CATION MOVEMENTS IN NORMAL AND SHORT-TERM DENERVATED RAT FAST TWITCH MUSCLE

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SUMMARY

1. The earliest known change in rat fast muscle following denervation is a fall in resting membrane potential unaccompanied by change in membrane resistance. The present study tested the hypothesis that increased Na permeability (P_{Na}) accounted for this early depolarization.

2. In all experiments, rat extensor digitorum longus muscles were studied *in vitro* at 25° C. Li uptake *in vitro*, used as a measure of P_{Na}, was greater in 1- and 2-day denervated muscles (and in 2-day denervated diaphragm) than in paired controls.

3. The extra Li taken up by denervated muscle was not sequestered in an extracellular or freely exchangeable compartment, nor was it irreversibly bound.

4. Measurements of resting membrane potential and of internal Na, K, and Li in Krebs solution before and 2 hr after replacement of NaCl by LiCl, were used to compute the ratios P_{Na}/P_K and P_{Li}/P_K for normal or denervated muscles. P_{Na} and P_{Li} were similar relative to P_K within each class of muscle.

5. Both P_{Na}/P_K and P_{Li}/P_K ratios were elevated more than twofold in denervated muscle, as were most estimates of relative P_{Li} approximated by the flux equation.

6. These data, and measurement of resting membrane potential of normal muscle in 1 mM external K-Krebs solution, support the view that an electrogenic Na–K pump does not substantially contribute to this potential of normal or denervated muscle, and that the early depolarization after denervation results from increased P_{Na}.

7. The Na–K pump of denervated muscle was as sensitive to ouabain as normal muscle. An effect of ouabain on P_{Na} may explain previously noted differential effects of ouabain on normal and denervated muscle.

INTRODUCTION

One of the earliest responses of mammalian muscle to denervation is a reduction in resting membrane potential (Albuquerque, Schuh & Kauffman, 1971) at a time when internal ion concentrations are essentially unchanged (see Results). This suggests that either passive ionic permeabilities or an electrogenic process is under close neuronal regulation. The aim of this investigation was to elucidate the underlying membrane property that is so rapidly affected by denervation.

The depolarization which develops in the first 3 days after denervation is nearly as great as that found at later times, but the mechanism may be different. The later depolarization is accompanied by increased membrane resistance, decreased K efflux, and increased Na permeability (Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970; Albuquerque *et al.* 1971; Klaus, Lullman & Muscholl, 1960; Creese, El-Shafie & Vrbova, 1968). In contrast, the earlier depolarization is not accompanied by any change in membrane resistance (Albuquerque & Thesleff, 1968; Albuquerque & McIsaac, 1970; Albuquerque *et al.* 1971), but little more is known.

It was suggested that the early depolarization after denervation resulted from a failure in a normally operating electrogenic pump. However, the supporting evidence is incomplete in that it rests entirely on membrane potential measurements after ouabain application (Locke & Solomon, 1967; McArdle & Albuquerque, 1975; Bray, Hawken, Hubbard, Pockett & Wilson, 1976). Furthermore, there is strong evidence that the resting potential of normal (as opposed to Na loaded) mammalian muscle results from purely passive mechanisms (Williams, Withrow & Woodbury, 1971*a*, *b*; Akaike, 1975). Therefore, the specific objective of this study was to determine if changes in passive permeability (in particular, increased Na permeability) could account for the depolarization of mammalian fast twitch muscle in the first 48 hr after denervation.

Most of the studies reported here entail measurement of net Li movements in normal and denervated muscle. Li was chosen as a cation with similar permeability properties to Na (Keynes & Swan, 1959; Yonemura & Sato, 1967) but with the advantage that net movements across the cell membrane appear to be less affected by active pump mechanisms and therefore reflect passive permeability more closely. The apparent compartmental distribution of sodium further complicates interpretation of Na flux data (Rogus & Zierler, 1973), whereas most of the Li which accumulates in the cell appears to be in the cytoplasm (see Results).

METHODS

Operations. Male Sprague–Dawley rats, 21–28 days old and weighing 45–64 g, obtained from Hilltop Laboratories, were anaesthetized with ether. Under sterile procedure, the peroneal nerve was severed as it descended beneath the peroneal muscles just below the knee. On the contralateral side, the nerve was exposed but not severed. The sham-operated muscle in all series was randomized between right and left sides. The entire operation took less than 10 min and the rats were awake 10 min later. At later times, the rats were re-anaesthetized and the extensor digitorum longus (EDL muscle) removed for incubation. Care was taken to keep muscles moistened with Na-Krebs solution (see below) during the dissection, and to preserve the blood supply until final removal from the animal. In one experimental series, the diaphragms of ether-anaesthetized rat pups were denervated by the thoracic approach (cf. Miledi & Slater, 1970).

Incubation. The dissected muscles were suspended vertically by thread from the proximal tendon on lucite frames, while the distal tendon, attached to weights of 300 mg, was set in place at a muscle length corresponding to this tension. In pilot experiments, it was found that maximal direct twitch tension was obtained in EDL muscles from forty-five to sixty-five g rats, at about 300 mg tension. The entire platform, holding the innervated and denervated muscles side-by-side, was then immersed in a lucite chamber containing 5 ml. solution (see below), which was then tightly sealed. The top was perforated to permit entry of a needle carrying 95 % O_2–5 % CO_2 which was blown on to the surface of the solution. The entire chamber at room temperature (25° C) was placed on a rotator to facilitate gaseous exchange during the incubation. In the experimental series with diaphragm muscles, the muscles were removed from ether-anaesthetized rats, and strips of innervated and denervated muscle, about 1 cm wide, were pinned side-by-side under slight tension to Silastic (Dow Corning) supports in a chamber containing 13 ml. solution bubbled with 95 % O_2–5 % CO_2. Methods of analysis were as for EDL muscles.

Solutions. The basic 'Na-Krebs' solution consisted of 135 mM-NaCl, 5 mM-KCl, 1 mM-Mg_2SO_4, 1 mM-Na_2HPO_4, 15 mM-$NaHCO_3$, 2·5 mM-Ca gluconate, 11-mM glucose, and D-tubocurarine, 1 μg/ml, to which horse serum (Grand Island Biological Co.) was added to a final concentration of 5 %. The measured pH of the Krebs solution bubbled with 95 % O_2–5 % CO_2 varied from 7·25 to 7·35 depending on degree of equilibration with CO_2. The D-tubocurarine was included to prevent any inadvertent spontaneous indirect stimulation due to tension on nerves resulting from the dissection. Horse serum was added to maintain internal Na and K (Akaike, 1971) and to preserve normal sodium permeability (Creese, 1968). [³H]inulin, or more usually [³H]methoxyinulin (ICN or New England Nuclear), both referred to as [³H]inulin, was added to a final concentration of 10 μc/ml. Before use, the radioactive inulin was either washed in methanol or in an Aminco UM-2 Diaflo apparatus to remove contaminants of molecular weight less than 1000.

'Li-Krebs' was the same as Na-Krebs except that NaCl was replaced by 135 mM-LiCl. In some experiments, as indicated, tetrodotoxin (kindly supplied by Dr N. Akaike, Sankyo Ltd) was added to Li-Krebs. In 'Tris-Krebs', all Na-containing ingredients of Na-Krebs were replaced by 150 mM-Tris (hydroxymethyl)amino-ethane adjusted to pH 7·4 with HCl. 'Cl-free Krebs' was made by replacing NaCl and KCl with equimolar methane-sulphonate salts of Na and K titrated to pH 7·4 with NaOH or KOH, respectively. The pH of the final Cl-free or Tris Krebs solution was checked and adjusted to 7·4 during aeration with 95 % O_2–5 % CO_2.

Analysis. At the end of the incubation, muscles were blotted gently, tendons were removed, the wet weight was obtained, and the muscles were dried overnight at

110° C, weighed, and dissolved in 50 μl. conc. HNO_3 in tightly capped vials. The resulting solution was diluted to 400 μl., and aliquots taken for scintillation counting or flame photometry. Samples of incubation fluid were also taken for counting. In earlier experiments, 5 ml. Aquasol (New England Nuclear) was used to count the tritiated inulin, but in later experiments more stable counts were obtained if 1 ml. water and 4 ml. Aquasol were used. At least 2000 counts were obtained per muscle, after which internal standards of [^{3}H]toluene were added and the samples re-counted to find the efficiency. Thus, samples from the right and left EDL of one rat and from the incubation fluid were all corrected to the same efficiency before calculation of the extracellular space. The inulin space, in l./kg wet wt., was (inulin c.p.m. per mg wet wt. of muscle)/(inulin c.p.m. per μl. incubation solution). In three experiments, Na_2 $^{35}SO_4$ (sp. act. 716 mc/m-mole, New England Nuclear) instead of [^{3}H]inulin was added to the incubation fluid to a final concentration of 1·6 μc/ml. and the muscles were incubated, processed, and analysed for sulphate-space as for inulin space.

The remaining dissolved muscle solution was diluted, filtered and read in duplicate for Li, K, or Na on a Baird-Atomic KY-4 flame photometer. Calibration standards were made up in solutions containing HNO_3 in the same proportions as in the experimental solutions. Internal cation concentration per litre fibre water were calculated from the formula $(X' - X'')/(1 - Y)$ where X' is the cation content per kg wet wt., X'' is the product of external X concentration times average extracellular space (l./kg wet wt.) and Y is the sum of extracellular space and (dry weight)/(wet weight).

In experiments on ^{22}Na exchange, the incubation fluid contained 2·5 μc/ml. carrier-free ^{22}NaCl (New England Nuclear). Preliminary experiments showed that the specific activity of muscle was 90–100% equilibrated with that of the bathing solution after 30–45 min incubation. Exchange of ^{22}Na with the extracellular Na-space was assumed to occur with the same time course as Li (Fig. 1) and therefore a 20 min incubation time in Na-Krebs (containing ^{22}Na) was employed to determine if the extracellular ^{22}Na spaces of innervated and denervated muscles were different. Processing, counting and standardization of muscle and bath samples was done as for tritium (except for different choice of counting windows).

Membrane potential. Paired innervated and denervated muscles were removed and set at 300 mg tension, as for incubations, then placed together in Krebs solution (not containing horse serum) and bubbled with 95% O_2–5% CO_2 at 22–25° C. Membrane potentials were recorded with 15–20 MΩ glass electrodes filled with 2·0 M-KCl at neutral pH. The fluid in the shank of the electrode could also be connected to the bath solution by a Krebs-agar polyethylene tubing conduit, thereby shunting the micro-electrode tip. The voltage difference before and after shunting was taken as the tip potential, and only pipettes with tip potentials less than 5 mV were used. Signals were led to a Bak amplifier and recorded on a Brush Mark 220 pen-writer. All penetrations of surface fibres which were abrupt and maintained a few seconds were included in the data reported.

Methodologic experiments. Paired muscles incubated varying lengths of time were used to determine equilibration of [^{3}H]inulin. Compared to the calculated inulin space after 60 min incubation time, the space at 15, 30 and 120 min was 58%, 81% and 93% respectively, with s.e. of the mean 3–10% (no. = 4 to 5 rats for each point). Therefore, at least 60 min were required for equilibration, but the space did not increase between 60 and 120 min. Because of the standard error in inulin equilibration, the calculated inulin space was averaged for each experimental group of innervated or denervated muscles, and the average used for calculations of internal cation concentrations. Inulin space was determined in about half the muscles in any series.

The values for inulin space in different experimental series varied from 0·18 to

0·24 l./kg wet wt., with the denervated muscle generally about 0·02 greater than innervated. In some but not all series, this difference was statistically significant. The values for control muscles agreed well with those of Kobayashi & Yonemura (1967) who found a relation between muscle wet weight (and age of rat) and inulin space of EDL muscles. Rogus & Zierler (1973) reported an extracellular space of 0·13 l./kg for rat EDL. Their suggestion that higher values reported by others were due to low molecular weight contaminants is not supported by the present experiments, in many of which inulin was washed by ultrafiltration to eliminate possible low molecular weight contaminants. Their lower value may have resulted from the inclusion of rats weighing up to 100 g (cf. Kobayashi & Yonemura, 1967) and possibly because equilibration in their larger muscles was incomplete at 60 min. On the other hand, in some cases in the present study, the calculated extracellular space was too large by at least a few per cent, since negative values of internal Na were occasionally obtained from the formula given above. Possibly a small degree of saturable binding of inulin explains these cases. Nonetheless, consistent results were obtained in any given experimental series, and the error introduced, if any, would not materially affect the basic conclusions (see also Discussion).

The *in vitro* incubation method described above was tested by comparing the Na and K contents of paired normal EDL muscles from the same rat. One of the muscles was always incubated for 60 min, and the other for 15, 30, or 120 min (four to six rats per comparison). K showed no difference between any of the time periods measured. There was an early gain of Na (7 m-mole/kg wet wt.) occurring in the first 30 min of incubation, but thereafter the muscles were maintained in a fairly steady state for the remainder of the 120 min incubation.

Statistics. The t test was used in all statistical analyses, and all results are expressed as mean ± S.E. of the mean.

RESULTS

Net Li movements after denervation

At various times after denervation, extensor digitorum longus (EDL) muscles were incubated for 2 hr *in vitro* in a Krebs solution in which 135 mM Na was replaced by Li (see Methods). Muscles denervated 5–18 hr showed no significant difference in Li uptake from paired contralateral controls (Table 1). However, at 24 and 48 hr, denervation resulted in significantly increased Li uptake (Table 1). The increase in Li uptake at 24–27 hr was significantly ($P < 0.001$) less than that at 48 hr.

In several experiments, tetrodotoxin (2×10^{-6} M) was added to the incubation medium in order to prevent any twitching due to depolarization of either nerve terminals or of muscle fibres. The addition of tetrodotoxin did not alter the *difference* in Li uptakes of normal and denervated muscle. However, in experiments at $15\frac{1}{2}$–$17\frac{1}{2}$ hr and at 24–27 hr, tetrodotoxin considerably reduced the Li uptake of *both* denervated and innervated muscles (Table 1). This is consistent with the known effect of tetrodotoxin in reducing resting sodium permeability in squid axons (Freeman, 1971). Nonetheless, at 24–27 hr, the two tetrodotoxin-treated denervated muscles still took up some 16 m-mole more Li/l. fibre water than paired controls (Table 1, footnote).

In one rat unilaterally denervated 26 hr, 5 % horse serum was omitted from the incubation medium. In this case the final Li concentration (m-mole/l. fibre water) was 90·2 in the denervated muscle and 57·8 in the contralateral control. Thus, the presence of serum did not qualitatively modify the effects of denervation on Li uptake. This point will be useful in data given later on. The measured internal Li concentrations varied from one experimental series to another (Table 1), and it is not apparent

TABLE 1. 2 hr Li uptake of denervated and control innervated muscles

Denervation time (hr)	Li (m-mole/l. fibre water ± s.e. of the mean)				
	Denervated	Innervated	Difference	n	P
5–5½	95·5	93·7	+1·8	3	n.s.
8–10	90·2 ± 11·4	100·5 ± 12·7	−10·3 ± 10·1	4	n.s.
15–18	30·2 ± 5·2*	37·4 ± 6·9*	−7·2 ± 5·1	4	n.s.
24–27	99·3 ± 4·3†	82·3 ± 3·4†	+17·0 ± 1·6	7	<0·01
45–50	104·4 ± 9·2	67·5 ± 7·6	37·0 ± 4·3	9	<0·001

* All these incubations in the presence of tetrodotoxin (2×10^{-6} M).

† Not including two experiments in presence of tetrodotoxin (2×10^{-6} M) in which average Li uptake was 43·8 and 59·8 for innervated and denervated, respectively.

TABLE 2. Li uptake of diaphragm from 14 to 16 day rats

Time after denervation (hr)	(m-mole/l. fibre water)		(left-right) Δ	n
	Innervated or denervated (left)	Innervated (right)		
0	79·2 ± 3·8	59·4 ± 4·8	19·8 ± 4·9	5
23–26	79·4 ± 7·6 (71–95)	61·0 ± 2·1 (58–65)	18·4 ± 8·1 (6–34)	3
46–49	132·4 ± 7·0 (119–147)	70·9 ± 2·3 (64–75)	62·0 ± 6·5* (45–72)	4

Data given as mean ± s.e. of mean with range of values for $n = 3$ or 4 in parentheses below. Denervated side was always left side. Mean and s.e. of Δ are from paired comparisons.

* This mean differs from first row Δ of 19·8, at $P < 0·002$.

whether the contralateral controls show a reduced Li uptake or whether the denervated muscles show an increase. However, in diaphragm muscle, more reproducible absolute values of Li uptake were found in different groups of animals, even though there appeared to be a consistent difference even in controls between left and right sides (Table 2). After denervation on the left side, no change in uptake relative to the left-right difference of control muscles was found at 1 day after denervation. However, the large increase found at 2 days (Table 2) cannot be accounted for by a decreased uptake on the innervated side.

Cation changes during incubation in lithium Krebs

Li, Na, and K were determined in normal and 24-hr denervated muscle after various incubation times in Li-Krebs (Fig. 1). In the first 15–20 min, Li mainly entered and occupied the extracellular and Na spaces. In this time, no significant difference in Li uptake appeared between innervated and denervated muscle. After 30 min in Li-Krebs, Na content was essentially zero (0·5 and 3·3 m-mole/l.f.w.) in four paired innervated and
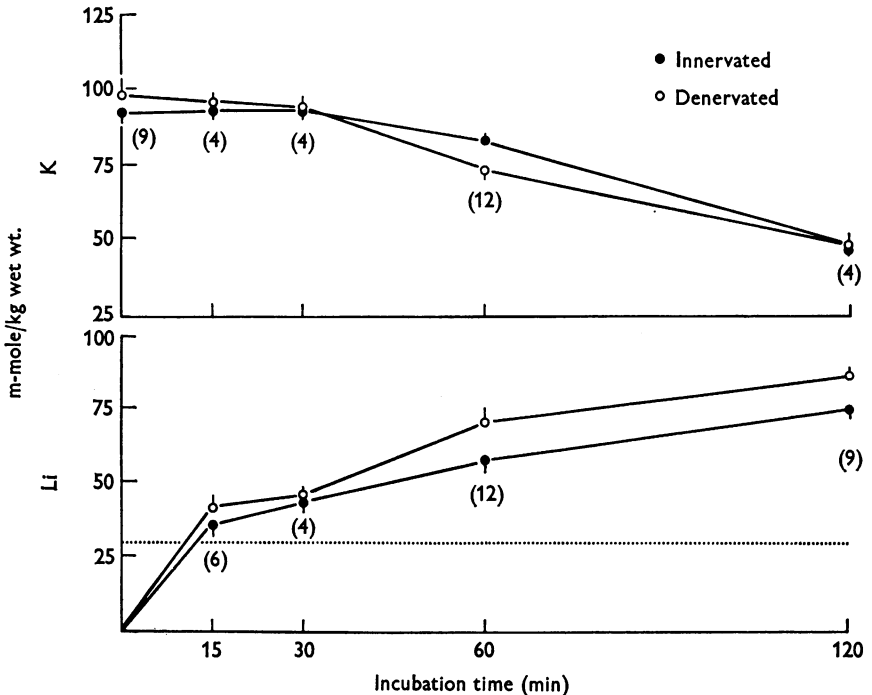

Fig. 1. Uptake of Li and loss of K during *in vitro* incubation of rat EDL muscles in Li-Krebs. Muscles were either denervated 24–29 hr *in vivo* before incubation, or were the contralateral innervated sham-operated controls. Numbers of muscles are in parentheses and vertical lines from each point are standard error of the mean. Interrupted horizontal line is amount of Li which would fill the entire extracellular space.

denervated muscles, respectively, while K content was virtually unchanged (Fig. 1). Thus, the initial events of Li uptake and replacement of Na in both extra- and intra-cellular space appeared to be identical in innervated and denervated muscle. By 1 and 2 hr, however, K loss accompanied further Li gain, and the difference in Li uptake between innervated and denervated muscle was established. This difference appears no greater at 2 hr than at 1 hr of incubation, but variation in the groups of rats used

for the different experimental points may account for this. Indeed, in 45–50 hr denervated rats, the *difference* in Li uptake from 1 to 2 hr incubation (26·3 m-mole/l. fibre water) was more than twice that occurring from 30 to 60 min (12·8 m-mole/l. fibre water).

On the location of Li taken up by muscle

In the above, it was assumed that inulin accurately measures the extracellular space, and that no new compartment or binding site appears in denervated muscle which would account for the increased Li uptake found after denervation. Several experiments were designed to test the validity of these assumptions.

First, there was no evidence for an increased Na space in denervated muscles. The Na content of fresh-dissected innervated muscles (36·5 m-mole/kg wet wt.) was essentially the same as that of the contralateral 24–29 hr denervated muscles (40·2 m-mole/kg wet wt.). The corresponding values at 45–50 hr of denervation were 43·4 and 44·5 m-mole/kg wet wt. ($n = 5$) for muscles incubated 20 min in Krebs solution.

Secondly, ^{22}Na uptake during incubation in Krebs solution was unchanged by denervation. The relative ^{22}Na content (denervated/innervated ± s.e.) after a 2 hr incubation in Krebs solution containing ^{22}Na was $1·01 \pm 0·09$ ($n = 4$) for 24–29 hr denervated muscle. After a 20 min incubation in the same solution, the relative ^{22}Na content of 45–50 hr denervated muscles was $1·04 \pm 0·06$ ($n = 5$). Thus, the increased uptake of Li in denervated muscle cannot be explained by expansion of the Na space.

Inulin, which was used for measurement of extracellular space, might penetrate into the T-tubular space only slowly, and therefore conceal an extracellular compartment to which Li had access. In three experiments, therefore, the [^{35}S]sulphate space after a 2hr incubation in Li-Krebs solution was measured in paired muscles 45–47 hr after denervation. As expected (Davson, 1970), the sulphate space (0·26 kg/l. wet wt. innervated, 0·30 denervated) was larger than the inulin space (0·18 innervated, 0·22 denervated) found in a parallel series of experiments. Hence, if the sulphate-space was used, the computed internal Li concentrations of denervated and innervated muscles were slightly lowered. However, the *difference* in uptake of Li between denervated and innervated muscles was hardly affected. For the same three pairs of muscles, the denervated EDLs took up 33·1 m-mole/l.f.w. more Li than controls, on the basis of the sulphate space, and 29·0 m-mole/l.f.w. more Li on the basis of the inulin space (average found in the parallel series).

Part of the added Li taken up by denervated muscle could be bound to internal sites, presumably proteins (Hinke, Caille & Gayton, 1973). Li uptake was measured in four pairs of innervated and paired 49–53½ hr

denervated muscles which had been immersed in cold (4° C) 50 % glycerin for 3 weeks. The glycerinated muscles were incubated for 2 hr in Li-Krebs at 24° C, and then washed in Na-Krebs for 10–30 min. The final Li content (m-mole/kg wet wt.) of innervated and denervated muscles was $3\cdot2 \pm 0\cdot1$ and $3\cdot1 \pm 0\cdot2$, respectively. Hence, a small amount of Li taken up may be bound to insoluble proteins, but the extent of binding is unaltered by denervation.

If Li taken up by the cell were mainly contained in an extracellular or tubular compartment, the half-time of washout would be less than 15 min (Rogus & Zierler, 1973), and Li content after a brief washout would become the same for innervated and denervated muscles. This was not the

TABLE 3. Correspondence between Li uptake and K loss

Time after denervation (hr)	Incubation (min)	Innervated		Denervated		
		ΔLi	ΔKi	ΔLi	ΔKi	n
22–29	60	$+47\cdot0 \pm 5\cdot2$	$-14\cdot0 \pm 3\cdot6$	$+59\cdot1 \pm 4\cdot9$	$-38\cdot9 \pm 5\cdot1$	12–13
24–27	120	$+79\cdot2 \pm 4\cdot2$	$-80\cdot7 \pm 0\cdot9$	$+94\cdot7 \pm 5\cdot1$	$-91\cdot9 \pm 6\cdot6$	4
48	120	$+67\cdot5$	$-75\cdot4$	$+104\cdot4$	$-92\cdot4$	2

mM/l. fibre water

ΔLi is simply the internal concentration after incubation in Li-Krebs for the durations indicated. ΔKi is the mean final value at the end of the incubation minus the mean value found in muscles either fresh-dissected or incubated in Na-Krebs for 20 min. Data (mean ± S.E. of the mean) are only from experiments where both Li and K were measured.

case. Two muscles denervated about 44 hr and paired controls were incubated in Li-Krebs for $2\frac{1}{2}$–3 hr, then rapidly washed with ten changes of Tris-Krebs over the next hour. The Li remaining in the denervated muscle at the end of the washout period exceeded that of the paired innervated muscle by $41\cdot1$ and $38\cdot8$ m-mole/l. fresh wt. in the two cases. This agreed well with the results in Table 1 (45–50 hr denervated pairs).

Finally, in incubations lasting 120 min (but not 60) the extra Li taken up by denervated muscle correlated with a greater loss of internal K (Table 3). Since K is thought to be completely dissociated in the cell water, the exchange of Li and K suggests that the *additional* Li in denervated muscle is also dissociated.

Resting membrane potential before and after Li uptake

In normal Krebs solution, the resting potential of denervated muscle was about 10 mV less than the control at about 1 day after operation and about 13 mV at 2 days (Table 4). Shortly after immersion in Li-Krebs

TABLE 4. Resting potential, internal cation concentrations, and $(P_{Na}$ or $P_{Li})/P_K$ in control and denervated muscles before and after incubation in Li-Krebs

$$\left(\text{Results are all in the form: } \frac{\text{Na-Krebs}}{\text{Li-Krebs}}\right)$$

Muscle	Type of Krebs solution	Time in Li-Krebs (min)	RMP* (mV)	[Na]$_i$	[Li]$_i$	[K]$_i$	$\dfrac{P_{Na}/P_K}{P_{Li}/P_K}$
					m-mole/l. fibre water		
Denervated 25½–26½ hr	Na	—	67·7 ± 1·1 (45/3)	14·7 ± 5·6 (9)	0	168·1 ± 8·4 (9)	0·044
	Li	30	50·9 ± 1·4 (18/3)	—	—	—	—
	Li	120	49·5 ± 1·1 (30/3)	0	99·3 ± 4·3 (7)	76·2 ± 6·6 (4)	0·048
Paired controls	Na	—	77·5 ± 0·5 (45/3)	8·3 ± 3·5 (9)	0	157·1 ± 5·0 (9)	0·016
	Li	30	66·6 ± 0·9 (18/3)	—	—	—	—
	Li	120	62·0 ± 0·6 (30/3)	0	82·3 ± 3·4 (7)	76·4 ± 0·9 (4)	0·013
Denervated 50–52½ hr	Na	—	64·4 ± 1·1 (40/3)	23·5 ± 11·5 (4)	0	164·9 ± 5·1 (4)	0·054
	Li	30	49·0 ± 1·4 (20/3)	—	—	—	—
	Li	120	43·9 ± 1·4 (26/3)	0	104·4 ± 9·2 (4)	72·5 (2)	0·068
Paired controls	Na	—	78·0 ± 0·9 (40/3)	23·7 ± 8·7 (4)	0	157·3 ± 4·8 (4)	0·015
	Li	30	71·6 ± 1·0 (20/3)	—	—	—	—
	Li	120	59·9 ± 0·9 (26/3)	—	67·5 ± 7·6 (9)	105·2 (2)	0·039

Membrane potentials were first recorded after muscles had been incubated 15 min in Na-Krebs, then at times indicated after switching the solution to Li-Krebs. Cation values are taken from parallel series of experiments, except that 5 % horse serum was present. In all experiments, paired innervated and contralateral control muscles were incubated together in the same chamber. Values for cations given above the ruled line are from fresh dissected muscles or from muscles incubated 20 min in Na-Krebs. Numbers of muscles are in parentheses. Correction for extracellular space based on inulin space of normal and denervated muscles incubated 1–2 hr.

* Resting membrane potential with, in parentheses (number of fibres/number of muscles).

(25–30 min), the resting potential declined in both denervated muscle and in controls (Table 4). It is likely that the surface fibres used for recording underwent the fastest change in Li and Na concentrations. However, by 2 hr, there should be considerably less difference in ion concentrations between the bulk of the muscle and surface fibres. Therefore, bulk values of cations, as well as measured resting potential were used to calculate either P_{Na}/P_K or P_{Li}/P_K ratios (where P is permeability) before and 2 hr after incubation in Li-Krebs. The constant field equation (Goldman, 1943; Hodgkin & Katz, 1949) was used in the form

$$V = \frac{RT}{F} \ln \frac{[K]_o + (P_X/P_K)[X]_o}{[K]_i + (P_X/P_K)[X]_i}, \qquad (1)$$

where X is either Na or Li in Krebs or Li-Krebs solutions, respectively, the subscripts i and o are for intra- and extracellular concentrations, respectively, V is the resting membrane potential, and R, T and F have their usual significance. Cl was assumed to be passively distributed (Williams *et al.* 1971*a, b*).

No direct data on chloride are presented here. However, the *excess* cation in denervated muscle (Li + K, denervated minus innervated), after incubation in Li Krebs, differed only slightly from the *excess* internal chloride, calculated from the resting membrane potential and assuming passive distribution. These discrepancies from the theoretical amount of chloride required to exactly balance the excess cation, could easily be accounted for by the combined errors of the four estimates (two each for Li and K) required for each value of cation excess.

In order to avoid foaming, the resting membrane potential was recorded using Krebs solution without horse serum, whereas the data on internal ion concentrations was taken from freshly removed muscle or muscle incubated in solutions containing 5 % horse serum. However, the error introduced was probably not substantial, because horse serum only increased RMP by 4 mV after 4 hr *in vitro* (Akaike, 1971), and P_{Na}/P_K ratios determined *in vivo* are close to those reported here (Williams *et al.* 1971*a, b*).

In both innervated and in 1 and 2 day denervated muscles, the computed ratio P_{Na}/P_K in a given series was generally close to that of P_{Li}/P_K, despite a variety of different membrane potentials and internal Li and K concentrations (Table 4). This result corroborated a major assumption in this study, namely that Li and Na permeabilities are similar to each other in both innervated and denervated muscle. The one exceptional case in which the two ratios were not in reasonable agreement, namely controls to 50–52½ hr denervated muscle, may be due to the small number of measured values of internal potassium. If the K values for controls to the 25½–26½ hr denervated muscle after 120 min in Li-Krebs were substituted, the resulting ratio became 0·017, in good agreement with the value for P_{Na}/P_K.

A second key point is that both P_{Na} and P_{Li} relative to P_K were elevated in denervated muscle compared to control (Table 4).

A similar result was obtained when relative P_{Li} (denervated/innervated) was derived from an average approximation of the flux equation (Hodgkin & Katz, 1949):

$$P_{\text{Li}} = M\frac{RT}{FV}\left(\frac{1 - \exp{(VF/RT)}}{[\text{Li}]_i \exp{(VF/RT)} - [\text{Li}]_0}\right), \qquad (2)$$

where P is permeability (cm/unit time), M is net movement of Li into the muscle fibres per unit time, and other terms are used as in eqn. (1). Absolute permeabilities were not computed because values for net Li flux and membrane potential were averaged over periods of 30–90 min, i.e. instantaneous values for Li influx and membrane potential were not available. Instead, only the *ratio* of approximate Li permeabilities of denervated and control muscles was computed.

For 24–27 hr denervated muscle, this permeability ratio was 2·2 and 1·25 for the incubation intervals of 30–60 min, and 60–120 min, respectively. The corresponding ratios for 45–50 hr denervated muscle were 2·82 and 2·51. Despite the lower value at one time period, the computations all affirm that Li permeability is indeed elevated in 1 and 2 day denervated muscle.

The data given above fit well with a passive mechanism for the resting membrane potential of both innervated and denervated muscle. Another test of this assertion was to replace chloride with methanesulphonate, to change external K from 5 to 1 mM, and to predict the resulting potential from eqn. (1) using the $P_{\text{Na}}/P_{\text{K}}$ ratio and internal ionic Na and K concentrations given in Table 4. Under these circumstances, the *Na-loaded* soleus muscle, which exhibits an electrogenic pump, deviates from eqn. (1) and a depolarization is seen (Fig. 4 in Akaike, 1975). However, for normal EDL muscle, the predicted resting membrane potential at $K_0 = 1$ mM was $-95·9$ mV, and the observed was $-95·4$ mV $\pm 1·0$ (12 fibres in two rats). Unfortunately, the same experiment could not be carried out in denervated muscle because of the pronounced twitching which occurred in Cl-free solutions, even in 5 mM-K.

Effects of ouabain and K-free solutions on cation movements

Ouabain causes the resting membrane potential of normal muscle to decline to a level comparable to that of denervated muscle, whereas the ouabain treated denervated muscle shows no change (Locke & Solomon, 1967; McArdle & Albuquerque, 1975; Bray *et al.* 1976). In order to relate these findings to the present results, two possibilities were explored: (1) that denervated muscle becomes relatively insensitive to ouabain, as it does to tetrodotoxin (Redfern & Thesleff, 1971), or (2) that ouabain increases P_{Na}, and thereby obscures the difference between innervated and denervated muscle.

In order to obtain base line data, innervated and 24–29 hr denervated muscles were incubated in Krebs solutions with 5 mM-K and no ouabain, and Na and K were measured at the end of the incubation. Incubation for 120 min in normal Krebs solution led to a slight gain in Na and no change in K, in innervated muscles from rats of the same body weight as those used in the remainder of the experiments itemized in Table 5. In another series using heavier (70 g) rats not shown in Table 5, both innervated and denervated muscles showed the same Na and K concentrations after 120 min in Krebs solution.

TABLE 5. Effects of ouabain and K-free solutions on Na and K concentrations of normal and 24–29 hr denervated muscle

| | m-mole/l. fibre water | | | | |
| | Na | | K | | |
	Denervated	Innervated	Denervated	Innervated	n
Fresh dissected	$14\cdot7 \pm 5\cdot6$	$8\cdot3 \pm 3\cdot5$	$168\cdot1 \pm 8\cdot4$	$157\cdot1 \pm 5\cdot0$	9
Krebs solution 120 min	—	$27\cdot9 \pm 2\cdot3$	—	$155\cdot8 \pm 3\cdot6$	5
Ouabain 7×10^{-4} M 90 min	$101\cdot4 \pm 13\cdot7$	$95\cdot4 \pm 11\cdot6$	$106\cdot9 \pm 6\cdot3$	$104\cdot0 \pm 5\cdot5$	4
Ouabain 10^{-4} M 120 min	$69\cdot9 \pm 4\cdot3$	$63\cdot7 \pm 5\cdot9$	$175\cdot0 \pm 4\cdot0$	$163\cdot4 \pm 4\cdot6$	5
K-free Krebs 90–120 min	$41\cdot3 \pm 16\cdot4$	$35\cdot9 \pm 14\cdot6$	$126\cdot6 \pm 11\cdot9$	$121\cdot6 \pm 11\cdot8$	5
K-free Krebs + ouabain 10^{-4} M 120 min	$94\cdot5 \pm 6\cdot2$	$98\cdot9 \pm 1\cdot9$	$113\cdot0 \pm 3\cdot2$	$99\cdot8 \pm 1\cdot4$	5

Data given as mean ± S.E. of the mean.

Ouabain (7×10^{-4} M) led to a net uptake of Na and loss of K, compared to control muscles (Table 5) in both normal and denervated muscle. Hence, the denervated muscle is sensitive to the drug. However, at 10^{-4} M-ouabain, there was no loss of K, even though Na gain was substantial. Even though incubation in the K-free solution had little effect on Na (although K declined), ouabain at 10^{-4} could still produce Na gain and K loss when potentiated by K-free solutions (Table 5). These results would be consistent with a unique effect of ouabain of increasing P_{Na} at 10^{-4} M and blocking Na-K exchange only with additional potentiation by K-free medium.

If ouabain increases P_{Na}, and if P_{Li} is similarly affected, then Li uptake should increase in the presence of ouabain. In fact, after 2 hr in Li-Krebs,

control muscles took up $77 \cdot 2 \pm 5 \cdot 2$ ($n = 7$) m-mole/l. fresh wt. Li in the absence, and $113 \cdot 2 \pm 3 \cdot 3$ ($n = 5$) m-mole/l. fresh wt. Li in the presence of ouabain (7×10^{-4} M). The contralateral muscles, which had been denervated 45–50 hr and were run in parallel with the controls, without and with ouabain, took up $115 \cdot 2 \pm 7 \cdot 3$ and $130 \cdot 7 \pm 5 \cdot 5$ m-mole/l. fresh wt., respectively. Thus, ouabain appeared to increase Li uptake of control muscles considerably, although denervated muscles were less affected. The latter result might be expected since P_{Li} is already increased two-to-three-fold in denervated muscle, and the internal Li concentration of $130 \cdot 7$ is approaching the limiting value of 135 m-mole/l. in the external solution.

DISCUSSION

The resting membrane potential of normal and denervated rat fast twitch muscle results from passive mechanisms with no substantial contribution of an electrogenic pump

The fall in resting membrane potential of denervated mammalian twitch muscle in the first 2–3 days after operation is not accompanied by any detectable change in specific membrane resistance. It was suggested that the early depolarization resulted from a faulty electrogenic pump, but the premise that an electrogenic pump contributes to the potential of normal muscle is not well supported. The 'constant field equation' adequately describes the RMP of mammalian muscle before and after alteration of internal Na and K (Williams *et al.* 1971 *a, b*) and over a wide range of external K concentrations (Thesleff, 1963; Akaike,1975). In confirmation, it was reported here that lowering external K to 1 mM led to a resting membrane potential predicted by the P_{Na}/P_{K} ratios computed at normal external K (5 mM), whereas a potential based on an electrogenic Na-K pump would have been depressed under this circumstance (Akaike, 1975). Finally, Akaike (1975) found in rat soleus, and also in rat EDL (personal communication) that the resting membrane potential, measured shortly after placement of the muscle in K-free Krebs solution, matched the predicted 'constant field' resting potential exactly. Although an electrogenic pump would be inhibited in K-free medium, residual K present in whole muscle could maintain some pump activity. Therefore, the results in K-free solutions argue against but do not entirely rule out an electrogenic pump component to the resting membrane potential.

In another line of evidence, it was found that after substitution of Li for Na and subsequent alteration in internal K, a P_{Li}/P_{K} ratio close to the normal P_{Na}/P_{K} ratio was computed. Yet replacement of Na by Li should have blocked an electrogenic pump, assuming the pump response to internal Li is as small as that of the Na-K exchange pump (Keynes &

Swan, 1959). Thus, the resting membrane potential of mammalian muscle is adequately predicted by the constant field equation under a wide variety of experimental circumstances, although it may show an electrogenic component under conditions of Na loading (Akaike, 1975). In addition, in frog muscle, the intracellular K *activity* can be used in the Nernst equation to predict the membrane potential with reasonable accuracy (Khuri, Hajjar & Agulian, 1972). On the assumption that rat muscle is similar in this respect, and that substantial cell water is not bound (Armstrong & Lee, 1971), the use of measured K concentrations in the 'constant-field equation' appears to be legitimate (see below for discussion of Na).

Supportive evidence for an electrogenic component to the resting membrane potential is firstly, a large temperature coefficient (Nakanishi & Norris, 1970) and secondly, the observation that ouabain rapidly depresses the resting membrane potential by some 15 mV. The temperature coefficient in the range 23–36° C, however, was not observed by many others (Li, 1958; Creese, Scholes & Taylor, 1958; Klein, Haddow, Kind & Cockburn,1968), and may be explained by temperature-dependent changes in membrane conductance observed by Boyd & Martin in cat muscle (1959). The effect of ouabain may also depend on a conductance change, in particular to Na, rather than on blockade of an electrogenic pump. Indeed, in both this work and in the report of Rogus & Zierler (1973), it was found that in rat EDL muscles, lower doses of ouabain appeared to increase Na uptake selectively, whereas higher doses impaired the Na-K pump as well. In the present study, ouabain at 10^{-4} M increased Na uptake with no effect on K loss until a higher dose was employed. Suggestive evidence was also given that ouabain increased Li uptake in normal muscle after incubation in Li Krebs, again by analogy pointing to an increased Na permeability. Furthermore, the resting membrane potential of surface fibres of one set of normal muscles (controls to 50 hr denervated muscles, Table 4) showed only a 6·4 mV depolarization 30 min after transfer to Li-Krebs, although under these conditions, internal Na was replaced by Li (see Results) and an electrogenic pump would have been blocked. If the pump were inactivated by denervation, the resting membrane potential of normal muscle in Li-Krebs solutions should at least have fallen some 15·6 mV to − 64·4, the resting membrane potential of the corresponding 50 hr denervated muscle (recorded in Na-Krebs solution). In sum, the resting membrane potential of normal muscle appears to result from passive mechanisms only. Therefore, it is likely that the membrane depolarization of denervated muscle can be explained in terms of the same mechanisms.

The early depolarization of denervated muscle results from increased Na permeability

If the resting membrane potential of normal muscle can be predicted from P_{Na}, P_K, and P_{Cl}, then, in the absence of substantial change in internal ionic concentrations, one or more of these permeabilities must change in the first 3 days after denervation in order to produce a depolarization of some 15 mV. Cl is an unlikely candidate because it appears to be largely passively distributed in mammalian muscle (Williams *et al.* 1971*a*). Also, some 90 % of the resting conductance derives from Cl conductance in rat diaphragm and EDL (Palade & Barchi, 1975; Camerino & Bryant, 1976). A decrease in P_{Cl} sufficient to depolarize the resting membrane potential by 15 mV would be readily apparent in the measurements of total membrane resistance, but these are unaltered shortly after denervation (Albuquerque & Thesleff, 1968; Albuquerque *et al.* 1971; Albuquerque & McIsaac, 1970). This leaves changes in P_{Na} or P_K as the likely causes of the early depolarization.

Studies of resting membrane potential of normal and denervated muscle before and after incubation in Li-Krebs provides evidence that the ratio P_{Na}/P_K or P_{Li}/P_K is elevated more than two-fold 1-2 days after denervation. The greater Li uptake of denervated muscle (Table 2) and computations of relative P_{Li}, denervated/innervated, both indicate that increased Na permeability rather than decreased K permeability forms the basis of the increased P_{Na}/P_K ratio. In this connection, it was shown that the extra Li taken up by denervated muscle did not reside in a freely exchangeable compartment (e.g. sarcotubule) nor was it irreversibly bound to cell protein. Ultrastructural data on 1-day denervated rat gastrocnemius suggests that sarcotubular area is slightly decreased (Stonnington & Engel, 1973) and therefore could not account for the increased Li uptake.

Evidence obtained by Albuquerque & Warnick (1972) in another context also suggests that increased Na permeability accounts for the early depolarization of denervated muscle. 1 day denervated rat EDL muscle showed a significant hyperpolarization either when external sodium was reduced to 5 mM or in the presence of tetrodotoxin, which in squid axon reduces resting Na permeability (Freeman, 1971). The negligible effects of low external Na or tetrodotoxin on *innervated* muscle may be due to the already low P_{Na}/P_K ratio, which minimizes the effects of further decrements in $[Na]_0$ or P_{Na}. By the same token, ouabain may increase P_{Na} of normal muscle far more than denervated if a common set of gates is involved, since a larger proportion of available gates may be open in the denervated muscle. It is unlikely that this differential response to ouabain was due to insensitivity to the drug following denervation, because equal

effects of ouabain on the non-electrogenic Na-K pump were observed in both denervated and control muscles (see Table 5).

It might be argued that the greater Li uptake of denervated muscle was due to fibrillation. However, the increased uptake was noted at 24 hr after denervation, when fibrillation has not yet appeared (Salafsky, Bell & Prewitt, 1968). In addition, fibrillation stops shortly after immersion of denervated muscle at room temperature (Miledi & Slater, 1968), yet Li influx in 45–50 hr denervated muscle relative to control is increased both from 30 to 60 min incubation and from 60 to 120 min. Finally, the increased P_{Li}/P_K ratio in denervated muscle could not result from fibrillation. Thus, different lines of evidence appear to rule out fibrillation as an important variable in the results reported here.

Certain qualifications should be made in the major conclusion that the increased P_{Na} accounts for the initial membrane depolarization after denervation. First, it is likely that the measured 'internal Na' is larger than the true sarcoplasmic activity (Caldwell, 1968; Rogus & Zierler, 1973). If the effective intracellular Na activity coefficient is taken as 0·2 (Armstrong & Lee, 1971) and that of K about 0·72 (see Discussion above), the 'constant field equation' still yields essentially the same P_{Na}/P_K ratios reported in Table 4. Also, if the Li taken up by the cell has an activity coefficient of 0·2 for the first 10 m-equiv/l. in the sodium space, and of a free solution (0·7) for the remainder, again the computed P_{Li}/P_K ratios agree well with those in Table 4. Thus, the finding of an elevated P_{Na}/P_K and P_{Li}/P_K ratio in denervated muscle is not substantially affected by the sequestration of sodium or a small fraction of the Li taken up.

Secondly, it is not clear whether all denervated mammalian muscles depolarize by virtue of increased Na permeability. Kovacs, Vissy & Went (1968) found *in vivo* that denervated EDL showed increased Li uptake 4 days after denervation, consistent with the *in vitro* results reported here. However, the denervated soleus muscle showed no change in Li uptake *in vivo*. This result may reflect reduced blood flow due to the inactivity of this normally vascular and tonically active red muscle. *In vitro* studies might decide the issue. In any event, the rat diaphragm, which has a majority of 'red' fibres by histochemical criterion (Gauthier & Padykula, 1966), shows an increased Li uptake *in vitro* following denervation (Table 2).

Thirdly, internal Na was not significantly altered 1 or 2 days after denervation, and yet P_{Na} is apparently increased some twofold. Therefore, increased Na efflux must balance the increased influx in denervated muscle but this prediction is difficult to verify experimentally. For instance, ^{22}Na fluxes are complicated by compartmentalization of Na (Rogus & Zierler, 1973). Another approach, namely, blocking the Na-K pump should

increase [Na]$_i$ more in denervated than in control muscle. However, the use of ouabain or K-free solutions introduce new variables such as altered P_{Na} or residual or leakage K preserving pump activity, respectively. Osmotic changes in K-free solutions may further complicate the analysis (Akiyama & Grundfest, 1971). Finally, measurement of [Na]$_i$ is only approximate because of uncertainties as to extracellular or osmotically inactive components, and small differences would be difficult to detect in bulk analyses. Indeed, consideration of these problems led to the choice of Li movements as the main focus in this study.

As noted in the introduction, the fall in resting membrane potential is a very early event following denervation (Albuquerque *et al.* 1971; Bray *et al.* 1976). On the basis of the present study, it appears that increased Na permeability, which underlies the reduced resting membrane potential is one of the most sensitive parameters of denervation. The earliest onset of increased P_{Na} would not be detected by the use of Li uptake studies, since the initial locus of change is confined to the end-plate region of the denervated muscle fibre (Albuquerque *et al.* 1971) and the membrane area showing increased P_{Li} would be small in proportion to the total. Indeed, it is possible, but unlikely, that the earliest depolarization differs in mechanism from that found 24 and 48 hr after denervation.

REFERENCES

AKAIKE, N. (1971). Maintenance of sodium and potassium ion in rat skeletal muscle fibres by serum. *Jap. J. Physiol.* **21**, 201–208.

AKAIKE, N. (1975). Contribution of an electrogenic sodium pump to membrane potential in mammalian skeletal muscle fibres. *J. Physiol.* **245**, 499–520.

AKIYAMA, T. & GRUNDFEST, H. (1971). The hyperpolarization of frog skeletal muscle fibres induced by removing potassium from the bathing medium. *J. Physiol.* **217**, 33–60.

ALBUQUERQUE, E. & McISAAC, R. J. (1970). Fast and slow mammalian muscles after denervation. *Expl Neurol.* **26**, 183–202.

ALBUQUERQUE, E. X., SCHUH, F. T. & KAUFFMANN, F. C. (1971). Early membrane depolarization of the fast mammalian muscle after denervation. *Pflügers Arch. ges. Physiol.* **328**, 36–50.

ALBUQUERQUE, E. X. & THESLEFF, S. (1968). A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscles of the rat. *Acta physiol. scand.* **73**, 471–481.

ALBUQUERQUE, E. X. & WARNICK, J. E. (1972). The pharmacology of batrachotixin. IV. Interaction with tetrodotoxin in innervated and chronically denervated rat skeletal muscle. *J. Pharmac. exp. Ther.* **180**, 683–697.

ARMSTRONG, W. McD. & LEE, C. O. (1971). Sodium and potassium activities in normal and 'sodium-rich' frog skeletal muscle. *Science, N.Y.* **171**, 413–415.

BOYD, I. A. & MARTIN, A. R. (1959). Membrane constants of mammalian muscle fibres. *J. Physiol.* **147**, 450–457.

BRAY, J. J., HAWKEN, M. J., HUBBARD, J. I., POCKETT, S. & WILSON, L. (1976). The membrane potential of rat diaphragm muscle fibres and the effect of denervation. *J. Physiol.* **255**, 651–667.

CALDWELL, P. C. (1968). Factors governing movement and distribution of inorganic ions in nerve and muscle. *Physiol. Rev.* **48**, 1–64.

CAMERINO, D. & BRYANT, S. H. (1976). Effects of denervation and cochicine treatment on the chloride conductance of rat skeletal muscle fibers. *J. Neurobiol.* **7**, 221–228.

CREESE, R. (1968). Sodium fluxes in diaphragm muscle and the effects of insulin and serum proteins. *J. Physiol.* **197**, 255–278.

CREESE, R., EL-SHAFIE, A. L. & VRBOVA, G. (1968). Sodium movements in denervated muscle and the effects of antimycin a. *J. Physiol.* **197**, 279–294.

CREESE, R., SCHOLES, N. W. & TAYLOR, D. B. (1968). Temperature and resting potential of diaphragm muscle in a CO_2-bicarbonate medium. *J. Pharmac. Exp. Ther.* **124**, 47–52.

DAVSON, H. (1970). *A Textbook of General Physiology*, p. 537. Baltimore: Williams & Wilkins.

FREEMAN, A. R. (1971). Electrophysiological activity of tetrodotoxin on the resting membrane of the squid giant axon. *Comp. Biochem. Physiol.* **40A**, 71–82.

GAUTHIER, G. F. & PADYKULA, H. A. (1966). Cytological studies of fiber types in skeletal muscle. *J. cell Biol.* **28**, 333–354.

GOLDMAN, D. E. (1943). Potential, impedance, and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

HINKE, J. A. M., CAILLE, J. P. & GAYTON, D. C. (1973). Distribution and state of monovalent ions in skeletal muscle based on ion electrode, isotope, and diffusion analyses. *Ann. N.Y. Acad. Sci.* **204**, 274–296.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

KEYNES, R. D. & SWAN, R. C. (1959). The permeability of frog muscle fibres to lithium ions. *J. Physiol.* **147**, 626–638.

KHURI, R. N., HAJJAR, J. J. & AGULIAN, S. K. (1972). Measurement of intracellular potassium with liquid ion-exchange microelectrodes. *J. appl. Physiol.* **32**, 419–422.

KLAUS, W., LULLMAN, H. & MUSCHOLL, E. (1960). Kalium-Flux des normalen und denervierten Rattenzwerchfells. *Pfülgers Arch. ges. Physiol.* **271**, 761–775.

KLEIN, R., HADDOW, J. E., KIND, C. & COCKBURN, F. (1968). Effect of cold on muscle potentials and electrolytes. *Metabolism* **17**, 1094–1103.

KOBAYASHI, N. & YONEMURA, K. (1967). The extracellular space in red and white muscles of the rat. *Jap. J. Physiol.* **17**, 698–707.

KOVACS, T., VISSY, A. & WENT, E. (1968). The effect of denervation on ion transport in tonic and tetanic skeletal muscles of the rat. *Acta physiol. hung.* **33**, 55–68.

LI, C. L. (1958). Effect of cooling on neuromuscular transmission in the rat. *Am J. Physiol.* **104**, 200–206.

LOCKE, S. & SOLOMON, H. C. (1967). Relation of resting potential of rat gastrocnemius and soleus muscles to innervation, activity and the Na-K pump. *J. exp. Zool.* **166**, 377–386.

McARDLE, J. J. & ALBUQUERQUE, E. X. (1975). Effects of ouabain on denervated and dystrophic muscles of the mouse. *Expl Neurol.* **47**, 353–356.

MILEDI, R. & SLATER, C. R. (1968). Electrophysiology and electron microscopy of rat neuromuscular junctions after nerve degeneration. *Proc. R. Soc. B.* **169**, 289–306.

MILEDI, R. & SLATER, C. R. (1970). On the degeneration of rat neuromuscular junctions after nerve section. *J. Physiol.* **207**, 507–528.

NAKANISHI, T. & NORRIS, JR., F. H. (1970). Effect of local temperature on the resting membrane potential in rat muscle. *Electroenceph. clin. Neurophysiol.* **28**, 633–636.

PALADE, P. T. & BARCHI, R. L. (1975). Chloride conductance in rat diaphragm fibers. *Neuroscience Abstracts* (Soc. for Neuroscience) **1**, 636.

REDFERN, P. & THESLEFF, S. (1971). Action potential generation in denervated rat skeletal muscle. *Acta physiol. scand.* **82**, 70–78.

ROGUS, E. & ZIERLER, K. L. (1973). Sodium and water contents of sarcoplasm and sarcoplasmic reticulum in rat skeletal muscle: effects of anisotonic media, ouabain and external sodium. *J. Physiol.* **233**, 227–270.

SALAFSKY, B., BELL, J. & PREWITT, M. A. (1968). Development of fibrillation potentials in denervated fast and slow skeletal muscle. *Am. J. Physiol.* **215**, 637–643.

STONNINGTON, H. H. & ENGEL, A. G. (1973). Normal and denervated muscle, a morphometric study of fine structure. *Neurology, Minneap.* **23**, 714–724.

THESLEFF, S. (1963). Spontaneous electrical activity in denervated rat skeletal muscle. In *The Effect of Use and Disuse in Neuromuscular Functions*, ed. GUTMANN, E. & HNIK, P., pp. 41–51, Amsterdam: Elsevier.

WILLIAMS, J. A., WITHROW, C. D. & WOODBURY, D. M. (1971*a*). Effects of ouabain and diphenylhydantoin on transmembrane potentials, intracellular electrolytes, and cell pH of rat muscle and liver *in vivo*. *J. Physiol.* **212**, 101–115.

WILLIAMS, J. A., WITHROW, C. D. & WOODBURY, D. M. (1971*b*). Effects of nephrectomy and KCl on transmembrane potentials, intracellular electrolytes, and cell pH of rat muscle and liver *in vivo*. *J. Physiol.* **212**, 117–128.

YONEMURA, K. & SATO, M. (1967). The resting membrane potential and cation movement in frog muscle fibers after exposure to lithium ions. *Jap. J. Physiol.* **17**, 687–697.