment on the electrical activity of normal and dystrophic mouse muscles. As in the present work, the differences described are minor, although these authors do state that the increase in resting potential produced by a reduction in K_o from 5.5 to 2.75 mM was larger in the dystrophic tissues. No indication of the statistical significance of this observation is given, however. In the present study no significant differences were found. That the responses of individual fibers in dystrophic muscles appear normal is not surprising, however, for it is well known that the number of fibers actually undergoing degenerative changes at any time is small. Those which have be-

come completely degenerate will not show any electrical activity, and hence will not be recognized. It is noteworthy that it is more difficult to locate a fiber in the diseased muscles, which must reflect the smaller number of functioning cells per unit of muscle mass. It is to be noted, too, that the observed potentials are unaffected in spite of the increased rate of K exchange which takes place in the dystrophic tissue (Burr and McLennan, 1961). This also is an expected finding (see *e.g.* Ussing, 1949).

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