THE EFFECT OF CHANGES OF ENVIRONMENT ON THE ELECTRICAL AND IONIC PATTERN OF MUSCLE

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In recent years the interrelationship between the ionic pattern and the electrical properties of nerve and muscle has been the subject of much inquiry (7, 14); for other references, see Grundfest *et al.* (10). The separation of Na^+ and K^+ by the living cell is held to result from a Donnan type distribution maintained by a metabolically mediated extrusion of Na^+ (1, 3, 30). The resting potential is said to arise from this unequal distribution of ions, particularly the K^+ gradient, in a fashion predictable by the Nernst equation. The effect of Na^+ and Cl^- on this potential has been precisely predicted by Goldman (9).

The active phase of nerve and muscle is ascribed to a specific alteration in the permeability of the membrane first to Na^+ ions and then to K^+ ions. The membrane is discharged to a value which is close to the Na^+ diffusion potential.

The validity of the Donnan theory of K^+ retention has been questioned by many authors. (For bibliography, see Shaw and Simon (25).) The nature of the electrical phenomenon has also been held in doubt (10, 11, 23). However, the mathematical treatment of these latter authors, in particular, has been based on the assumption of a Donnan distribution of K^+ , with a Na^+ "pump." It would consequently seem of prime importance to test these basic assumptions, so that future work will not be based on doubtful premises.

Apparatus and Methods

The resting and action potentials were measured with 3 molar KCl microcapillary liquid junctions. Tip diameter was about $0.5\ \mu$ and electrical resistance varied from 10 to 20 megohm.

The recording equipment consisted of an input cathode follower electrometer (6 AK 5) with a second cathode follower as a buffer stage. The Ag/Ag Cl electrode fitted into a clip attached to the end of the grid pin of the input valve. The input tube was placed in a double shielded can in the same manner as described by Hodgkin and Katz (16). The output from the input stage was connected to two d.c. amplifiers in parallel. One was used to drive a chart recorder for accurate reading of all d.c. levels and the second was connected to a single beam cathode ray oscilloscope.

Voltage calibration was carried out with precision resistors and a standard cell. A fixed voltage (90 mv.) could always be introduced through the indifferent electrode. During an experiment calibration was carried out at regular intervals. A multivibrator circuit was used for time calibration.

A square wave voltage generator was used for stimulation. Its output was isolated from the bath by a special transformer. This generator also provided a square pulse which could be introduced through the indifferent electrode to check the input time constant of the apparatus with the electrode in position, and hence the resistance of the tip. The time constant varied from 75 to 150 μ sec. Low resistance Ag/AgCl electrodes were used at both liquid-metal interfaces. Movable platinum electrodes were used for stimulation.

All the manipulation was carried out under microscope control. The muscle was placed in the bottom of a perspex dish on the stage of a Zetopan microscope. The input head with its attached microtip was held fixed at an angle of 45°. Movement of the microscope stage in 3 dimensions was available and was found satisfactory for impalement of the fiber.

The sartorius muscle of the toad *Bufo marinus* was used throughout this work. Muscles were dissected with great care, and all insertions were left intact. The fibers lying on the top 4 to 5 layers were studied. Unfortunately, this muscle in the toad is thoroughly invested with connective tissue, which varies considerably among different specimens and at different times of the year. At times it is "stringy" and difficult to penetrate with all but the sharpest tips. At other times it is "sticky" and seems to block tips frequently. This problem did not arise in studies on several varieties of frogs, where connective tissue appeared to be looser and superficial. However, frogs were not available in the numbers necessary for the ionic analyses.

The ionic analyses were carried out using a Beckman flame spectrophotometer, and the methods used were similar to those described previously (25). In all experiments concerned with ionic changes 12 pairs of muscles were used. Mean values were taken, and the significance of differences between control and treated determined by a "t" test. Controls were in all cases companion muscles and, unless otherwise stated, were immersed in normal Ringer for a period of time equivalent to that of the treated. In all tables of ionic content the wet weight figures have been referred to the intracellular level. Consequently a correction has been made for the effect of alteration of the composition of the extracellular fluid. This effect has not been allowed for in dry weight figures, which will include alterations in extracellular composition as well as intracellular changes. The extracellular space has been taken as 15 per cent, except in muscles showing swelling of more than 15 per cent of the total volume. In these cases the extracellular volume has been assumed to be 10 per cent. No correction has been made for the distribution of solids between the two phases.

The justification for assuming 15 per cent extracellular space has been discussed previously (25), when it was pointed out that the maximum value of the extracellular space in low Na^+ muscles was set at that value which would give zero intracellular Na^+ (a negative value being impossible). This maximum value was of the order of 10 per cent. The maximum value which was found in low Na^+ muscles has been confirmed in muscles of low Cl^- content. Plasma Cl^- and muscle Cl^- have been estimated in the same animal, and the lowest muscle Cl^- obtained was 10.6 m.eq./

kg., with a plasma Cl of 86.8 m.eq./liter. The maximum extracellular space in this example, assuming zero intracellular Cl, would be 12.2 per cent (unpublished results). This value agrees well with that calculated by Fenn *et al.* (5), using the same method; *viz.*, 14.5 per cent.

The fact that a similar upper limit to the extracellular space in some muscles is set by two different methods is some justification for choosing a value around 15 per cent. Undoubtedly the extracellular space is a variable, but, if in some muscles it has a maximum value of 10 per cent (it is natural to suppose that some muscles would have a value lower than this), then it is reasonable to assume a mean of 15 per cent. A value as high as 30 per cent could possibly be achieved by a few muscles.

Figures for bioelectric potentials were obtained from the mean of many penetrations, and are shown $\pm$ the standard error, with the number of observations given in parentheses. Muscles were placed in the test solutions for at least 1 hour before the listed potentials were obtained. Although the potentials reached a constant value after the first 10 minutes, ionic equilibrium throughout the muscle would not be complete in less than 1 hour. Surface fibers, in which potentials were recorded, would achieve a steady state more rapidly. Muscles were analyzed for Na^+ and K^+ at the end of each experiment.

Ringer solutions used have been described by Shaw, Holman, and Mackenzie (24), and were buffered with phosphate or bicarbonate. To maintain the bicarbonate Ringer at physiological pH it is necessary to aerate continuously with carbogen (5 per cent CO_2 in oxygen). It was not always convenient to do this when working with microtips, so most of this work was carried out with phosphate-buffered Ringer. In low Na Ringer the osmotic pressure was made up with sucrose.

RESULTS

The Effect of Bicarbonate and Phosphate Buffers on the Resting Potential and the Ionic Pattern.—The resting potential (R.P.) measured with the sartorius muscle immersed in phosphate Ringer gave a value of 92 mv. $\pm$ 0.5. A series of impalements in bicarbonate Ringer gave 94 mv. $\pm$ 0.5. When CO_2 was bubbled through phosphate Ringer (the pH did not fall below 6.8) a small but statistically insignificant increase in the level of the resting potential was found. These results are at variance with those of Desmedt (4) who found a lower resting potential in bicarbonate Ringer. Ling and Gerard (20) had also described a depolarizing effect of CO_2 on frog muscle.

The effect of these buffers on the Na^+ and K^+ content of the muscle was determined by the usual procedure. Six pairs of muscles were used, and one of each pair was soaked in either of the two solutions for 4 hours. On analysis no significant difference in Na^+ and K^+ content was found between the two groups.

The Effect of Hydrogen Ion Concentration.—A series of estimations of the R.P. were carried out in phosphate Ringer, the pH of which was varied between 6.5 and 8.5. Solutions were checked on a Jones pH meter before addition and after removal from the tissue bath. No significant change in R.P.

was observed within this range of hydrogen ion concentration, either by the examination of scatter diagrams of individual experiments or from calculation of mean values. When, however, the hydrogen ion concentration was increased to pH 5.8 the R.P. fell off rapidly, and after 10 minutes an average value of 60 mv. was obtained.

The effect of hydrogen ion concentration on the ionic content of muscle has been reported elsewhere (25) and is in distinction to the findings with the intracellular electrode. The graph of K^+ content against pH shows an increase in K^+ level with decreasing hydrogen ion concentration, and shows no marked alteration in shape in the region of pH 5.8. The Na^+ curve passes through a minimum at pH 7.2, and rises continuously at higher and lower hydrogen ion concentrations.

The Effect of Alteration of the External K Level.—The accumulation of K^+ in the cell has in the past been held to be due to a Donnan distribution (1, 3), which resulted in a potential predicted by the Nernst or Goldman equations (9). This concept rests on two experimental procedures. First, Conway found that alteration in the level of external K^+ resulted in an alteration in internal K^+ such as could be theoretically predicted. Second, it has been found (2, 13, 17, 20, 22) that an alteration in the external K^+ resulted in a concomitant variation in resting potential. In these experiments, and in others of a similar nature, no analyses were made to determine the ratio K^+_{in}/K^+_{out} , indeed it was assumed that K^+_{in} did not change, although Conway has shown that this was not so.

The problem has been reinvestigated, as our earlier results (29) had shown that if a muscle were soaked in K^+ -free solution there was no change in the internal K^+ compared with the companion muscle in normal Ringer. This finding is, of course, at variance with Conway's theory.

The plan of our experiments is set out in the diagram below:

The experiments have been divided into two series. In one series the Na^+ content of the Ringer has been reduced by an amount osmotically equivalent to the added K^+ (the Cl^- remaining constant), and in the second series the Na^+ content has been held constant and the K^+ and Cl^- content raised. The effects of this alteration in tonicity are reported below. The procedure

was further subdivided so that (a) the muscles were removed from the animal and placed immediately in the high K^+ Ringer (non-equilibrated) and (b) they were placed in normal Ringer for 2 hours, and then removed to the high K^+ Ringer (equilibrated) for a further 3 to 4 hours. Control muscles were analyzed at the end of the equilibration period (2 hours). Muscles placed in normal Ringer lose K^+ during the first 2 hours, and then tend to achieve a steady state with regard to their ionic content. It was our intention to see whether we could influence this loss, or alter the steady state level of the cell. It had been shown (1, 19, 21) that there was a "threshold" level of K^+ in the Ringer which would prevent this initial leakage.

The results of our experiment are set out in Table I. In the first part of this table the muscles were placed in Ringer which had been rendered hypertonic by the addition of KCl to normal Ringer. The effects of these solutions are shown with and without previous equilibration. It will be seen that there has been no volume change, although the tonicity of the solution has been increased by as much as one-third. Raising the external K^+ level to 12.5 and 25 m.eq./liter has not prevented the leakage of K^+ from the cell, nor caused a return of K^+ to the cell, in the non-equilibrated and equilibrated series respectively. At 50 m.eq./liter (18 times normal) there has been a moderate increase in the K^+ level compared with muscles in normal Ringer, in both series.

There has been no change in the Na^+ level of the tissue, save for a slight drop in the unequilibrated series at the highest level of external K^+ .

The second part of Table I shows the results of experiments in which, as KCl was added to the Ringer, an equivalent amount of NaCl was subtracted. These findings differ in three respects from those of the previous series.

First, there was a definite increase in volume in the non-equilibrated series of about 17 per cent, when the Na^+ in the Ringer was lowered to 100 m.-eq./liter or less. The degree of swelling was less in the equilibrated series, being 11.7 per cent as compared with 17.0 per cent.

Second, when the results are considered on a wet weight basis there has been no change in the internal K^+ level, save for a slight gain at the highest external K^+ level. As the muscles had increased in volume there was, however, an actual migration of K^+ across the membrane, which was evident from the results referred to a dry weight basis.

The third point brought out in these experiments is the effect of high K -low Na Ringer on the internal Na^+ level. In both series there was a marked decrease in the Na^+ content of the cell, which was greater than would have been expected from the actual decrease in the Na^+ level of the Ringer. This effect is shown in Table II. The normal ratio Na^+_{out}/Na^+_{in} is approximately 3, but under these experimental conditions it has increased to as much as 7.6.

A direct comparison of the effects of normal and reduced Na^+ in high K^+ Ringer is shown in Table III. The results, which are derived from 6 pairs of muscles, confirm those which had been obtained separately above. The

TABLE I
The Effect of High K Ringer on Ionic Content of Muscles

Na level of Ringer not altered							
K in Ringer, <i>m.eq./liter</i>			Equilibrated		Not equilibrated		
			12.5	50	12.5	25	50
Volume change, <i>per cent</i>			+1.7	+0.6	+2.3	-1.6	+0.6
Na, <i>m.eq./kg.</i>	Wet weight	Control	36.1	43.2	43.0	34.7	29.3
		Treated	35.8	44.0	39.4	30.5	24.2*
	Dry weight	Control	260	302	272	246	230
		Treated	259	323	267	216	200
K, <i>m.eq./kg.</i>	Wet weight	Control	86.3	67.7	90.0	80.3	69.0
		Treated	85.2	81.4*	91.2	84.9	94.4*
	Dry weight	Control	379	313	385	353	288
		Treated	386	413*	392	380	438*

Na level of Ringer reduced proportionately							
K in Ringer, <i>m.eq./liter</i>			Equilibrated		Not equilibrated		
			25	50	12.5	25	50
Volume change, <i>per cent</i>			+0.7	+11.7	+4.4	+15.2	+17.0
Na, <i>m.eq./kg.</i>	Wet weight	Control	38.5	37.6	44.8	51.4	40.1
		Treated	30.8*	16.7*	34.8*	39.1*	15.8*
	Dry weight	Control	284	274	356	371	380
		Treated	209*	105*	306*	219*	175*
K, <i>m.eq./kg.</i>	Wet weight	Control	79.9	90.2	80.0	81.9	85.1
		Treated	85.8	99.0*	91.2	86.7	102*
	Dry weight	Control	372	400	449	350	365
		Treated	423*	528*	497*	478*	601*

In this and other tables of ionic content wet weight values are calculated at intracellular level on a basis of 15 per cent extracellular volume. Each figure is the mean of at least 12 observations. Only figures marked with an asterisk are significantly different from control ($P < 0.05$). K content of Ringer and volume changes refer to treated muscles only.

muscles in the low Na Ringer increased in volume by 15.2 per cent. There was no difference in the K^+ content on a wet weight basis, but dry weight figures indicate that some K^+ had crossed the membrane in the low Na^+

muscles. (Dry weight figures in this series may be compared directly, as the extracellular K^+ included in the figures is the same in both cases.) The latter muscles also showed an extrusion of Na^+ , increasing the ratio Na^+_{out}/Na^+_{in} , as was found previously.

Bioelectric Changes.—The concept of a Donnan type distribution of K^+ has led to the belief that the resting potential is a " K^+ diffusion potential," modified by the effect of Na^+ and Cl^- permeability (9). The effect of

TABLE II
The Effect of High K—Low Na Ringer on Ratio $\frac{Na_{out}}{Na_{in}}$

K in Ringer <i>m.eq./liter</i>	Na in Ringer <i>m.eq./liter</i>	Ratio $\frac{Na_{out}}{Na_{in}}$	
		Equilibrated	Not equilibrated
12.5	117.5	—	3.6
25	105	2.5	4.6
50	80	3.3	7.6

TABLE III
A Comparison of the Effect of High K—Low Na Ringer with High K—Normal Na Ringer on Ionic Content

Na in Ringer <i>m.eq./liter</i>	Volume change <i>per cent</i>	Na, <i>m.eq./kg.</i>		K, <i>m.eq./kg.</i>	
		Wet weight	Dry weight	Wet weight	Dry weight
130	Nil	44.4	331	96.8	459
80	+15.2	20.0	192	93.4	559*

One muscle was placed in Ringer containing K 50 m.eq./liter and Na 130 m.eq./liter, and the companion placed in K 50 m.eq./liter and Na 80 m.eq./liter, for 4 hours. The difference in wet weight K found is not significant.

* Significant.

alteration of the external K^+ level on the R.P. has been one of the strongest arguments in the justification of this belief.

This experiment has been repeated by us, and the results of the measurements of the R.P. in concentrations of K^+ varying from 0 to 54 m.eq./liter are given in Fig. 1. At a concentration of more than 13.5 m.eq./liter K^+ A.P. were obtained only during the first few minutes. After this time the muscle was inexcitable but the R.P. remained constant for several hours. It will be noted that it is only after this point that there is a linear relationship between the R.P. and the log of the external K^+ concentration.

This investigation was now extended along the lines set out in the plan

on page 266. The results may be seen in Table IV. The following points should be noted: (1) The observed R.P. are always more than 35 per cent higher than the potential predicted from the Nernst equation on the basis of the ionic analyses which were carried out at the end of each experiment. (2) The observed R.P. were the same irrespective of whether the muscles had been previously equilibrated in normal Ringer or not. (3) In high

FIG. 1. The effect of variation in the external K^+ level on the resting potential. The vertical lines represent confidence limits at 5 per cent. There was a minimum of 30 readings for each point.

K^+ Ringer there was no significant difference in R.P., whether Na^+ was normal or reduced by an amount equivalent to the added K^+ .

The ratio Na^+_{out}/Na^+_{in} was not always greater in reduced Na^+ solutions, as would have been expected from the results presented in the previous section. This may have been due to the fact that these results were collected from experiments carried out over several months, when the initial ionic content of the muscles would be expected to vary (see reference 26). In order to obviate this difficulty, experiments were performed using paired muscles. One member was placed in normal Na -high K Ringer, and the companion

muscle was placed in low Na-high K Ringer. The results of these experiments are set out in Table V. It will be seen that in 4 of the 5 experiments the low Na-high K Ringer-treated muscles showed an increase in Na^+ gradient. The

TABLE IV
The Effect of High K Ringer on the Resting Potential

Not equilibrated						
K in Ringer <i>m.eq./liter</i>	Na in Ringer <i>m.eq./liter</i>	Resting potential		K <i>m.eq./kg.</i>	Na <i>m.eq./kg.</i>	Ratio $\text{Na}_{\text{out}}/\text{Na}_{\text{in}}$
		Calculated	Observed			
13.5	130	45.8	57.4 ± 1.2 (15)	83.8	45.0	2.9
		37.1	52.4 ± 1.7 (5)	60.0	54.9	2.4
13.5	117	44.3	53.2 ± 2.2 (7)	80.2	49.0	2.4
		21.6	57.0 ± 1.8 (9)	51.2	55.0	2.1
30	130	27.2	36.9 ± 0.7 (29)	87.5	51.8	2.5
30	100	27.0	36.3 ± 0.26 (30)	84.6	22.3	4.6
50	130	18.9	31.5 ± 0.6 (15)	106	23.0	5.7
50	80	21.0	33.0 ± 1.8 (9)	115	11.5	6.9
Equilibrated						
27	130	36.3	38.5 ± 0.4 (20)	114	57.1	2.3
30	130	28.6	38.0 ± 1.1 (29)	93.4	47.1	2.8
32	130	29.6	37.6 ± 0.7 (11)	105	40.0	3.2
		29.5	40.0 ± 0.9 (17)	104	56.0	2.5
32	98	26.3	35.0 ± 0.6 (12)	92.0	23.1	3.3
		27.4	32.1 ± 0.6 (21)	96.5	15.3	6.5
30	100	27.6	37.7 ± 0.4 (31)	89.6	34.6	2.9
50	130	19.4	32.5 ± 0.4 (26)	108	23.6	5.5
		18.4	29.3 ± 0.5 (23)	104	20.0	6.5
50	80	13.3	28.7 ± 0.5 (21)	84.6	15.7	5.1
		9.5	31.0 ± 1.1 (21)	72.8	14.9	5.4

In this and subsequent tables bioelectric potentials are shown $\pm$ s.e. The number of observations is given in parentheses. Ionic contents are referred to intracellular levels.

drop in R.P. in this series compared with that found in the normal Na-high K-treated muscles is small, but was statistically significant at the 5 per cent level.

The significance of these results for the prediction of the R.P. by the Goldman equation will be referred to in the discussion.

The Effect of Alteration of the External Na Level.—It would appear that up to the present time there has not been an adequate investigation of the alterations in internal Na^+ in response to alterations of the level of this ion in the external environment. Fenn (5) did not calculate his Na^+ contents on an intracellular basis. If one makes this correction then his results are in agreement with those listed below. This would seem of great importance in the elucidation of the nature of Na^+ extrusion from the cell, and also in determining the relationship between the Na^+ gradient and the A.P. It is held that

TABLE V
The Effect of Alteration in Na Gradient on the Resting Potential in High K Ringer
K 46 m.eq./liter ($\times 17$).

Na_{in} Ringer	Resting potential	Na, m.eq./kg. wet weight	K, m.eq./kg. wet weight	Ratio
130	29.1 ± 0.6 (30)	38.2	82.2	3.4
85	29.0 ± 0.8 (25)	10.3	102	8.2
130	32.8 ± 0.8 (26)	45.9	63.0	2.8
85	29.5 ± 0.6 (28)	9.9	96.2	8.6
130	33.6 ± 0.6 (29)	50.4	99.5	2.6
85	32.5 ± 0.8 (25)	17.8	96.5	4.8
130	31.9 ± 0.7 (28)	57.8	103	2.2
85	28.5 ± 0.9 (24)	41.4	92.2	2.1
130	31.5 ± 0.7 (31)	41.0	96.7	3.2
85	30.0 ± 0.5 (31)	10.6	101	7.6

Ionic contents refer to intracellular levels. Ratio means $\frac{\text{Na}_{\text{out}}}{\text{Na}_{\text{in}}}$.

during the passage of the spike the membrane is discharged to a value close to that of the " Na^+ diffusion potential." This concept has rested largely on experiments relating the alteration in external Na^+ to the rise of the overshoot (15, 16) (see also reference 4). It has been assumed in these experiments that the internal Na^+ level remains constant.

We have investigated fully the effect of alteration of external Na^+ on the ionic and potential patterns in the same manner as was done with K^+ , *i.e.* muscles were soaked in high and low Na^+ Ringer, with and without previous equilibration in normal Ringer.

Ionic Changes.—The external Na^+ level has been varied between 45 and 650 m.eq./liter. In low Na^+ solutions the osmotic pressure has been made up

with sucrose. High Na^+ solutions (> 130 m.eq./liter) were of necessity hypertonic.

Low Na Ringer (Unequilibrated).—As will be seen from Table VI muscles placed in reduced Na Ringer lose Na^+ in proportion to the decrease in the Na^+ level of the external solution. Thus, after 2 to 4 hours' soaking, when the muscle has reached a steady state, the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ is the same or slightly greater than that of the other member of the pair which was soaked in normal Ringer. There has been no concomitant change in K^+ level of the muscle.

Low Na Ringer (Equilibrated).—When a muscle is soaked in Ringer it tends to gain Na^+ , until a steady state level is achieved, with a ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ approximately equal to 3. If this muscle is now removed to a Na^+ -deficient

TABLE VI
The Effect of Reduced Na Ringer on Ionic Content

Na in Ringer, m.eq./liter.....	Equilibrated				Not equilibrated			
	43.4 ($\times 0.33$)	Ratio	65 ($\times 0.50$)	Ratio	43.4 ($\times 0.33$)	Ratio	65 ($\times 0.50$)	Ratio
Na, m.eq./kg.								
Control.....	52.0	2.5	39.2	3.3	46.1	2.8	31.9	4.5
Treated.....	21.2	2.0	21.5	3.0	12.6	3.4	12.9	5.0
K, m.eq./kg.								
Control.....	87.0		82.0		81.2		93.2	
Treated.....	86.0		90.0		85.0		92.6	

Ratio means ratio $\frac{\text{Na}_{\text{out}}}{\text{Na}_{\text{in}}}$.

solution Na^+ is extruded from the cell, against a concentration gradient, until the Na^+ ratio is almost completely reestablished. These results are set out in Table VI. The control group of 6 muscles was always removed from the Ringer solution for analysis at the end of the equilibration period. As in the unequilibrated series there was no change in K^+ levels.

The relation of this Na^+ extrusion to the Steinbach (30) "pump" is discussed in two other papers (27, 28).

High Na Ringer (Equilibrated and Unequilibrated).—The results of soaking muscles in high Na Ringer are set out in Table VII. It will be seen that as the osmotic pressure of the external solution is increased there is a progressive shrinkage, which does not however, exceed 22 per cent, even when the Na^+ concentration has been increased by 500 per cent. It is also evident that the internal Na^+ level has been elevated in proportion to the rise in external Na^+ . As was found in the low Na^+ series the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ has remained

constant at a value of approximately 3. In Ringer with more than 390 m.eq./liter Na^+ the muscles became bunched up and unexcitable after about half an hour.

TABLE VII
The Effect of High External Na on Ionic Content of Muscles

Unequilibrated										
Na in Ringer, m.eq./liter.....	156 ($\times 1.2$)	Ratio	173 ($\times 1.33$)	Ratio	390 ($\times 3$)	Ratio	520 ($\times 4$)	Ratio	650 ($\times 5$)	Ratio
Volume change, per cent.....	-5.5		-6.8		-15.0		-17.7		-22.9	
Na, m.eq./kg. wet weight										
Control.....	49.5	2.8	47.0	2.9	44.0	3.1	36.0	3.9	36.5	3.8
Treated.....	62.5	2.7	67.0	2.7	121	3.4	138	4.0	222	3.1
K, m.eq./kg. wet weight										
Control.....	81.0		77.5		76.5		91.0		70.8	
Treated.....	76.5		71.3*		51.6*		58.0*		4.5*	
Equilibrated										
Na in Ringer, m.eq./liter.....	260 ($\times 2$)	Ratio	650 ($\times 5$) (3 hrs.)	Ratio	650 ($\times 5$) (5 hrs.)	Ratio				
Volume change, per cent.....	-14.4		-22.4		-15.9					
Na, m.eq./kg. wet weight										
Control.....	52.8	2.8	25.8	5.0	21.0	6.2				
Treated.....	83.0	3.3	177	3.7	234	2.8				
K, m.eq./kg. wet weight										
Control.....	79.7		95.0		101					
Treated.....	57.5*		37.6*		16.0*					

K contents marked with an asterisk are significantly different from control ($P < 0.05$).

Ratio means $\frac{\text{Na}_{\text{out}}}{\text{Na}_{\text{in}}}$.

As the Na^+ level of the Ringer is raised there is a concomitant decrease in the K^+ level of the cells. This is just evident when the Na^+ is increased by 33 per cent, and results in a loss of 90 per cent of the cellular K^+ when the external Na^+ is increased by 500 per cent. In some muscles at this level of

Na^+ , there was no K^+ intracellularly. Thus there had been an extrusion of K^+ against a concentration gradient. A similar K^+ extrusion was previously noted by us (24).

Bioelectric Changes.—The effect of alteration of external Na^+ level on the height of the overshoot has been investigated by several authors over a range of concentrations from less than normal Ringer to 120 per cent the normal level (14–16, 22). They have reported a decrease in the height of the overshoot in

TABLE VIII
The Effect of Reduced Na Ringer on the Resting Potential and Overshoot
Unequilibrated series.

Na 65 m. eq./liter (× 0.5)									
Ex- peri- ment	Time in low Na Ringer	Potential				Analysis			
		Control		Treated		Control		Treated	
		R. P.	Overshoot	R. P.	Overshoot	Na, m.eq./ kg.	K, m.eq./ kg.	Na, m.eq./ kg.	K, m.eq./ kg.
	min.								
1	0-10	82.3±0.7 (17)	36.3±0.8 (17)	78.8±1.3 (11) 80.5±1.4 (12)	23.5±2.0 (11) 27.3±1.1 (12)	47.0	93.6	29.2	84.6
2	0-10	92.6±1.6 (12)	33.3±1.8 (12)	86.2±1.2 (8) 86.4±1.2 (18)	26.0±2.8 (8) 19.5±1.3 (18)	42.6	100	24.6	108
3	0-10 60-90	94.6±0.7 (20)	33.6±1.1 (20)	85.7±0.9 (14) 86.7±0.7 (29)	22.4±2.1 (14) 20.1±1.3 (29)	54.0	79.5	29.3	103
Na 46.0 m. eq./liter (× 0.33)									
1	0-10 60-90	—	—	85.1±1.3 (15) 85.7±0.8 (15)	22.5±0.9 (15) 17.1±2.2 (15)	24.5	80.0	9.2	95.0
2	0-10 60-90	91.3±1.6 (16)	42.7±1.7 (16)	77.0±1.6 (16) 83.8±1.0 (22)	15.4±1.6 (16) 14.6±1.4 (22)	59.5	91.5	51.6	83.4
3	0-10 60-90	92.5±0.8 (16)	36.8±1.3 (16)	81.3±1.1 (23) 79.6±0.9 (20)	21.8±1.3 (23) 15.2±6.9 (20)	51.0	85.0	26.7	97.2

Ionic contents refer to intracellular levels.

reduced Na^+ Ringer, with a slope of 58 mv. for a tenfold decrease in Na^+ level. A slight increase in the overshoot in 120 per cent Na Ringer was also noted.

We have investigated the effect of alteration of the external Na^+ on the R.P. and A.P. with a procedure similar to that used in the previous section.

Low Na Ringer (Unequilibrated).—The sartorius muscle was removed from the animal and immediately placed in reduced Na Ringer. R.P. and A.P. were recorded at once, and at intervals over the next 90 minutes. Control readings on the paired muscle were taken after 2 hours' soaking in normal Ringer. At the end of the experiment both muscles were analyzed. The results are set out in Table VIII.

It will be seen that the R.P. is several millivolts lower in reduced Na Ringer, although there has been no alteration in the intracellular K^+ level. The overshoot fell as soon as the muscle was placed in low Na Ringer, and remained

TABLE IX
The Effect of Reduced Na Ringer on the R.P. and Overshoot
Equilibrated—Na 65 m.eq./liter ($\times 0.5$).

Experiment	Time in Ringer	Potential			Analysis			
		Resting potential	Overshoot		Control		Treated	
			Found	Calculated	Na, m.eq./kg.	K, m.eq./kg.	Na, m.eq./kg.	K, m.eq./kg.
1	2 hrs. normal Ringer (a)	89.5 $\pm$ 1.4 (8)	36.0 $\pm$ 2.6 (8)	30.7				
	0-10 min. half Na Ringer (b)	85.4 $\pm$ 1.1 (10)	29.6 $\pm$ 1.5 (10)	13.8				
	60-90 min. half Na Ringer (c)	87.9 $\pm$ 1.7 (10)	28.1 $\pm$ 1.6 (10)	20.2	38.4	89.4	29.3	95.0
2	(a)	87.9 $\pm$ 1.3 (13)	40.6 $\pm$ 1.9 (13)	30.0				
	(b)	84.1 $\pm$ 0.9 (10)	35.7 $\pm$ 1.5 (10)	12.6				
	(c)	84.5 $\pm$ 1.7 (7)	31.1 $\pm$ 1.1 (7)	22.8	39.7	106	26.3	94.0
3	(a)	89.7 $\pm$ 1.9 (3)	42.2 $\pm$ 3.2 (3)	33.9				
	(b)	88.6 $\pm$ 1.1 (12)	30.2 $\pm$ 2.2 (12)	16.4				
	(c)	90.0 $\pm$ 1.9 (10)	28.0 $\pm$ 2.4 (10)	24.1	33.9	104	25.0	100
4	(a)	90.7 $\pm$ 1.8 (9)	40.6 $\pm$ 2.5 (8)	44.6				
	(b)	84.1 $\pm$ 1.5 (12)	23.3 $\pm$ 1.9 (11)	27.2				
	(c)	81.8 $\pm$ 1.5 (10)	21.1 $\pm$ 2.5 (10)	36.2	22.1	90.0	15.4	96.0
5	(a)	90.8 $\pm$ 3.1 (9)	37.9 $\pm$ 2.1 (9)	34.7				
	(b)	81.6 $\pm$ 1.8 (11)	29.4 $\pm$ 1.3 (11)	17.3				
	(c)	87.0 $\pm$ 1.3 (10)	18.5 $\pm$ 1.1 (10)	39.2	32.8	64.2	13.7	85.0

Ionic contents refer to intracellular levels. Calculated overshoot from $58 \log \frac{Na_{out}}{Na_{in}}$.

In (b), 0 to 10 minutes in half Na Ringer, Na_{in} is assumed to be equal to that of control muscle.

at this level, or in some cases declined further during the next 90 minutes. In all but one experiment the Na^+ ratio, as determined by analysis at the end of the experiment, remained at the normal value. The results for the height of the overshoot recorded in one-third Na Ringer showed considerable scatter and in some cases the fiber would not "fire" on stimulation.

Low Na (Equilibrated).—The muscle was removed from the animal and

placed in normal Ringer for 2 hours. During this period measurements were made on R.P. and A.P. The Ringer was then replaced with half Na Ringer and potentials were again measured for a further 90 minutes. The paired

FIG. 2. The effect of half Na^+ Ringer on the configuration of the action potential. (a) A.P. in normal Ringer; (b) A.P. within 5 minutes of placing in half Na^+ Ringer; (c) A.P. after 90 minutes in half Na^+ Ringer. In this and subsequent photographs the upper horizontal line represents zero potential.

muscle was soaked in Ringer for 2 hours, and then removed for analysis. The extent of the Na^+ extrusion into the half Na Ringer was gauged from the difference in analysis of the two muscles.

The results of 5 typical experiments may be seen in Table IX. When the

TABLE X

The Effect of High Na Ringer on the Resting Potential and Overshoot
 Equilibrated series. Na 156 m.eq./liter ($\times 1.2$).

Time in Ringer	Potential		Analysis		
	Resting potential	Overshoot	Na, m.eq./kg.	K, m.eq./kg.	Na _{out} / Na _{in}
2 hrs. in normal Ringer (a)	88.2 $\pm$ 1.0 (16)	37.3 $\pm$ 1.1 (16)			
0-10 min. in high Na (b)	86.1 $\pm$ 1.4 (18)	33.8 $\pm$ 1.4 (18)			
90-120 min. in high Na (c)	88.2 $\pm$ 0.9 (20)	39.4 $\pm$ 0.8 (20)	87.5	93.3	1.8
(a)	91.2 $\pm$ 1.1 (13)	30.0 $\pm$ 1.5 (13)			
(b)	87.0 $\pm$ 1.6 (12)	30.0 $\pm$ 1.7 (12)			
(c)	92.7 $\pm$ 1.3 (12)	27.0 $\pm$ 1.1 (12)	81.0	87.0	2.0
(a)	89.4 $\pm$ 1.2 (12)	45.8 $\pm$ 1.6 (12)			
(b)	87.5 $\pm$ 0.8 (11)	40.1 $\pm$ 0.9 (11)			
(c)	—	—	66.0	105	2.4
(a)	90.0 $\pm$ 0.9 (20)	41.0 $\pm$ 0.9 (20)			
(b)	89.1 $\pm$ 0.8 (14)	41.1 $\pm$ 1.3 (14)			
(c)	87.8 $\pm$ 1.0 (25)	35.4 $\pm$ 1.1 (25)	70.5	84.5	2.2
(a)	85.9 $\pm$ 1.3 (14)	40.4 $\pm$ 0.9 (14)			
(b)	91.2 $\pm$ 0.9 (12)	41.2 $\pm$ 1.0 (12)			
(c)	89.6 $\pm$ 1.0 (10)	36.2 $\pm$ 1.6 (10)	72.5	80.4	2.2
(a)	84.3 $\pm$ 0.8 (18)	41.1 $\pm$ 1.0 (18)			
(b)	86.0 $\pm$ 1.1 (12)	41.3 $\pm$ 1.8 (12)			
(c)	87.9 $\pm$ 0.8 (12)	36.7 $\pm$ 1.8 (12)	73.4	80.0	2.1
(a)	90.5 $\pm$ 0.9 (17)	27.8 $\pm$ 1.2 (17)			
(b)	89.7 $\pm$ 1.1 (13)	30.2 $\pm$ 1.4 (13)			
(c)	89.0 $\pm$ 1.2 (17)	33.5 $\pm$ 0.8 (17)	61.2	92.7	2.5
(a)	88.3 $\pm$ 1.2 (15)	26.5 $\pm$ 0.8 (15)			
(b)	88.5 $\pm$ 1.0 (21)	27.8 $\pm$ 0.8 (21)			
(c)	87.7 $\pm$ 0.9 (19)	32.3 $\pm$ 1.2 (19)	90.0	98.5	1.7
(a)	91.5 $\pm$ 3.3 (8)	36.1 $\pm$ 3.4 (8)			
(b)	90.4 $\pm$ 1.2 (9)	38.5 $\pm$ 1.9 (9)			
(c)	91.0 $\pm$ 1.5 (11)	36.7 $\pm$ 1.9 (11)	47.2	104	3.3

Ionic contents are referred to intracellular levels.

TABLE XI
The Effect of High Na on the Resting Potential and the Overshoot Equilibrated

Na in Ringer	Time in Ringer	Potential		Analysis		
		R. P. Resting potential	Overshoot	Na, m.eq. /kg.	K, m.eq. /kg.	Na _{out} / Na _{in}
<i>m.eq./liter</i>						
173 (× 1.33)	2 hrs. normal	91.7 ± 1.9 (6)	36.6 ± 2.5			
	0-10 min. high Na	90.7 ± 1.7 (13)	34.6 ± 1.6 (13)			
	120-180 min. high Na	95.3 ± 1.2 (15)	34.8 ± 1.1 (15)	61.0	100	2.85
	2 hrs. normal	91.0 ± 1.4 (9)	38.7 ± 2.7 (9)			
	0-10 min. high Na	89.3 ± 1.8 (14)	37.5 ± 2.4 (14)			
	120-180 min. high Na	86.5 ± 2.6 (11)	34.8 ± 2.3 (11)	68.3	95.4	2.7
195 (× 1.5)	2 hrs. normal	90.4 ± 1.4 (7)	47.0 ± 1.8 (7)			
	0-10 min. high Na	90.8 ± 0.9 (17)	46.0 ± 1.0 (17)			
	100-120 min. high Na	91.7 ± 1.0 (11)	36.0 ± 1.5 (11)	66.6	102	2.9
	2 hrs. normal	93.5 ± 1.1 (8)	42.6 ± 0.9 (8)			
	0-10 min. high Na	96.1 ± 1.2 (12)	40.5 ± 2.5 (12)			
	100-120 min. high Na	92.4 ± 0.9 (10)	33.0 ± 1.2 (12)	73.5	94.1	2.8
260 (× 2.0)	2 hrs. normal	93.6 ± 2.3 (7)	40.3 ± 2.0 (7)			
	0-10 min. high Na	95.6 ± 1.5 (10)	38.7 ± 2.7 (10)			
	100-120 min. high Na	88.8 ± 1.4 (14)	31.3 ± 1.6 (14)	103	114	2.7
390 (× 3.0)	2 hrs. normal	94.8 ± 0.9 (9)	37.9 ± 2.1 (9)			
	0-10 min. high Na	97.2 ± 2.0 (10)	38.3 ± 2.6 (10)			
	120-180 min. high Na	91.4 ± 1.0 (15)	25.3 ± 1.3 (15)	133	73.9	3.1
520 (× 4.0)	—	—	—			
	0-10 min. high Na	82.6 ± 1.9 (13)	43.8 ± 1.7 (13)			
	200-240 min. high Na	24.5 ± 1.8 (16)	—	279	18.9	2.0
650 (× 5.0)	2 hrs. normal	91.3 ± 1.1 (19)	32.3 ± 1.3 (10)			
	0-10 min. high Na	92.0 ± 2.2 (10)	33.3 ± 1.9 (10)			
	30-40 min. high Na	45.3 ± 4.5 (13)	26.0 ± 3.4 (8)	282	33.5	2.4
	2 hrs. normal	94.7 ± 1.7 (14)	29.1 ± 1.5 (14)			
	0-10 min. high Na	89.2 ± 2.5 (16)	27.3 ± 2.7 (16)			
	30-40 min. high Na	45.4 ± 4.5 (11)	29.9 ± 4.2 (8)	314	39.2	2.2

Ionic content refers to intracellular levels.

Ringer was changed from normal to half Na⁺, the Na⁺ ratio would initially be half that in normal Ringer. The results show that the overshoot has only decreased by about 25 per cent. During soaking the Na⁺ ratio has returned close to its original value; but the overshoot has not altered. Indeed the po-

tential has in all cases declined further by a few millivolts. As was seen in the unequilibrated series there has been no change in the intracellular K^+ throughout these experiments. On the other hand there has been a small but statis-

FIG. 3. The effect of $Na^+ \times 2$ Ringer (260 m.eq./liter) on the configuration of the A.P. (a) A.P. in normal Ringer; (b) A.P. within 5 minutes of placing in high Na^+ Ringer; (c) A.P. after 200 minutes in high Na Ringer.

tically significant drop of 3 mv. in R.P. on changing the Ringer, which was partially reversed on further soaking. The measurements of the overshoot have been corrected for the effects of extrafibrillary potentials (26), which were of similar magnitude to those obtained with isolated muscles in normal Ringer. The nature of the extrafibrillary potentials will be discussed fully at a later date. They are similar to the distortions of the action potential de-

scribed by Easton (6). Penetration of the membrane by the capillary electrode was more difficult in low Na Ringer.

We have also compared the configuration of the action potential in normal Ringer with that in half Na Ringer. In some cases (about 10 per cent) there is no difference in configuration; in others the rate of rise is the same, but the duration of the A.P. is longer; and in some cases (about 50 per cent) the foot, or "take off," is slower. See Fig. 2.

FIG. 4. The effect of $\text{Na}^+ \times 5$ Ringer (650 m.eq./liter) on the configuration of the A.P. (a) A.P. in normal Ringer; (b) A.P. within 5 minutes of placing in high Na^+ Ringer; (c) A.P. after 16 minutes in high Na^+ Ringer; (d) A.P. after 35 minutes in high Na^+ Ringer.

High Na Ringer (Equilibrated).—Other workers (14–16, 22) have found that slight increases in the external Na^+ have resulted in concomitant increases in the A.P. This has been assumed to follow from an increase in the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$, but such an increase has never been demonstrated.

The results obtained on placing the muscle in solutions with excess Na^+ are given in Tables X and XI. At an external level of 156 m.eq./liter Na^+ (120 per cent) the R.P. and overshoot have remained constant at the level recorded in normal Ringer. Potentials were measured over a period of at least 2 hours, and were found to be unchanged. These results were duplicated in 133 per cent Na Ringer, but when the Na^+ concentration was raised to 150

per cent the overshoot tended to fall after 2 hours in the high Na^+ solution. This fall was more marked at higher Na^+ levels, and at levels greater than 300 per cent A.P. were obtained only during the first 30 to 40 minutes in high Na^+ . The R.P. remains essentially constant until we reach 300 per cent external Na^+ , and beyond this point falls rapidly after the muscle has been in the high Na^+ for more than 25 minutes. It should be noted, however, that even in 500 per cent Na^+ it is possible to obtain R.P. and A.P. of normal height and dimensions during this period. After this initial period when normal potentials are obtained, there is a rapid fall in both R.P. and A.P., and after approximately 5 minutes the muscle bunches, and ceases to fire. The R.P. at this point is between 30 and 40 mv., and it is noteworthy that almost normal A.P. have occasionally been obtained from fibers with a R.P. as low as 35 mv. After the muscle has become unexcitable the R.P. falls to a level of 20 to 30 mv., where it remains constant for as long as 4 hours, although there is then very little K^+ in the cell.

The configuration of the A.P. may be seen in Figs. 3 and 4. Accompanying a decrease in height of the A.P. there is an increase in duration, and a marked increase in the value for the after-potential.

DISCUSSION

In previous papers (25, 29), evidence was presented which tended to refute the hypothesis that the accumulation of K^+ in the cell was due to some type of Donnan mechanism. In particular we were able to show that the complete elimination of K^+ from the external Ringer did not result in the loss of intracellular K^+ greater than that of a control muscle soaked in normal Ringer. In this paper we have investigated the effect of increasing the level of the external K^+ . The earlier work of this nature was performed by Boyle and Conway (1). Their results showed that the K^+ entered the cell in proportion to the increase in external K^+ , from which they drew the conclusion that the intracellular K^+ level could be attributed to the electrochemical gradient, the product $\text{K}^+_{\text{in}} \times \text{Cl}^-_{\text{in}}$, being equal to the product $\text{K}^+_{\text{out}} \times \text{Cl}^-_{\text{out}}$.

More recently, Netter *et al.* (19, 21), using a different technique, *i.e.* perfusion of the whole hind limbs rather than immersion of a single muscle, have presented results which substantially confirm those of Boyle and Conway. Our experiments differ from those of the above workers in that we have employed the toad whilst the frog has served as the experimental animal for other workers. In a small series of experiments we have found that the results obtained with the toad could be duplicated with the tissues of the frog *Hyla caerulea*. The effect of alteration of the external K^+ level can be studied in four ways: the additional K^+ may be added directly to the Ringer or an equivalent amount of Na^+ may be removed; the muscle may then be placed in these altered Ringer solutions immediately after dissection (unequilibrated), or after

equilibration in normal Ringer. During the equilibration period there has, of course, been some loss of K^+ and gain of Na^+ , until a steady state is reached. Boyle and Conway (1) placed their muscles directly into Ringer from which a constant amount of Na^+ had been extracted, regardless of the alteration of external K^+ . Netter *et al.* (19, 21) also perfused their muscles without previous equilibration, but did reduce the Na^+ in proportion to the added K^+ . The results of these latter authors will be discussed in a later paper along with our own results obtained with perfusion methods.

Our results are directly opposed to those of Boyle and Conway. We have followed this problem in the four directions outlined above, and we have consistently found that the intracellular K^+ is uninfluenced by the level of the external K^+ , until this concentration reaches 50 m.eq./liter. Even at this value the amount of K^+ entering the cell is only a fraction of that to be expected if the K^+ accumulation were due to a Donnan mechanism.

The above results have been obtained either with normal or low Na Ringer, when the calculations are made on a wet weight basis. The normal Na-high K Ringer does not produce a change in cellular volume (so the wet weight and dry weight results are comparable). The low Na-high K solutions induce a swelling of the cells which does not amount to more than 17 per cent even when the external K^+ level is 50 m.eq./liter. This swelling is much less than that obtained by Boyle and Conway under similar conditions. When one allows for this swelling, *i.e.* considers the results on a dry weight basis, there is an actual entry of K^+ into the cell which amounts to 40 per cent of the original content at 50 m.eq./liter external K^+ . When the muscles are equilibrated in normal Ringer prior to soaking in low Na-high K Ringer the swelling and the increase in internal K^+ are reduced.

We shall now consider the alterations in Na^+ resulting from alterations in the external K^+ level in the unequilibrated series. In normal Na-high K Ringer there has been no alteration in Na^+ content except at the highest K^+ level in the unequilibrated series. At this level the entry of Na^+ which normally takes place on soaking has been hindered. However, high K^+ does not cause a reduction in Na^+ level once it has entered, since there was no difference in internal Na^+ between control and treated, when the muscle had previously been equilibrated in normal Ringer.

When the muscles are placed in low Na-high K Ringer, the level of internal Na^+ falls, so that the ratio Na^+_{out}/Na^+_{in} is greater in these muscles than in controls soaked in normal Ringer.

A perusal of Tables VI and VII shows that the isolated cell maintains a ratio Na^+_{out}/Na^+_{in} , approximately equal to 3 over a great range of external Na^+ (45 to 650 m.eq./liter). This it does by either extruding or imbibing Na^+ as the situation demands. In the presence of low Na^+ (45 m.eq./liter) the muscle contracts normally, although when stimulated the A.P. is some-

what reduced in height (see below). However, at the highest levels of Na^+ the muscle becomes non-excitable and bunched up after 30 minutes, and the intracellular K^+ falls greatly, in some cases being extruded, until there is none in the fiber. In spite of this apparently severe damage to the cell the normal Na^+ ratio is maintained. This contrasts with the previous finding (27) that the extrusion of Na^+ into low Na Ringer is inhibited in the cold. In this case the cell has not been able to maintain the usual ratio of 3. The only other deviation from this general finding was mentioned in the previous section, where a ratio greater than 3 was found in muscles treated with low Na-high K Ringer. It should be pointed out that this constancy of Na^+ ratio is only found in soaked preparations. Wide fluctuations, both greater and less than this ratio, are found in the intact animal (26).

The main conclusion to be drawn from these results is that the cell is able to maintain its internal K^+ at an *absolute level* despite great fluctuations (0 to 50 m.eq./liter) of the external K^+ . This is in contrast to the capacity of the cell to maintain an *absolute ratio* $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ in the face of large variations in the external Na^+ .

It is of interest that the only change in intracellular potassium obtained by alteration of the external ionic pattern was due to very high external sodium. Also the only alteration in Na^+ ratio was produced by high K-low Na Ringer, whereas normal K-low Na Ringer had no effect on this ratio.

In an earlier paper (26) we have shown that there is no simple correlation between the internal K^+ and Na^+ content of the cell, and the R.P. and overshoot respectively. This non-correlation between ionic ratios and bioelectric potentials was demonstrated from a consideration of variations in the internal ionic constitution whilst the external environment was held constant. In this paper the effect of variations of the external ionic environment on the bioelectric potentials has been considered. Our results substantially confirm those of our earlier paper, that there is no simple physicochemical relationship or conception which can connect the ionic gradients and the bioelectric potentials.

We have studied the alteration in R.P. following raising or lowering of the external K^+ level, and have obtained results which essentially confirm those of other workers (16, 17, 22). However, when these findings are considered in conjunction with the ionic analyses which were carried out in parallel with the bioelectric studies, it is clear that they do not support the existing theories. It is commonly said that alteration in the external K level results in a series of values for the R.P. which, when plotted against the log of the external K^+ concentration, has a slope of 58, except at low values of external K^+ . From this, it is assumed that the R.P. is a "potassium potential." That the linearity of the above relationship does not hold over that range of external K^+ which is compatible with the appearance of an action potential is ignored.

When the K^+ was reduced below the normal Ringer level (even to zero) there was not a theoretical elevation of the R.P. This disparity has commonly been ascribed to a leakage of K^+ from the cell, which reduces the ionic gradient (4). The results of our ionic analyses show that there is no greater loss of K^+ into a K-free Ringer than there is into normal Ringer (29). Thus, in a K-free Ringer (the preparation having reached a steady state) the R.P. should be infinite, whereas the highest potential we have ever measured under this condition was 112 mv.

As the external K^+ concentration is raised there is still no marked alteration in the level of the R.P., the value for which only begins to decrease with a slope of 58 when the external K^+ is raised about fivefold. At this point there is a marked diminution in the ability of the muscle to produce an action potential on stimulation. When the K^+ is further increased, the muscle fails to produce any electrical response to stimulation. Although at this point the R.P. is falling off with the predicted slope the absolute values do not agree with those predicted by the Nernst equation, in fact the observed values are always more than 35 per cent higher than the calculated. If one calculates the K^+_{in} on a "fiber-water" basis the two values approach one another more closely but are still significantly different at the 1 per cent level. (See Table IV.) In agreement with results mentioned earlier in this paper the analyses of the individual muscles whose fibers had been penetrated showed that no K^+ had entered the cells except at the very highest levels of external K^+ (50 m.eq./liter).

The R.P. of muscles soaked in high K Ringer were similar, whether the excess K^+ was added to normal Ringer or whether an equivalent amount of Na^+ was replaced. Nor were the results affected by equilibration of the muscles in normal Ringer prior to immersion in the high K^+ solution.

In one respect our results with high K^+ solution were of particular interest. Muscles immediately immersed in Ringer containing 50 m.eq./liter K^+ with an equivalent amount of Na^+ subtracted yielded R.P. slightly but significantly lower than those of the companion muscles placed in high KCl-normal Na Ringer. Analyses showed that the only difference between the two muscles was that the former contained much less Na^+ , and consequently the ratio Na^+_{out}/Na^+_{in} was increased (Table V). Whether the small drop in R.P. found between these series is of the order of magnitude predicted by the Goldman equation must await further work, including in particular the estimation of Cl^- . It must be noted that there is a marked difference in Cl^- content between the two Ringer solutions.

We have shown previously (26) that R.P. measured in the intact animal did not show any concomitant variation with the ratio Na^+_{out}/Na^+_{in} , although the value of this ratio varied from infinity to 1:2.

In distinction to the resting potential, the action potential is considered

to be a "sodium potential." The evidence for this assumption has again been provided by a study of the effect of alteration of the external Na^+ , and it has always been assumed that the internal Na^+ remained constant.

The results of our potential measurements in general agree with those obtained by other authors. But the results of our analyses carried out on the penetrated muscles lead us to a different interpretation. We have previously pointed out that the level of the internal K^+ remains almost constant despite large fluctuations of the K^+ environment. Such is not the case with Na^+ for, when the level of the external Na^+ is altered, the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ remains constant at a value of about 3. Therefore other workers have been in error in assuming that the internal level was unaltered. Accordingly there should be no alterations in the magnitude of the overshoot with variation in the external Na^+ . In agreement with other authors we find an immediate decrease in the overshoot when a muscle is placed in low Na Ringer, although this drop is not as great as that predicted by theory. As the Na^+ ratio returns to normal during the next hour there is no alteration in the size of the overshoot although a return to normal as the Na^+ ratio increases might be expected. It is noteworthy that Fatt and Katz (8) were able with crustacean muscle to obtain large values of the overshoot in solutions in which Na^+ had been replaced by other cations. The result of increasing the external Na^+ has been said to lead to an increase in the magnitude of the overshoot. The relative increase in external Na^+ was, however, small (125 per cent).

We have not observed an increase in A.P. in any of the high Na^+ series, neither in the mean value obtained during the first 10 minutes, nor in isolated potentials measured during this period. Work with radioactive Na^+ has suggested that the rate of diffusion of the ion through the muscle is much greater than the rate of exchange across the membrane (12, 18). Consequently, one would expect that soon after the muscle had been placed in high Na Ringer there would have been a transient increase in Na^+ gradient. This was never reflected in high A.P.

Exposure of the muscle to Na^+ levels of 400 to 500 per cent caused damage to the fibers. Normal R.P. and A.P. were obtained for up to 30 minutes, but after this time both potentials fell off rapidly and the tissue became unexcitable.

At the present time, it would appear that the majority view is that the resting and action potentials are due to the ionic gradients of K^+ and Na^+ respectively. These gradients are maintained by the active pumping out of Na^+ ions. The K^+ gradient is passively set by the activity of the Na^+ pump. More recently doubt has been cast on the above theory (10, 11, 25, 26, 29). Parlin and Eyring (23) have proposed a more radical theory that the resting potential results from the activity of the Na^+ pump. Grundfest (10, 11) also leans toward this view. We do not propose in this paper to put forward any hypothesis to account for either the bioelectric potentials or the maintenance

of the ionic gradients. This will be discussed in a later paper on the basis of results at present under review. We should, however, like to stress that the interpretation of our results is completely at variance with the majority view expressed above. The theory of Eyring is, by its nature, less easily checked by physical measurements, but it would seem difficult to reconcile with our previous finding (26) that the ratio of $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$ varies from infinity to 1:2 in the intact animal, while the resting potential and internal K^+ remain essentially constant.

SUMMARY

The resting and action potentials of sartorius muscles of the toad, *Bufo marinus*, have been measured under varying conditions of external environment. At the same time, analyses for Na^+ and K^+ content were carried out.

There was a slight elevation of 2 mv. when the measurements were made in phosphate-Ringer instead of in bicarbonate-Ringer. The R.P. was independent of the hydrogen ion concentration between pH 6.5 and 8.5, although at these pH's there was marked alteration in the level of Na^+ and K^+ in the muscle.

Alteration of the external K^+ level between 0 and 50 m.eq./liter has little influence on the internal K^+ concentration. When the log of the external K^+ concentration is plotted against the R.P. there is not a linear relationship until the external K^+ is raised above 12 m.eq./liter, at which point the cell is unexcitable. Above this value a straight line with a slope of 58 mv. per ten-fold change in concentration is obtained, but the absolute values at any point are about 35 per cent higher than those which would be given by the Nernst equation.

Alteration of the external Na^+ level within a range of 45 to 650 m.eq./liter resulted in marked changes in the internal Na^+ content, without, however, having any effect on the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$. This ratio has remained at about 3 in spite of marked fluctuations in the absolute value of the internal and external Na^+ levels. When the Na^+ level is lowered there is a decrease in the height of the action potential although there is no alteration in the ratio $\text{Na}^+_{\text{out}}/\text{Na}^+_{\text{in}}$. As the Na^+ level is raised the height of the action potential is not affected even in the presence of a fivefold increase in Na^+ in the Ringer.

The results do not support the conclusion that the bioelectric potentials can be calculated from the ionic ratios by means of simple physical chemical hypotheses such as the Nernst or Goldman equations.

The maintenance of the normal K^+ content of the cell cannot be accounted for by a Donnan mechanism. No definite evidence has been produced to explain the mechanism of a Na^+ "pump." In other words, the concept of a Na^+ pump requires that there shall be a physico- or organochemical mechanism which will distinguish between Na^+ and K^+ (or other) ions. There is evidence that Na^+ can be extruded against a concentration gradient. On the other hand

the cell is able to maintain a constant ratio of external to internal Na^+ even when the cell has been severely damaged by very high external Na^+ levels.

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