Potassium Exchange and Afterpotentials in Frog Sartorius Muscles Treated with Glycerol

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ABSTRACT The potassium exchange properties of glycerol-treated sartorius muscles of the frog were determined. Potassium (^{42}K) uptake, efflux, and net flux were measured in the presence of glycerol and at various times after exposure to glycerol and return to isotonic Ringer solution. Potassium uptake was not altered by the presence of glycerol but was reduced on the average 53% after glycerol treatment. Efflux transiently increased in the presence of glycerol and was reduced 37% after glycerol removal. Consequently, there was a net loss of intracellular potassium as well as a gain of sodium. In contrast to the irreversible alterations of potassium exchange induced by glycerol treatment, action potentials with normal negative afterpotentials (N.A.P.) were elicited 4–5 hr after glycerol removal. The reappearance of the N.A.P. was associated with a return of the membrane potential to normal values (90 ± 2 mv). However, the response of these muscles to reduced extracellular potassium was anomalous. In K^+-free Ringer solution the average resting membrane potential was 74 ± 3 mv and a positive afterpotential of 11 ± 3 mv was associated with the action potential.

INTRODUCTION

The membrane of frog striated muscle cells has been shown to exhibit anomalous or asymmetric properties. Katz (1949), Hodgkin and Horowicz (1959 *b*), and Adrian and Freygang (1962 *a,b*) have shown that there is a fall in membrane conductance for potassium for outward membrane current and a rise in the conductance for inward current. This rectification was in the direction opposite to that predicted by the constant-field equation for a muscle with constant potassium and chloride permeability and was called anomalous rectification.

Hodgkin and Horowicz (1960) reported that the change in membrane potential of single muscle fibers produced by a decrease in the potassium concentration of the bathing fluid was significantly slower than the change pro-

duced by a rise in the potassium concentration. The asymmetry between the on-and-off effects, observed in the presence or absence of chloride, could not be explained by the logarithmic relation between potential and concentration. They postulated that the asymmetry was due to the existence of a "special region" which would retain potassium ions for a short time.

Adrian (1961) studied KCl movements in muscle when the concentration in the surrounding medium had been changed and found that the equilibrium values for the intracellular potassium and chloride concentrations could be predicted by a Donnan system according to Boyle and Conway (1941), but the rate of change of inward movement of KCl was faster than for outward movement.

Freygang, Goldstein, and Hellam (1964) and Freygang, Goldstein, and Peachey (1964) found that the negative afterpotential that follows a train of impulses in frog muscle fibers decays more slowly than the afterpotential that follows a single impulse. This "late afterpotential" is prolonged in hypertonic and low chloride solutions but shortened in potassium-free solution. These findings and the phenomena of anomalous rectification were explained in terms of the theoretical three-compartment model of Adrian and Freygang (1962 *a,b*). The model consists of three membrane-bound compartments: compartment (1), the extracellular fluid; compartment (3), the sarcoplasm; and compartment (2), the intermediary space which may be the middle part of the triads of the sarcoplasmic reticulum. It was assumed that the membrane between compartments (1) and (2) was permeable only to potassium and sodium and that this membrane as well as the one between compartments (2) and (3) was relatively impermeable to anions. Chloride movement was restricted to the membrane between compartments (1) and (3). The membrane between compartments (2) and (3) was considered to be permeable only to potassium.

Although this model adequately described some of the asymmetric behavior of the muscle membrane, it may require some modification in view of some discrepancies which have been found. It has been reported that the central element of the triad is permeable to sulfate ions and sucrose (Adrian, 1964) and to ferritin (Huxley, 1964). Several fluorescent dyes have access to some component of the triads and the "dye space" has been estimated to be 1–2% of the fiber volume (Endo, 1966). Therefore, the intermediary space may also be permeable to chloride ions. However, it has recently been proposed that the lumen of the transverse tubular system is lined with fixed negative charges (Rapoport, Peachey, and Goldstein, 1969; Rapoport, 1969). Chloride ions might then be restricted on the basis of electrostatic repulsion.

In order to fully assess the model in terms of the known skeletal muscle structure, the permeability properties of each of the membranes should be determined. It has not, however, been possible to fully differentiate between

the transport properties of the various membranes of skeletal muscle. Recently, a method has become available which selectively disrupts the transverse tubular system in frog sartorius muscles (Howell and Jenden, 1967; Eisenberg and Eisenberg, 1968; Howell, 1969). In this method, originally described by Fujino, Yamaguchi, and Suzuki (1961), muscles were first exposed to Ringer solution made hypertonic with glycerol for 1 hr and subsequently returned to isotonic Ringer solution. Sartorius muscle treated in this manner lost the ability to develop twitch and contracture tension associated with elevated potassium. The resting membrane potential of glycerol-treated muscles was lower than that of normal muscles and both the negative afterpotential associated with a muscle action potential and the late negative afterpotential following a train of impulses were absent (Gage and Eisenberg, 1969 *b*). Eisenberg and Gage (1969) have also been able to differentiate between the individual ionic conductances with this preparation and have found that potassium conductance of the surface membrane is 28 μmhos/cm^2 while that of the tubular membrane is 55 μmhos/cm^2.

In the present investigation an attempt was made to differentiate between the exchange of potassium ions across cell surface membrane and the exchange through the tubular system. When the T tubules are disrupted by treatment with glycerol, potassium influx, efflux, and net flux should be restricted to the surface membrane. Therefore, comparison of flux measurements of glycerol-treated muscles with those of intact muscles should yield some insight into the potassium exchange properties of the muscle cell membranes.

METHODS

Specific methods were similar to those described by Sjodin and Henderson (1964). The sartorius muscle of the frog, *Rana pipiens*, was used throughout. The normal Ringer solution was composed of (mM): NaCl 105, KCl 2.5, $CaCl_2$ 2.0, tris (hydroxymethyl) aminomethane (Tris) 1.0. In addition to the above ions, glycerol Ringer solution contained 400 mM glycerol, unless otherwise specified. The Tris buffer maintained the pH of the Ringer solution at 7.4. All experiments were carried out at room temperature (20–22°C) unless otherwise specified.

Carefully dissected muscles were mounted on platinum wire frames and uptake and efflux of radioactive potassium (^{42}K) measured using a Packard Auto-Gamma spectrometer with a well-type detector. Uptake and efflux of ^{42}K were measured prior to exposure to glycerol, in the presence of glycerol, and after glycerol treatment. Specific experiments will be described where appropriate in the text.

At the termination of all experiments the muscles were removed from the platinum frames, blotted gently on filter paper, and wet weight determined in tared platinum crucibles. The muscles were then dried in an oven at 120°C for 1 hr and reweighed. Finally, the muscles in platinum crucibles were ashed for 10 hr at 550°C.

The ash was diluted to a volume of 10 ml by the addition of deionized water and analyzed for potassium and sodium content by flame photometry.

Resting and action potential measurements were made with microelectrodes pulled from 2 mm kimax tubing with a Nastuk type puller (Alexander and Nastuk, 1953). Microelectrodes with low tip potentials and resistance between 5 and 10 megohms were connected by means of a chlorided silver wire to a high impedance neutralized input capacity amplifier. Action potentials were displayed on a Tektronix 502A oscilloscope and representative action potentials recorded on Polaroid film with a Tektronix C-12 camera.

RESULTS

The Influence of Glycerol on Potassium (^{42}K) Uptake and Efflux

In a typical uptake-efflux experiment muscles were placed in 25 ml of Ringer solution containing ^{42}K for a period determined on the basis of the desired per cent of tracer equilibration. The muscles were removed from the isotope solution and counted for 1 min at specified intervals during the uptake period. At the termination of uptake, the muscles were placed in a series of tubes containing 5 ml of normal isotope-free Ringer solution. In this way, efflux of ^{42}K could be monitored for any desired interval and modifications of the Ringer solution could be made as desired.

The following types of experiments were carried out to determine the influence of glycerol on the uptake and efflux of potassium.

A. Pretreatment of muscles with glycerol followed by uptake and efflux in normal Ringer solution.
B. Uptake of ^{42}K in the presence of glycerol followed by efflux in normal Ringer solution.
C. Uptake of ^{42}K in normal Ringer solution followed by exposure to glycerol in some period of efflux.

An experiment using two sartorius muscles from the same frog and combining methods B and C is presented in Fig. 1. Uptake of potassium in micromoles per gram is presented in the upper half of the figure. The open circles represent the potassium uptake of one muscle in the presence of 400 mM glycerol and the solid circles represent the uptake of the other muscle in normal 2.5 mM potassium Ringer solution. There is very little change in the rate of uptake in the presence of glycerol. The results obtained in additional experiments of this type are presented in column 3 of Table I. Comparison of the mean value determined for influx of potassium in the presence of glycerol (column 3) with that for influx in normal 2.5 mM potassium Ringer solution by the usual t test indicated that there is no significant difference between the two means ($0.4 < P < 0.3$).

The rate constants for efflux of the same pair of muscles are shown in the lower half of Fig. 1. The rate constant of the muscle previously exposed to glycerol (open circles) was 50% lower than its mate (solid circles) during the 1st hr of efflux. This reduced rate of efflux persisted throughout the 4 hr period

of measurement. The other member of the pair was exposed to glycerol only during the period marked by the arrows. While in glycerol there was a transient increase in the rate constant during the first 15 min of exposure which reversed before the end of the 1 hr exposure. The final 2 hr of efflux was carried out in normal Ringer solution. There was again a 50% reduction in the

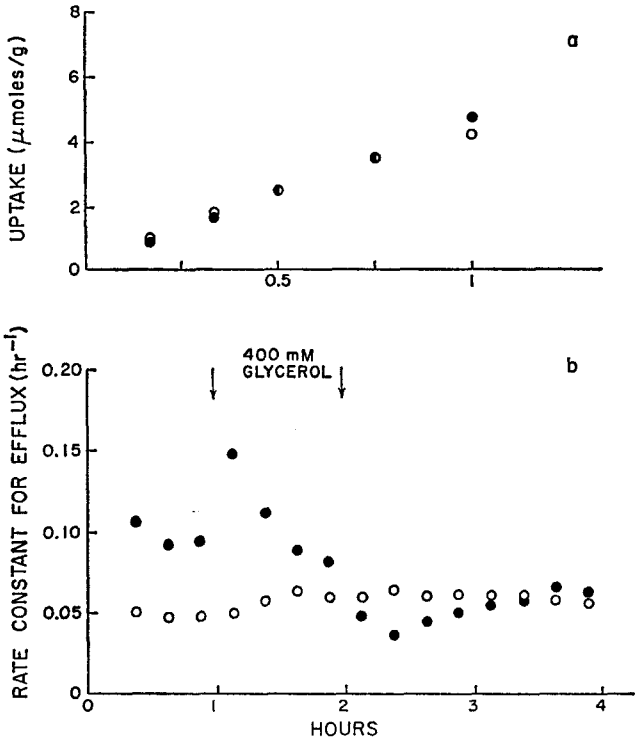

FIGURE 1. The influence of 400 mM glycerol on potassium (^{42}K) uptake and efflux is illustrated. a, the (^{42}K) uptake of a pair of muscles from the same animal in 2.5 mM potassium Ringer solution, (○) plus 400 mM glycerol. b, the rate constants for efflux (hr^{-1}) of the same pair of muscles measured in 2.5 mM potassium Ringer solution. Glycerol (400 mM) was added to the efflux solution of the muscle represented by (solid circles) during the period indicated by the arrows.

rate constant for potassium efflux, which was maintained for the final 2 hr efflux period.

Several efflux experiments of the type represented by the muscle depicted in Fig. 1 (solid circles) are summarized in Table II. The values in column 2 are average rate constants determined from the 1st hr of efflux in 2.5 mM potassium Ringer solution. The "in glycerol" value represents the rate constant determined during the first 15 min of exposure to glycerol. All of the muscles listed in Table II were exposed to 400 mM glycerol Ringer solution

TABLE I

THE EFFECT OF 400 mM GLYCEROL ON THE UPTAKE OF POTASSIUM

Muscle No.	Influx		
	Preglycerol	In glycerol	Postglycerol
	$\mu M/g\text{-}hr$	$\mu M/g\text{-}hr$	$\mu M/g\text{-}hr$
623AH9	10.4		
623A'H9	10.4	11.4	
623BH9	9.4	8.8	
623B'H9	9.3		
623CH9	8.6	8.6	
623C'H9	8.0		
624AH9	9.8	9.0	
624A'H9	9.0	6.8	
624BH9	9.9	8.1	
624B'H9	8.6		
71AH9			2.4
71A'H9	7.0		
71BH9			3.7
71B'H9	7.9		
17AH0	8.4		
17A'H0			4.3
113AM0	8.0		
113A'M0		7.9	3.9
113BM0	9.5		
113B'M0		7.4	3.2
114AM0	11.6		
114A'M0		8.9	2.8
114BM0	11.6		
114B'M0		9.3	4.4
430AH9	6.0		
430A'H9		5.6	
430BH9		7.5	
430B'H9			4.0
429CD9	7.8		
429C'D9			5.5
429DD9			7.1
429D'D9		9.5	
55AH9	7.67		
55A'H9			4.5
55BH9	7.4		
55B'H9			5.85
Mean (±SD)	8.9 ± 1.4	8.4 ± 1.4	4.2 ± 1.2

for 1 hr. The last column in Table II contains the average rate constant determined after glycerol treatment in glycerol-free Ringer solution. The period of postglycerol efflux varied from 1 to 4 hr. Statistical analysis by means of the t test indicated that the mean values of all three columns were significantly different from one another ($P < 0.01$).

The fourth column of Table I contains influx measurements obtained from muscles which were either pretreated with glycerol before the beginning of the uptake experiment or exposed to glycerol during the initial part of the uptake measurement and subsequently returned to Ringer solution. Thus, they are all influx measurements in normal 2.5 mM potassium Ringer solution postglycerol. The mean of these values was significantly different from the mean of the influx measurements in normal Ringer solution and the mean of the measurements made in the presence of glycerol ($P < 0.01$).

An experiment of this type is presented in Fig. 2 a. The muscle represented by the open circles was exposed to 400 mM glycerol Ringer solution for 1 hr

TABLE II

THE EFFECT OF 400 mM GLYCEROL ON THE EFFLUX RATE CONSTANT OF ^{42}K

Muscle No.	Rate constant for ^{42}K efflux		
	Preglycerol	In glycerol	Postglycerol
	hr^{-1}	hr^{-1}	hr^{-1}
423A'H9	0.134	0.193	0.069
423BH9	0.100	0.153	0.057
423B'H9	0.101	0.151	0.075
429AH9	0.094	0.147	0.060
623B'H9	0.145	0.228	0.090
623C'H9	0.118	0.175	0.065
624B'H9	0.112	0.163	0.066
129AH0	0.096	0.152	0.060
129A'H0	0.102	0.177	0.062
25AM0	0.105	0.180	0.071
25BMO	0.103	0.166	0.078
25B'M0	0.093	0.143	0.080
Mean (±SD)	0.110 ± 0.017	0.169 ± 0.022	0.069 ± 0.009

and returned to normal Ringer solution for 24 hr before uptake of radioactive potassium was measured. The muscle represented by the solid circles was obtained from the same frog and maintained in normal 2.5 mM potassium Ringer solution for the same period. Influx, determined by the slope of the uptake curve for the 1st hr, was 41% lower after exposure to glycerol (open circles) than that for the control muscle (solid circles). This value compares favorably with the mean reduction of influx following glycerol treatment in Table I (53%).

The rate constants for efflux of the same pair of muscles are presented in Fig. 2 b. The average efflux rate constant of this muscle which was exposed to glycerol 24 hr before uptake was 25% lower than that of the control muscle. The mean value of the efflux rate constants obtained early (between 1 and 4 hr) postglycerol was 38% lower than the mean of the controls (Table II).

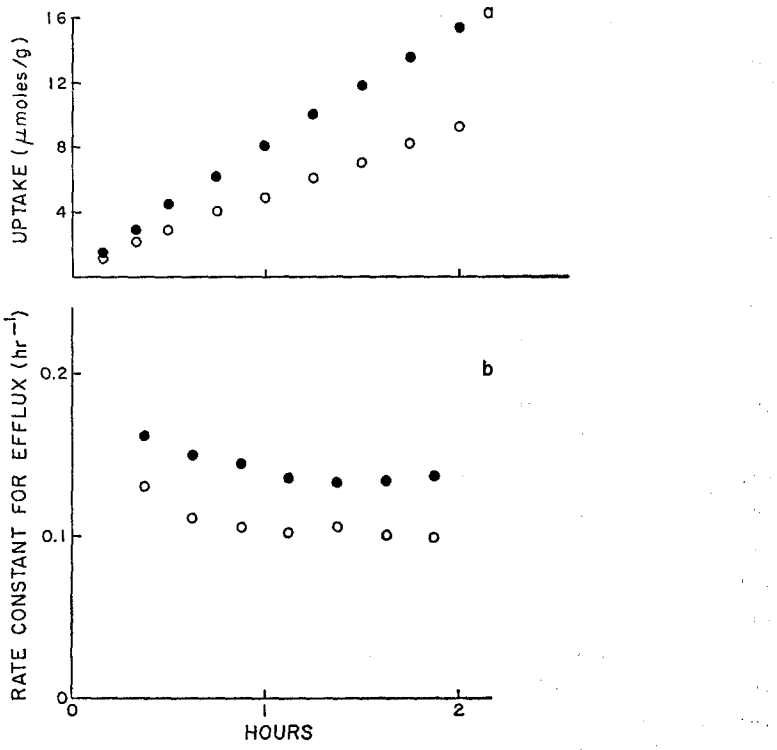

FIGURE 2. Uptake and efflux of ^{42}K measured with a pair of muscles from the same frog. The muscle represented by (open circles) was exposed to Ringer solution plus 400 mM glycerol for 1 hr and returned to isotonic Ringer solution for 24 hr before uptake was measured. The other member of the pair (solid circles) was equilibrated in isotonic Ringer solution for the same period of time before uptake measurements. a, uptake of both muscles in micromoles per gram and b, rate constant for efflux (hr^{-1}).

The main influence of prior exposure to 400 mM glycerol on potassium exchange was a significant reduction in both influx and efflux. The data of Tables I and II suggested that the reduction of influx (average 53%) was greater than the reduction of efflux (average reduction of rate constant 38%). This differential action on the tracer fluxes should result in a net loss of intracellular potassium. Analytical measurements of the potassium content of glycerol-treated muscles indicated that this was the case.

The Influence of Glycerol on the Net Intracellular Distribution of Potassium and Sodium

Measurements were made of the intracellular sodium and potassium contents of all muscles used for tracer experiments, as well as in similar experiments not involving isotopes. In these experiments one muscle of a pair from the same animal was maintained in normal 2.5 mM potassium Ringer solution for the

same period of time that its mate was exposed to glycerol or to glycerol and subsequently to Ringer solution. Several of these experiments are listed in Table III. The first column contains the muscle number. The primed number (e.g. 226A'MO) in each case refers to the muscle of the pair which was treated with the glycerol concentration listed in the second column for 1 hr. At the termination of each experiment all muscles were washed for 10 min in a sodium-, potassium-free Ringer solution (tonicity was maintained with Tris) prior to weighing and ashing. Thus, the extracellular space was cleared of sodium and potassium.

The first four pairs of muscles in Table III were sacrificed immediately after the usual 1 hr exposure to glycerol (primed numbers only). In order to maintain the tonicity of the Tris solution with that of the glycerol Ringer solution, the 10 min Tris wash of these muscles also contained the same glycerol concentration as indicated in column 2. The percentage dry weight (DW) over wet weight (WW) in column four is a measure of the change in water content of the muscles. An increase of this value as compared with the control muscle of the same pair would indicate a net loss of muscle water and a decrease would indicate a net gain of water during the experiment. The values obtained for the control muscles in isotonic Ringer solution were of the same order of magnitude as those previously determined (Adrian, 1960). Adrian obtained a mean value of 21.5%. The mean value of all the control muscles in Table III was 21.0%. Therefore, it is apparent that each of the four muscles exposed to the glycerol Ringer solution for 1 hr lost cell water. The analytically determined amounts of potassium and sodium of the muscle ash are listed in columns 5 and 10, respectively. Columns 6 and 11 contain the intracellular potassium and sodium concentration, respectively, expressed as micromoles per gram of tissue wet weight. These values indicate a concentrating influence of the hypertonic glycerol solution on intracellular potassium and sodium as would be expected. However, the tracer data (Tables I and II) suggested that there should be a net loss of potassium in the presence of glycerol, since efflux was increased while influx remained essentially unchanged.

The values for the intracellular potassium and sodium concentration in columns 8 and 13, respectively, were obtained by assuming that the percentage DW/WW of the muscles exposed to glycerol would have been the same as the control muscles in the absence of glycerol. Since the dry weight was known, it was an easy matter to obtain the wet weight corrected on the basis of the control muscle. When the intracellular potassium was calculated on this basis (column 8) it was suggested that a net loss of potassium had occurred. No consistent change in intracellular sodium was apparent in these four pairs of muscles.

The primed numbered muscles of the next four pairs in Table III (Nos. 210A'MO-211D'MO) were exposed to normal Ringer solution plus the glyc-

TABLE III

THE INFLUENCE OF GLYCEROL ON THE INTRACELLULAR DISTRIBUTION OF K^+ AND Na^+

Muscle No.	Glycerol	Time in Ringer post-glycerol	DW*/WW	Potassium					Sodium				
							Corrected					Corrected	
				μmoles	μmoles/g	mmoles‡/kg f.w.	μmoles/g	mmoles‡/kg f.w.	μmoles	μmoles/g	mmoles‡/kg f.w.	μmoles/g	mmoles‡/kg f.w.
	mM	*hr*											
226AM0			20.6	4.69	88.53	154.13			0.310	5.91	10.28		
226A′-M0	350	0	25.6	4.27	99.63	189.90	80.2	152.87	0.408	9.52	18.14	7.67	14.61
227AM0			19.2	6.40	77.92	123.98			0.714	8.13	13.81		
227A′M0	350	0	22.8	6.44	83.92	151.92	70.8	128.17	0.821	10.70	19.36	9.04	16.38
226BM0			20.2	7.35	84.46	146.04			0.790	9.07	15.68		
226B′M0	450	0	27.5	7.08	105.92	209.72	78.0	154.44	0.837	12.54	24.83	9.23	18.28
227BM0			18.2	4.83	72.82	121.88			0.730	11.02	18.44		
227B′M0	450	0	24.0	4.96	86.07	159.63	65.1	120.74	0.638	11.07	20.53	8.37	15.52
210AM0			21.44	3.84	65.87	122.99			0.451	7.74	14.45		
210A′M0	350	0.25	20.27	4.20	65.06	118.89	72.10	131.70	0.479	7.41	13.53	8.22	15.01
211CM0			21.4	5.04	83.74	156.31			0.631	10.48	19.56		
211C′M0	350	0.25	20.06	4.58	71.28	129.75	76.00	138.30	0.744	11.57	21.06	12.32	22.43
210BM0			23.90	3.32	66.13	129.42			0.486	9.68	18.94		
210B′M0	450	0.25	17.73	2.80	41.73	72.87	55.80	97.45	0.500	7.45	13.01	9.95	17.36
211DM0			23.0	4.29	77.68	149.41			0.827	14.98	28.81		
211D′M0	450	0.25	20.06	3.88	58.46	106.41	70.25	128.00	0.827	12.47	22.70	14.98	27.28
211AM0			19.9	5.40	72.14	130.97			0.659	8.81	15.99		
21A′M0	350	1.0	14.64	5.72	53.71	88.99	76.50	126.74	1.00	9.39	15.56	13.38	22.17
211BM0			19.65	6.24	72.14	130.34			0.741	8.57	15.48		
21B′M0	450	1.0	13.48	5.60	45.23	75.53	64.80	105.21	1.192	9.63	15.66	13.80	22.42
56AH9			19.0	5.05	88.60	152.63			0.290	5.09	8.77		
56A′H9	400	4.0	11.5	4.65	79.08	125.67	81.5	129.45	0.750	12.76	20.28	13.20	21.00
114AM0			21.3	4.70	72.81	151.61			1.128	17.49	36.42		
114A′M0	400	4.0	17.1	3.50	48.38	90.74	54.30	101.90	1.802	24.89	46.68	27.95	52.47
321AD9			20.6	5.6	77.99	143.03			0.90	12.53	22.98		
321A′D9	400	4.0	17.4	4.5	53.13	92.17	62.60	108.86	2.40	28.33	49.15	33.40	58.00
326AD9			21.1	5.96	74.38	126.48			0.24	3.00	5.50		
326A′D9	400	4.0	16.4	4.82	48.20	82.25	61.50	105.00	2.37	23.70	41.44	29.65	50.64
328AH9			25.4	4.91	72.42	145.92			0.68	10.03	20.21		
328A′H9	400	4.0	18.6	3.08	37.47	66.45	44.80	79.46	3.75	45.62	80.91	55.35	98.17

* DW = dry weight; WW = wet weight.

‡ Assuming an extracellular space of 25%, f.w. = fiber water.

erol concentration listed in column 2 for 1 hr followed by 15 min in isotonic Ringer solution. Instead of reversal of the shrinkage which occurred in the presence of glycerol, these muscles were swollen on return to Ringer solution (column 4). This is consistent with the findings of Caputo (1968) who found an increase of fiber volume under similar conditions. The increase of intracellular water should dilute the intracellular sodium and potassium content. This was clearly the case with the muscles previously exposed to 450 mM glycerol, but the swelling and dilution were not as great with the 350 mM glycerol-treated muscles.

The remaining muscles in Table III were returned to isotonic Ringer solution for longer periods of time after the 1 hr exposure to glycerol. Each of these muscles gained significant amounts of water which was not removed by longer soaking periods in isotonic Ringer solution. In addition to the dilution of intracellular potassium, there was also a reduction in the amount of intracellular potassium in each of these muscles. Conversely, each of these muscles gained considerably more sodium than the respective control muscles. Calculations based on the radioactive tracer data predicted that the net efflux of potassium from muscles exposed to 400 mM glycerol should be about 3 μmoles/g-hr greater than from muscles maintained in normal Ringer solution for the same period of time. If the value for intracellular potassium given in column eight (Table III) was subtracted from the respective value of the control muscle in each case and divided by total experimental time, a value for the net flux was obtained. There was reasonable agreement with the net flux determined by tracers in roughly half the experiments which involved washing in isotonic Ringer solution, postglycerol, for an hour or longer. Notable exceptions in Table III were muscles 211A'MO, 56A'H9, and 328A'H9. Possible reasons for these discrepancies will be discussed further in the text.

Anomalous Effects of Glycerol on Potassium Efflux Measurements

In roughly 25% of the experiments involving measurements of the rate constants for efflux of ^{42}K from muscles previously exposed to 400 mM glycerol, there was a transient increase of the rate constant of loss which occurred between 1 and 2 hr after the period of exposure to glycerol. A typical experiment demonstrating this behavior is depicted in Fig. 3. The muscle represented by the open circles in the figure was exposed to 400 mM glycerol during the final hour of uptake immediately preceding the efflux measurement, while its mate (solid circles) was used for an additional hour of uptake measurements in isotonic Ringer solution before efflux began. There was approximately a doubling of the efflux rate constant of each muscle approximately 1 hr after exposure to glycerol and a subsequent decline to normal values within 3 hrs. Muscles which exhibited this type of response had a considerably greater net loss of intracellular potassium. Potassium uptake was never influenced in this way by

glycerol. It was difficult to elucidate the precise mechanism for this anomalous effect, but a causal relationship with the concentration of glycerol used has been found.

The rate constants for ^{42}K efflux of two muscles from the same frog exposed to different concentrations of glycerol are shown in Fig. 4. The 1st hour of efflux was in Ringer solution. For the period indicated by the arrows the muscle represented by the solid circles was exposed to Ringer solution plus

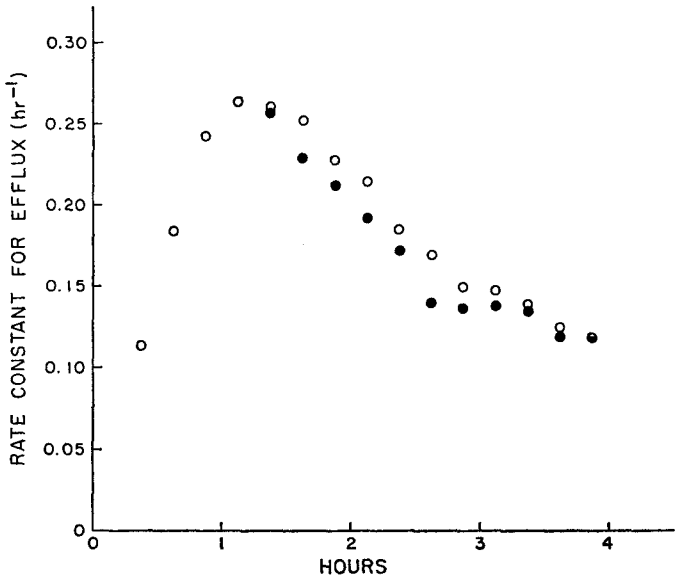

FIGURE 3. Rate constant for ^{42}K efflux of a pair of muscles from the same frog. The muscle represented by (open circles) was exposed to Ringer solution plus 400 mM glycerol during the final hour of uptake, while its mate (solid circles) was maintained in isotonic Ringer solution for an additional hour of uptake measurement before efflux monitoring began.

450 mM glycerol and the muscle represented by the open circles was exposed to Ringer solution plus 350 mM glycerol. The efflux rate constants of the muscle exposed to 450 mM glycerol were slightly higher than for the one exposed to 350 mM glycerol. On return to isotonic Ringer solution, both muscles exhibited the characteristic reduction in efflux rate constants. However, 2 hr later, the muscle treated with the higher concentration of glycerol exhibited a transient increase in its rate constant while the rate constant of the other muscle returned essentially to control levels. It was a general finding that the rate constants for efflux (postglycerol) of muscles exposed to less than 375 mM glycerol were always significantly lower than those for the control period (preglycerol) while the rate constant of 25% of those treated with higher concen-

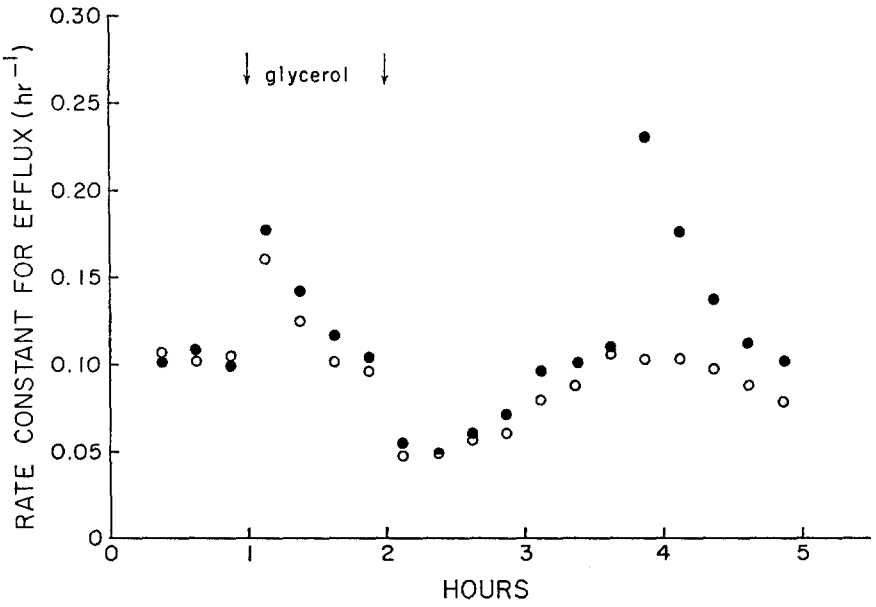

FIGURE 4. Rate constants for ^{42}K efflux of a pair of muscles from the same frog. Potassium (^{42}K) uptake was measured for 18 hr prior to the beginning of efflux. During the time indicated by the arrows the muscle represented by (solid circles) was exposed to Ringer solution plus 450 mM glycerol while the other member of the pair (open circles) was exposed to 350 mM glycerol containing Ringer solution. Efflux rate constant measurements before and after this period were in isotonic 2.5 mM potassium Ringer solution.

TABLE IV

THE CONCENTRATION DEPENDENCE OF GLYCEROL EXPOSURE

Muscle No.	Glycerol	Rate constant 2 hr post-glycerol*	$\frac{DW}{WW}$	K^+		Na^+	
	mM			μM	μM/g	μM	μM/g
128AH0	350	Dec.	18.1	5.33	67.47	0.85	10.76
128A'H0	450	Inc.	16.0	4.43	52.61	1.81	21.50
128BH0	350	Dec.	19.2	4.72	69.72	0.62	9.16
128B'H0	450	Dec.	19.8	4.27	70.70	0.54	9.77
129BH0	350	Dec.	17.2	4.76	61.10	1.08	13.86
129B'H0	450	Inc.	16.0	4.76	55.67	2.14	25.03
26AMO	375	Dec.	17.7	4.27	65.66	1.13	17.45
26A'M0	400	Inc.	16.2	3.54	49.93	1.86	26.28
26BM0	400	Dec.	18.7	4.70	65.06	0.999	13.82
26B'M0	400	Inc.	17.0	5.19	62.58	1.431	17.24
128BM0	400	Inc.	14.8	4.67	49.95	2.01	21.50
128B'M0	400	Inc.	15.3	4.34	49.89	2.63	30.23

* Dec. indicates a decrease as compared with control values; inc. indicates an increase as compared with control values

trations exhibited transient increases of efflux rate constants 1–2 hr postglycerol.

The accumulated data of a few experiments of this type are presented in Table IV. The third column indicates whether the efflux rate constant 2 hr after glycerol treatment was greater or less than the measurements made in the same solution prior to exposure to glycerol. Thus, each muscle served as its own control for this measurement. In each case in which there was a transient increase of the potassium efflux rate constant, there was a greater water gain, potassium loss, and sodium gain.

The muscles which exhibited a transient increase in potassium efflux rate constants, postglycerol, should have a greater net loss of potassium if there was no associated change in influx. This was borne out by the data in Table IV. Therefore, it is possible that the net flux determined by analytical methods for the muscles of Table III which did not agree with that determined by tracer was in error because of this transient increase of potassium efflux.

The Influence of Temperature and Extracellular Potassium Concentration on Resting and Action Potential Measurements of Glycerol-Treated Muscle Fibers

Resting membrane potentials and action potentials of surface muscle fibers of the sartorius were recorded under various conditions involving glycerol. Stimulation was either direct or through the sciatic nerve. The response of the muscles reported here was independent of the glycerol concentration between 350 and 450 mM.

Howell (1969) demonstrated that muscles maintained for periods as long as 72 hr in cold glycerol Ringer solution failed to twitch when returned to Ringer. Reducing the temperature to 0–2°C during the removal of glycerol did not prevent the loss of contractile response to stimulation. Although no measurements were made of the mechanical response of glycerol-treated muscles in this study, it was apparent from visual observation that the contractile response was absent under the same conditions as those reported by Howell (1969). However, it was consistently observed that exposing muscles to cold glycerol Ringer solution at 8°C for 1 hr and subsequently to Ringer solution at the same temperature for 1 hr prevented the depolarization and loss of the negative afterpotential which have been reported to occur after glycerol removal (Gage and Eisenberg, 1969 *b*). The action potentials shown in Fig. 5 were recorded from a typical muscle which was treated for 1 hr at 8°C with glycerol and subsequently returned to isotonic Ringer at the same temperature. The record in Fig. 5 A is a representative response of all fibers tested between 1 hr and 20 min and 1 hr and 30 min after exposure to glycerol. The resting membrane potential of this fiber was −101 mv, the overshoot, +38 mv, and the negative afterpotential, 14 mv. Muscles treated in this manner at 20°C would normally be depolarized to between −80 and −70

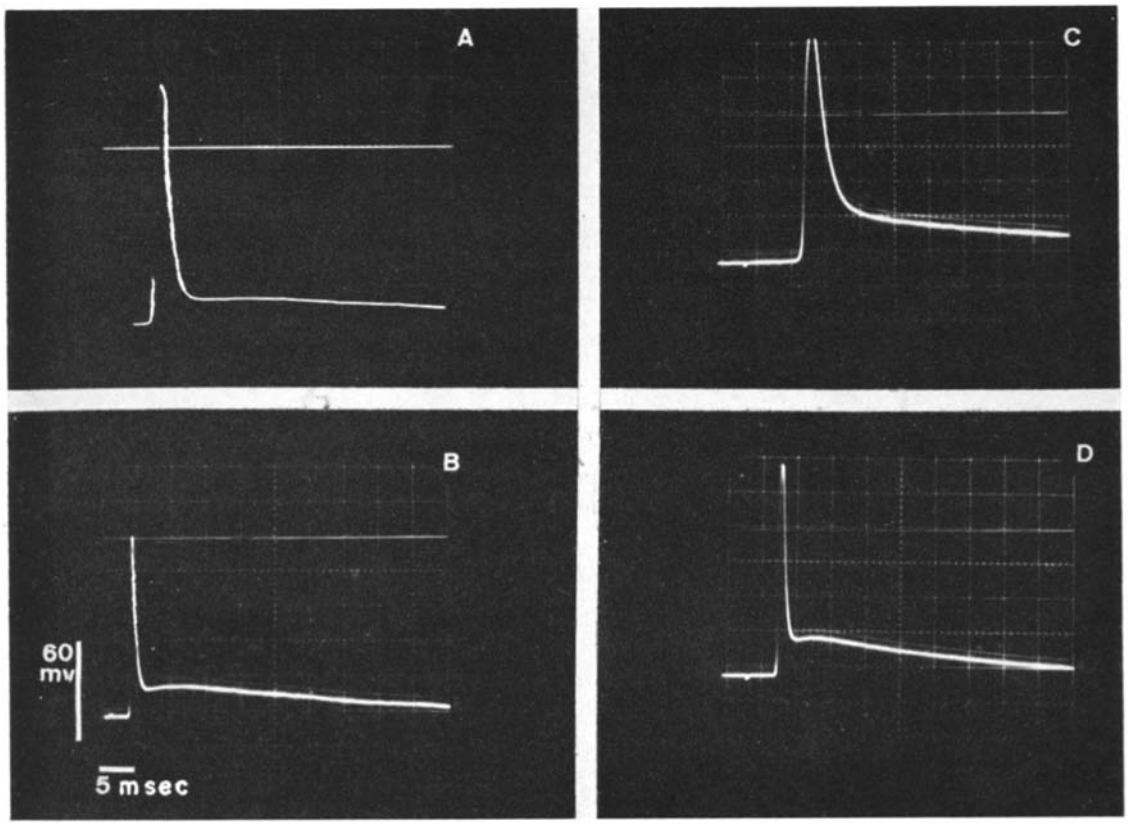

FIGURE 5. Action potential records from different surface muscle fibers of one sartorius muscle, representing the average response at a particular time. The muscle was treated for 1 hr at 8°C with 400 mM glycerol containing Ringer solution and subsequently returned to isotonic Ringer solution at the same temperature. Record A, isotonic 2.5 mM potassium Ringer solution 8°C, 1 hr and 25 min after glycerol treatment. Record B, 2 hr and 30 min postglycerol. T = 29°C. Record C, 1 hr after returning the muscle to isotonic Ringer solution at 8°C. Record D, 30 min after record C at 26°C. Calibration markers in lower left corner.

mv and the negative afterpotential would be absent (Gage and Eisenberg, 1969 *a* and Fig. 6 C). The record in Fig. 5 B was taken from another surface fiber of the same muscle 1 hr later at 29°C. The membrane potential was still −100 mv and the negative afterpotential was 18 mv, but there was essentially no overshoot of the action potential. The muscle was then returned to cold Ringer solution for 1 hr and the record in Fig. 5 C taken after 1 hr at 8°C. The resting membrane potential of this representative fiber at this time was −85 mv, the negative afterpotential, 26 mv, and the overshoot greater than +40 mv. The increased duration of the action potential on cooling is consistent with previous findings (MacFarlane and Meares, 1958). The record in Fig. 5 D

was recorded 30 min after the record in Fig. 5 C (4 hr after glycerol treatment) at 26°C. The membrane potential of this fiber was −84 mv, the overshoot, +39 mv, and the negative afterpotential, 21 mv. These values are within the range of those found for muscles not exposed to glycerol. Although these muscles failed to contract when stimulated electrically, there was apparently no significant influence of glycerol on their action and resting potential characteristics.

If muscles were exposed to glycerol for 1 hr at room temperature followed by immersion in cold (< 10°C) isotonic Ringer for extended periods, action potential characteristics remained normal as long as the muscle remained in the cold. However, when these muscles were warmed the fibers were depolarized and action potentials were not followed by negative afterpotentials which is a characteristic result of glycerol treatment at room temperature.

In the course of these experiments it was found after long periods of rinsing in normal Ringer solution following glycerol treatment that there was a considerable degree of reversibility of the changes in the action potential characteristics induced by glycerol. A representative experiment of this type is presented in Fig. 6. The record in Fig. 6 A is from a typical fiber of a muscle bathed in Ringer solution. The record in Fig. 6 B is a representative response of a different fiber from the same muscle in the presence of glycerol. There was an increase in the action potential duration but essentially no other changes in the action potential. The distortion following the action potential was due to dislodgement of the microelectrode by the mechanical response. The record in Fig. 6 C is from another fiber 2 hr after return to isotonic Ringer solution. As previously described by Gage and Eisenberg (1969 *b*), the muscle was depolarized by this treatment and there was very little evidence of a negative afterpotential. There was considerable variability of the membrane potentials at this time and some action potentials were followed by positive afterpotentials. In general, there was always an increase in the magnitude of the overshoot of the action potential. Subsequently, the muscle was washed for an additional 2 hr in Ringer solution. The record in Fig. 6 D is a typical action potential recorded 4 hr postglycerol. The average membrane potential of all the fibers tested at this time was 90 ± 2 mv and the average negative afterpotential of this muscle was 10 ± 3 mv. The overshoot and duration were essentially normal. This reversibility of the glycerol effect on the action potential contour was routinely found in all the muscles tested in this procedure. The data from these experiments are tabulated in Table V. There was no visual evidence of a return of contractility associated with reversal of the electrical event.

Although the average resting membrane potential of these muscles always returned to 90 ± 2 mv 4-5 hr after glycerol treatment, their response to altered extracellular potassium concentrations was not the same as muscles not exposed to glycerol.

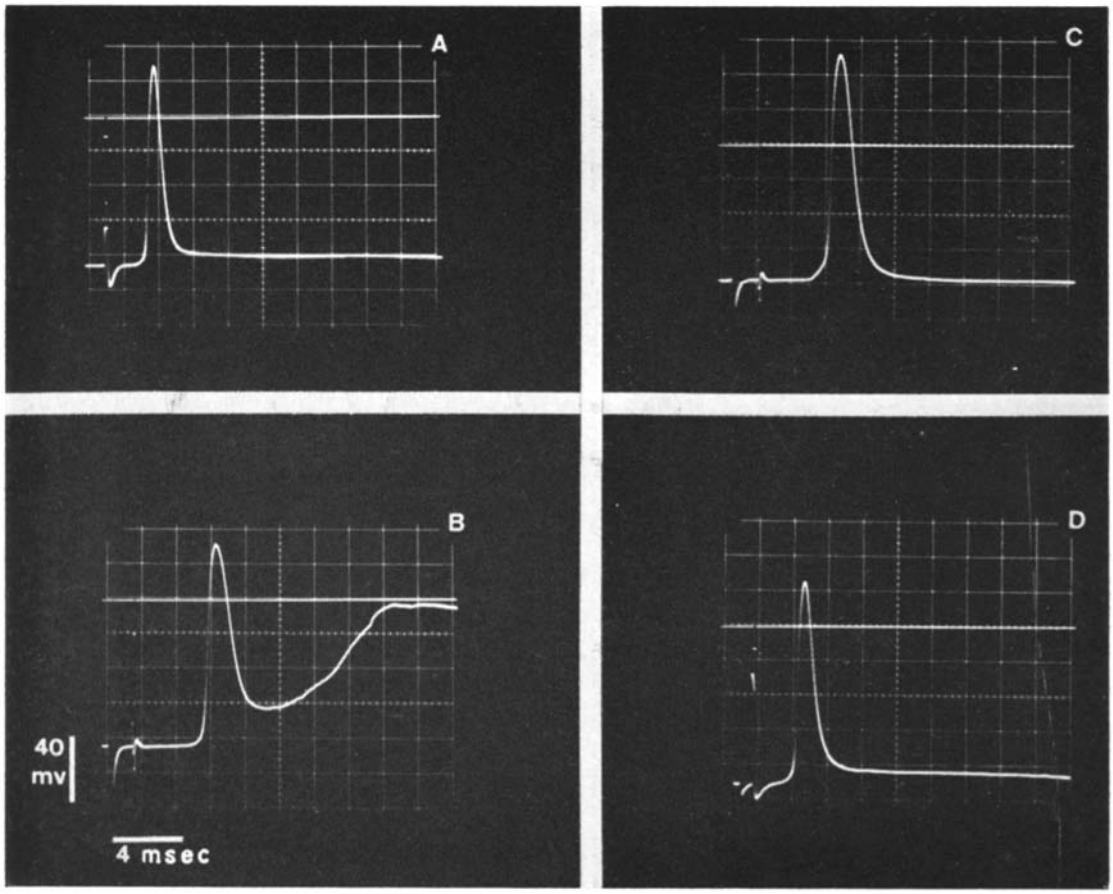

FIGURE 6. Action potential records from different surface muscle fibers of one sartorius muscle representing the average response at a particular time. Record A, isotonic 2.5 mM potassium Ringer solution. Record B, Ringer solution plus 400 mM glycerol, 30 min. Record C, 2 hr after returning the muscle to isotonic Ringer solution. Record D, 4 hr postglycerol. T = 22°C throughout the experiment. Calibration markers in lower left corner.

Normal muscles exposed to potassium-free Ringer solution hyperpolarize (resting membrane potentials > -100 mv) with an associated increase in the size of the negative afterpotential (Desmedt, 1953). On the contrary, resting membrane potentials of muscles treated with glycerol either were unchanged (1-2 hr after exposure to glycerol) or were depolarized (4 or more hr postglycerol). In addition, the action potential was followed by a significant positive afterpotential in both cases. A typical experiment of this type is presented in Fig. 7. This muscle was treated with 400 mM glycerol for 1 hr, 9 hr before the recording reproduced in Fig. 7 A. The resting membrane potential of the

TABLE V

THE INFLUENCE OF GLYCEROL ON RESTING MEMBRANE POTENTIALS AND AFTERPOTENTIALS

Solution*	No. of muscles	No. of fibers	Resting‡ E_m	Afterpotential§
			mv	*mv*
Isotonic Ringer	18	58	91 ± 1	−15 ± 2
Plus 400 mM glycerol	24	85	89 ± 3	−14 ± 2
1–2 hr postglycerol	35	156	76 ± 4	+2 ± 4
4+ hr postglycerol	32	181	90 ± 2	−11 ± 3
K^+-free Ringer‖	23	77	74 ± 3	+11 ± 3
5.0 mM KCl Ringer¶	14	62	76 ± 2	−5 ± 2

* 2.5 mM KCl Ringer solution unless specified.

‡ Resting membrane potential (mean ± SE).

§ Positive values indicate positive afterpotentials and negative values indicate negative afterpotentials (mean ± SE).

‖ After exposure to glycerol solution.

¶ 4 or more hr after exposure to glycerol.

fiber recorded in Fig. 7 A was −90 mv and the action potential was followed by a negative afterpotential of 12 mv. This representative action potential at this time indicated that there was complete reversal of the influence of glycerol on the action potential contour. The record in Fig. 7 B shows the effect of potassium-free Ringer solution on the same muscle. The fiber was depolarized to −74 mv and there was a 10 mv positive afterpotential instead of an increase in the negative afterpotential. The record in Fig. 7 C was taken from another fiber in the same muscle 30 min after the normal 2.5 mM KCl was added to the Ringer solution. The average membrane potential of 10 fibers tested at this time was −91 ± 2 mv and the negative afterpotential was 12 ± 2 mv. 23 additional muscles were tested for their response to reduction of extracellular potassium and the average membrane potential was found to be −74 ± 3 mv with an average positive afterpotential of 11 ± 3 mv (Table V).

In contrast to the response of these muscles to reduction of extracellular potassium, elevation of the potassium concentration in the bathing medium always resulted in a depolarization and reduction in the magnitude of the negative afterpotential as was the case for muscles not exposed to glycerol (Persson, 1963). The record in Fig. 7 D was from a different fiber of the same muscle that was used to demonstrate the response to reduction of the potassium concentration. 1 hr after the elevation of the potassium concentration to 5.0 mM the average membrane potential of this muscle was −74 ± 3 mv and the negative afterpotential was 5 ± 1 mv. The average membrane potential measured for glycerol-treated muscles in 5.0 mM potassium Ringer solution was found to be −76 ± 2 mv and the average negative afterpotential was 5 ± 2 mv (Table V). Further increases in the potassium concentration up to

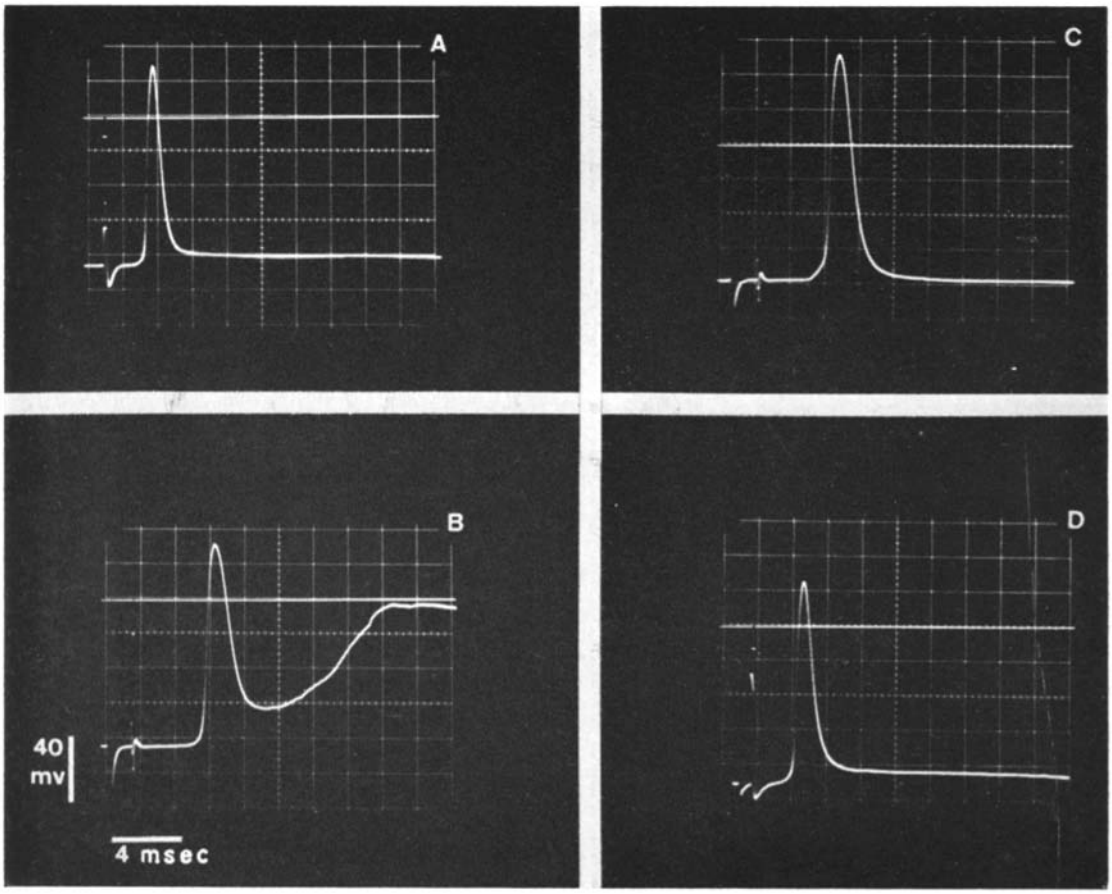

FIGURE 7. Representative action potential records from different surface muscle fibers of one sartorius muscle which was treated with 400 mM glycerol for 1 hr, 9 hr before the record in A. Record A, isotonic 2.5 mM potassium Ringer solution. Record B, potassium-free Ringer solution 1 hr. Record C, 2.5 mM potassium Ringer solution 30 min after the record in B. Record D, 5.0 mM potassium Ringer solution 1 hr. Calibration markers in lower left corner.

50 mM led to further depolarizations in the same manner as has been demonstrated in normal muscle fibers (Adrian, 1956).

DISCUSSION

Very good agreement between ^{42}K kinetics of individual frog semitendinosus fibers (Hodgkin and Horowicz, 1959 *a*) and kinetics measured with whole sartorius muscles (Sjodin and Henderson, 1964) has been documented. Control measurements in this study agreed very well with these previous publications. Therefore, it can be concluded that tracer measurements of the whole sartorius muscle reflect ionic fluxes of the individual fibers.

It has been estimated that the ratio of the areas of tubular and surface membranes (in a fiber with a diameter of 50 μ) is between 3.5 and 4.5 (Peachey, 1965; Peachey and Schild, 1968). If potassium exchange across these various membranes of frog skeletal muscle is symmetrical such that the permeability properties of the surface membrane are identical with those of the tubular membrane, it would be expected that the potassium fluxes would be reduced by a factor proportional to the ratio of tubular to surface area of the membrane when the tubules are disrupted by glycerol treatment. In this study it was found that potassium (^{42}K) influx of glycerol-treated muscles was 53% lower than influx measurements determined for control muscles. The ratio of tubular to surface exchange would then be roughly one to one. Since the tubular area was estimated to be roughly four times the surface area for individual fibers, the data for tracer influx would indicate that about 50% of the potassium influx pathways were located in one-fifth of the total muscle surface area. On the other hand, it was found that ^{42}K efflux was only reduced 37% in glycerol-treated muscles, indicating that there may be more efflux pathways per unit surface membrane area than there are influx pathways.

A possible source of error might have resulted from incomplete disruption of the tubules of deep fibers. Eisenberg and Eisenberg (1968) have demonstrated that only 2% of the tubular system of the surface fibers remains connected to the extracellular space after glycerol treatment and that even these extend only a few micra into the fiber. They indicated that deep fibers appeared to have more tubules remaining, although these were not examined in detail. If the glycerol effect is due to the disruption of tubular membranes, incomplete disruption of extensive numbers of tubules in deep fibers would lead to a significant development of twitch when the whole muscle was stimulated electrically. Howell (1969) has demonstrated that twitch and KCl contractures were completely absent from glycerol-treated sartorius muscles. Visual examination of the muscles used in this study indicated that there was no evidence of twitch or tetanus associated with maximal electrical stimulation. Therefore, it may be concluded that the uncoupling produced by glycerol treatment was due either to something other than tubular disruption or that the tubular disruption was very nearly complete in all the fibers of the bundle.

If it can be assumed, then, that the percentage of fibers with intact tubules was very low as compared with those with disrupted tubules in the whole sartorius, the figures for the distribution of tracer fluxes across the membranes involved would be relatively accurate. There would then be a considerable asymmetry between the potassium exchange of the tubular membrane and the surface membrane. Eisenberg and Gage (1969) also found that their measurements of the potassium conductance of the tubular and surface membranes of glycerol-treated muscle fibers were not in agreement with predictions on the basis of the ratio of the surface areas.

It was noted that in 25% of the muscles examined there was a transient but very significant increase of potassium efflux following exposure to glycerol. This phenomenon may be related to the type of tubular disruption. Thus if disruption involved blockage of potassium exchange through tubular membranes the over-all tracer fluxes would be reduced as was generally found. On the other hand, if disruption of the tubules actually caused a destruction of tubular membranes and breakdown of permeability barriers, an increase of potassium exchange through this pathway would be expected. Greater destruction would be expected at higher glycerol concentrations due to the increased osmotic shock on return to Ringer solution.

Despite the net loss of potassium found in muscles treated with glycerol, reversibility of the depolarization produced by this procedure occurred in all muscles tested. With the return of the resting membrane potential to between −88 and −92 mv, action potentials elicited by direct or indirect electrical stimulation were followed by normal negative afterpotentials. Calculations based on the intracellular distribution of sodium and potassium from Table III indicated that the potassium equilibrium potential (E_K) of each of the glycerol-treated muscles was lower than normal and the calculated resting membrane potentials (E_m) were always less negative than those of the respective control muscles. For example the E_K of muscle 328AH9 was −100 mv while the E_K of 328A'H9 was −83 mv. The calculated E_m of the same pair of muscles was −92 mv (328AH9) and −75 mv (328A'H9).

There was obviously a considerable difference between the calculated resting membrane potentials and those observed experimentally. The muscles were capable of repolarizing to near normal values without a reversal of the net loss of intracellular potassium. Since the concentration ratio between intra- and extracellular potassium is the prime determinant of the resting membrane potential under normal conditions (Hodgkin and Horowicz, 1959 *b*), other factors must be considered to explain the repolarization in glycerol-treated muscle fibers. One possibility is that of redistribution of intracellular potassium in the glycerol-treated muscles. Sartorius muscles exposed to glycerol containing Ringer solution and returned to normal Ringer solution always gained considerable cell water. The increase in cell water was not reversed by long periods of washing in isotonic Ringer solution. However, the increase in cell water may not be uniform throughout the fiber, since it has been reported that there was considerable vacuolization in the sarcoplasm of muscle fibers after washout of nonelectrolytes (Krolenko, Adamjan, and Shwinka, 1967). There also appeared to be considerable vacuolization in the electron micrographs of glycerol-treated muscles in the study of Howell (1969). If the increase in cell water occurred primarily in these vacuoles, calculations of intracellular ion concentration on the basis of total cell water could be in error. In fact, extravacuolar concentrations of ions may be much higher than those calculated in this study for whole sartorius muscles.

The reappearance of the negative afterpotential of glycerol-treated muscles was associated with the repolarization. Therefore, regardless of the fact that the disruption of the tubules is irreversible in glycerol-treated muscle fibers, these muscles can generate normal action potentials. It is possible that additional reversible changes in the surface membrane also take place after exposure to glycerol. In any event, the mechanism responsible for the negative afterpotential in striated muscle is probably located in the surface membrane.

It was observed that removal of the extracellular potassium chloride from the medium bathing glycerol-treated muscles resulted in a depolarization of the muscle membrane and that action potentials were followed by positive afterpotential. A possible explanation for this anomalous depolarization may be that the sodium permeability or the sodium extrusion mechanism or a combination of the two may be altered after glycerol treatment. It is known that reduction of the extracellular potassium in normal frog sartorius muscles inhibits the sodium extrusion mechanism and that elevation of extracellular potassium stimulates it (Desmedt, 1953). It has also been shown that muscle fibers producing large outward movements of sodium may have substantially more negative membrane potentials than fibers not moving sodium (Adrian and Slayman, 1966). Conversely, it may be possible that reduction of sodium extrusion may result in internal potentials which are more positive than under normal conditions. The data in Table III indicate that glycerol-treated muscles gained more sodium than control muscles. Obviously, there was some change in the sodium exchange mechanism. Calculation of the resting membrane potential on the basis of the Goldman, Hodgkin, Katz equation (Hodgkin and Horowicz, 1959 *b*), assuming a sixfold increase in the sodium permeability coefficient in potassium-free solution, indicated that glycerol treated muscles would be depolarized to −78 mv. Possibly a smaller increase in sodium permeability would be sufficient to produce the same degree of depolarization if the sodium extrusion mechanism were also inhibited in the potassium-free situation.

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