THE NON-CORRELATION OF BIOELECTRIC POTENTIALS WITH IONIC GRADIENTS*

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Present day theories postulate that the resting potential (R.P.) is essentially determined by the ionic gradients between the interior of the cell and its exterior. This asymmetry is said to be produced at least partly by a Donnan effect (1) and partly by a specific metabolically mediated Na^+ extrusion (2). Thus, Hodgkin and Katz (3) were able to account for the dependence of the resting potential in the squid giant axon on the external K^+ by adapting the constant field equation of Goldman (4). Data on resting potentials of muscle fibers (5, 6) are in agreement with this analysis.

Alteration of the external ionic environment was also a principal tool in the explanation provided by Hodgkin and Katz (3) for the overshoot of the axonal spike. According to these authors this response is initiated by a small decrease of the resting potential which causes in turn a striking, specific alteration in the permeability of the membrane first to Na^+ and then to K^+ ions. The suggestion has been made that the rising phase of the action potential is due to the penetration of a small amount of Na^+ ions, discharging the membrane to a value which is generally close to the sodium diffusion potential. Goldman (4) predicted that the effect of K^+ and Cl^- permeability would be to reduce the spike height when the Na^+ gradient is steep.

The theory that the bioelectric potentials are directly related to the ionic ratios across the membrane has resulted almost entirely from a consideration of the results of the alteration of the external environment. Only recently have workers turned their attention to the effect of change of the internal ionic composition. Desmedt (7) has studied the effect of increased internal Na^+ on the potentials, and his results are in agreement with the classical theory. However, a close examination of his paper reveals inconsistencies, which will be discussed later.

Grundfest *et al.* (8) have devised a microinjection technique to bring about alteration of the internal ionic constitution, and they have found that alterations of the internal K^+ or Cl^- concentrations do not cause the change in resting potential expected on the basis of a Donnan mechanism.

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The work to be described in this paper takes advantage of the naturally occurring variation in the internal Na^+ and K^+ level of the sartorius muscle of the toad, *Bufo marinus*. In an earlier communication (9), it was shown that there is a temporal variation of several hundred per cent in the Na^+ content. There is a small, but by no means concomitant K^+ variation. In this paper these ionic variations will be correlated with the electrical potentials.

Methods

Material.—The experiments reported here were made throughout 1955 on the toad *Bufo marinus*, batches of which were obtained at fortnightly intervals. The brain and spinal cord of the animals were destroyed. The skin over the sartorius muscle was slit and pinned back to form a bath. In the earliest experiments the "bath" was filled with paraffin oil, but later a small amount of Ringer was placed in the bath (if there was insufficient extracellular fluid to cover the muscle). Isolated muscles were prepared by careful dissection.

Solutions.—Either toad Ringer or phosphate Ringer was used. The composition of these solutions is given in an earlier paper (9) with the exception that the total phosphate was reduced to 3 mM/liter in the phosphate Ringer, while the Na^+ was kept at 130 mM/liter in both solutions.

Soaking Procedure.—Isolated muscles were soaked in 30 ml. solution until they had achieved a steady state with respect to Na^+ and K^+, a process taking from 2 to 3 hours (9).

Apparatus and Electrical Recording.—Penetrations were done under microscope control with Ling-Gerard type microtips, filled with 3 M KCl.

Recording equipment consisted of a D.C. amplifier with a high impedance low capacity cathode follower (1000 meg. and 1 $\mu\mu$ fd.) input. The rise time of the apparatus was frequently checked with a square wave derived from the stimulator and injected in series with the microtip. The over-all rise time lay between 75 and 125 μsec.

Electrolyte Estimation.—See Shaw and Simon (9).

RESULTS

Individual Variation in Ionic Content.—We have analyzed 320 muscles during the last 4 years. The results are given in Figs. 1 and 2 and Table I. The mean value of the total Na^+ is 33.4 m.eq./kg. but 57 per cent of our muscles contain less than 29 m.eq./kg., and 7 per cent more than 46 m.eq./kg. In contrast to this wide variation the K^+ content is comparatively constant. The mean is 87.7 m.eq./kg., the lowest figure 71.0 m.eq./kg., and the highest 112 m.eq./kg. We feel confident from this fact, and other evidence (9), that the variation in the Na^+ analyses is not due to analytical inaccuracy.

A similar variation in the Na^+ and constancy of the K^+ content, has been found in the results of our analyses of nerve (9). In addition the Na^+ content of muscles of one toad tends to follow the content of the same ion in the nerve of the same animal (see Table I).

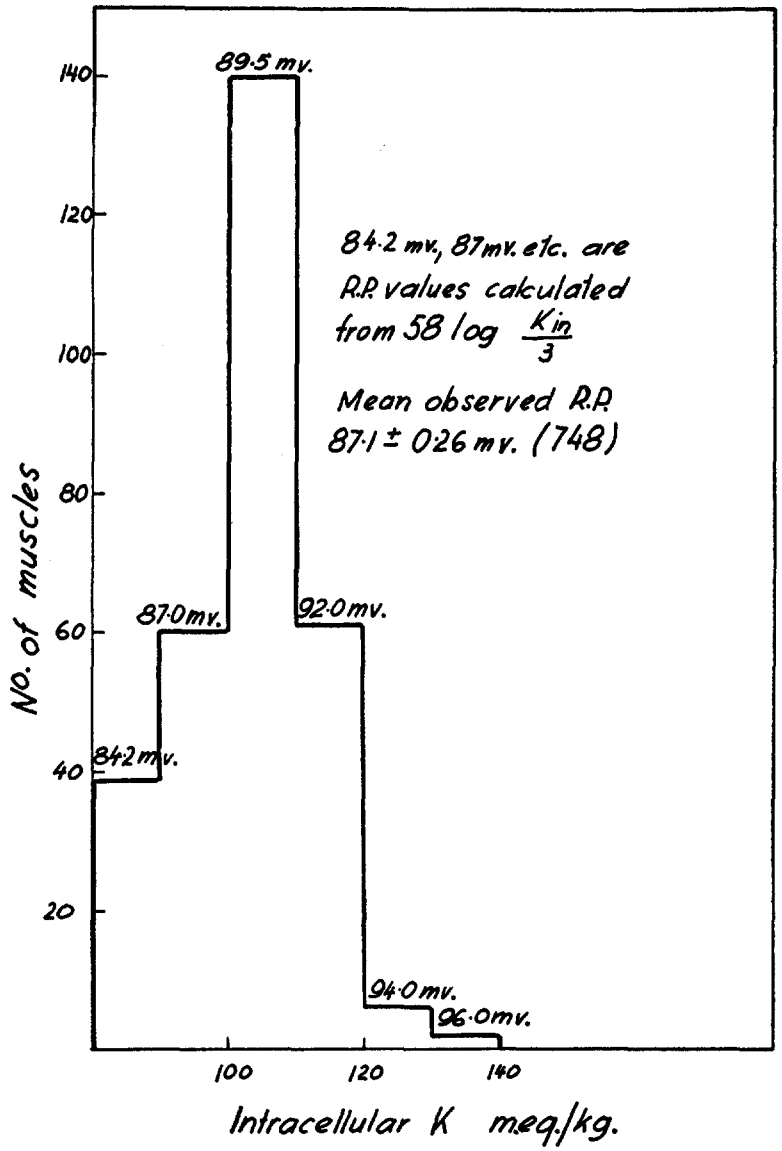

FIG. 1. The distribution of intracellular Na^+ in sartorii. This figure is compiled from analyses carried out over 4 years.

Analyses of plasma have also been made from time to time, but these show that the Na^+ content is constant at 130 ± 2.0 (16 observations) m.eq./liter and the value for K^+ is 3.0 ± 0.08 (18 observations).

The point now arises whether the intracellular Na^+ content shows a simi-

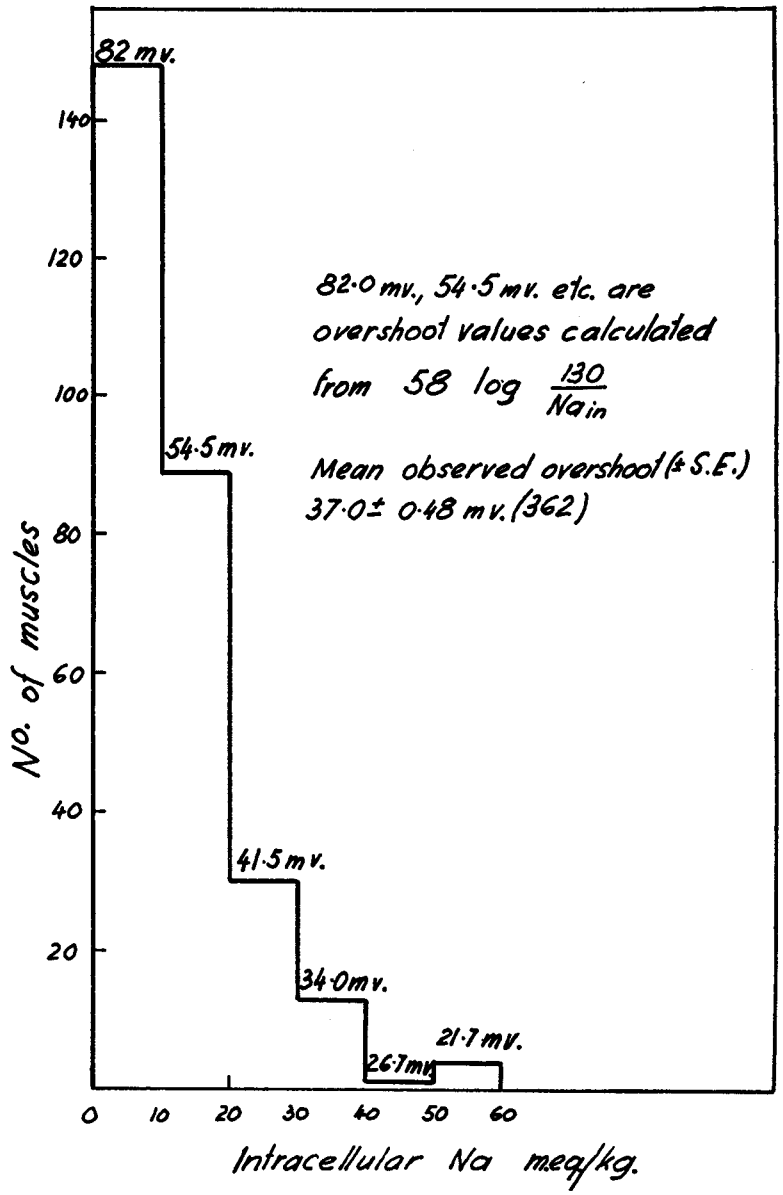

FIG. 2. The distribution of intracellular K^+ in sartorii. This figure is compiled from analyses carried out over 4 years.

lar variation to that of the total Na^+. This would be so if the extracellular volume remained constant.

Other possible inaccuracies have been considered; for instance, our analyses have not been carried out on fat-free tissue, but several estimations of the fat content of sartorius muscles gave values of less than 1 per cent. Variations in the water balance of the animal could also not offer an explanation, because (*a*) the animals were kept moist and (*b*) similar variations were

TABLE I

Temporal Variation in Ionic Content

Month	Sartorii				Sciatics			
	H_2O *gm./kg. wet weight*	Na, *m.eq./kg. wet weight*	Intracellular Na, *m.eq./kg.*	K, *m.eq./kg. wet weight*	H_2O, *gm./kg. wet weight*	Na, *m.eq./kg. wet weight*	Intracellular Na, *m.eq./kg.*	K, *m.eq./kg. wet weight*
July	814	20.8 ± 4.4 (12)	0	70.9 ± 2.3	790	60.6 ± 2.7 (12)	0	24.4 ± 1.7
Mar.	786	20.8 ± 1.7 (8)	0	88.2 ± 2.7	775	91.5 ± 9.3 (8)	45.0	34.1 ± 4.8
Sept.	789	26.9 ± 1.2 (12)	7.3	98.0 ± 1.1	742	74.0 ± 2.1 (12)	10.0	37.1 ± 1.0
Nov.	813	27.4 ± 1.7 (8)	7.9	90.0 ± 1.4	773	71.8 ± 1.3 (8)	5.6	30.7 ± 0.8
Oct.	840	29.0 ± 1.8	9.8	78.3 ± 4.3	798	73.4 ± 4.4 (4)	8.8	37.8 ± 1.7
Jan.	806	30.6 ± 1.2	11.6	85.4 ± 2.6	772	68.5 ± 3.2 (8)	0	22.8 ± 3.8
Oct.	816	32.9 ± 1.2 (10)	14.3	74.6 ± 1.4	797	68.6 ± 2.0 (10)	0	25.3 ± 1.4
Aug.	797	33.7 ± 3.9 (10)	15.3	89.4 ± 2.8	796	63.3 ± 2.0 (10)	0	36.4 ± 2.1
Apr.	806	33.9 ± 1.2 (10)	15.6	92.6 ± 2.2	799	85.6 ± 3.4 (10)	232	34.2 ± 1.5
May	825	36.9 ± 3.6 (10)	19.1	82.8 ± 4.1	818	85.2 ± 2.3	22.4	41.3 ± 1.0
Oct.	826	45.1 ± 2.7 (6)	27.6	68.8 ± 3.1	802	90.1 ± 1.1 (4)	42.2	40.4 ± 2.5
Dec.	816	54.3 ± 2.6 (8)	39.6	90.4 ± 2.0	821	112.4 ± 6.6 (8)	86.8	34.7 ± 0.9

The intracellular Na in sartorii has been calculated on the basis of 15 per cent extracellular volume and in the sciatics on the basis of 50 per cent extracellular volume. The figures in parentheses refer to the number of observations. This table was compiled from experiments carried out over 15 months.

obtained when the results of the analyses were calculated on a dry weight basis.

We have not carried out any determinations of extracellular volume of muscle. This is because there is at present no method for determining this value with any degree of satisfaction. Most of the results in the literature suggest that it is around 15 per cent (7, 14). Errors in estimation of the extracellular volume will produce greater variation in the calculated intracellular Na^+ than in the calculated intracellular K^+ due to the very much greater Na^+ content of extracellular fluid. If variation in extracellular space is to account for the differences in Na^+ content, then those mus-

cles showing a low Na^+ content, and having presumably a low extracellular space, should have a raised K^+ content, and conversely those muscles showing a high Na^+ content, and so having a high extracellular space, should have a low K^+ content. As can be seen from Table I this correlation is not found. Furthermore, it is possible to calculate the variation in extracellular space that would be necessary if there is in fact a constant intracellular level of Na^+. We shall assume this level to be approximately 29 m.eq./liter Na, a value which, when substituted in the Nernst equation gives the observed

TABLE I *a*

Individual Variation in Ionic Content of Sartorii

Total water, *gm./kg. wet weight*	Na, *m.eq./kg. wet weight*	Intracellular Na	K, *m.eq./kg. wet weight*
817	15.0	−5.9	76.8
816	16.9	−3.6	79.2
808	19.4	−0.7	72.5
799	20.5	0.6	77.2
807	18.3	−2.0	74.4
810	20.5	0.6	73.4
812	17.3	−3.2	82.2
813	20.0	0.0	78.6
818	21.9	2.2	81.4
826	26.8	8.0	76.5
815	30.6	12.5	72.5
830	22.8	3.3	80.0

These figures correspond to July group in Table I. Paired muscles were taken from 6 toads.

result of 37 mv. for the overshoot. The range of total Na^+ content varies from 16 to 75.0 m.eq./kg. (wet weight). It is obvious for the low figure that even if there were no extracellular space there would not be sufficient Na^+ to obtain the ratio required by the equation. In muscles with the high Na^+ we must assume an extracellular space of 33 per cent to give an intracellular level of 29 m.eq./kg. Returning to a consideration of the low Na^+ (16 m.eq./kg) muscles, it is easily seen that if a constant value of 13 per cent for the extracellular volume is assumed (14), then these muscle fibers will, of course, contain a negative concentration of Na^+ (Table I *a*). This is impossible, so we are forced to assume that the extracellular volume is a variable with a minimum value of 10 per cent. At this value for the volume of the

extracellular space there would be little or no intracellular Na^+. This is demonstrated in Table I *a*, in which the intracellular Na^+ for muscles of the month of July has been calculated on the basis of 15 per cent extracellular volume, giving negative values in some individual muscles. Thus the maximum value of the extracellular space in these muscles can be calculated as that volume which would give zero intracellular Na^+. It will be seen in Tables I and I *a* that there is no correlation between variation in Na^+ and water content of the tissue, which remains constant over the whole series.

Further evidence that there is a genuine variation in the intracellular Na^+ is provided by the behavior of the muscle when soaked in Ringer. Those muscles which are low in Na^+ gain more Na^+ than those of average content, while the high Na^+ muscles extrude Na^+ into the Ringer solution. That is, all muscles tend to assume a similar Na^+ concentration when soaked in Ringer. This phenomenon has also been observed with sciatic nerves (9).

Bioelectric Potentials.—We have found a value of 87.4 ± 0.38 mv. for the resting potential (491 observations), and of 37.0 ± 0.32 mv. for the overshoot (433 observations) for isolated muscles soaked in Ringer. These values are in agreement with those obtained by other workers in this field, and are also reasonably close to the potentials predicted by the Nernst equation on the basis of the ionic gradients across the membrane found in soaked muscle.

In view of the fact that there is a larger variation in the intracellular Na^+ content *in vivo* than there is in the isolated soaked muscle, and that the isolated muscle gains Na^+ and loses K^+, we thought it advisable to compare the potentials measured in the intact animal with those obtained when the muscle is isolated. We were impressed with the constancy, first, of the resting potential in both the *in vivo* and isolated preparations, and second, with the even more remarkable similarity of the overshoot in both preparations, although in the *in vivo* preparations there were great variations in the ratio $\frac{Na^+_{out}}{Na^+_{in}}$. Over a period of a year the overshoot measured in dissected muscles bathed in Ringer solution gave a mean value of 37.0 ± 0.32 mv. (433 observations in 24 muscles). When we came to measure the potentials in the sartorius muscle *in situ* in a pithed toad (the capillary circulation could be observed under the microscope), it was found that the scatter of the overshoots was much greater than that observed with isolated preparations. The maximum potentials obtained (there were a few as high as 50 mv.) were no greater than those found in the isolated preparation, but there were many values in the region of 10 to 20 mv., values never obtained with the isolated preparation. The configuration of these action potentials was sometimes abnormal, and led us to suspect the presence of an artifact. A perusal of the paper by Easton (11) suggested to us that in the *in vivo* preparation there was interaction between the fiber in which the electrode was situated and adjacent fibers.

This hypothesis was checked by withdrawing the electrode just outside the fiber and recording a potential on restimulation. This resulted in a diphasic wave. This irregularity corresponded in time and amplitude to a similar irregularity which could be observed on the resting potential line when the fiber failed to "fire," and parts of it could be detected in the latent period or afterpotential when the fibre did fire. We therefore assumed that on some occasions this "extrafibrillary potential" would occur during the action po-

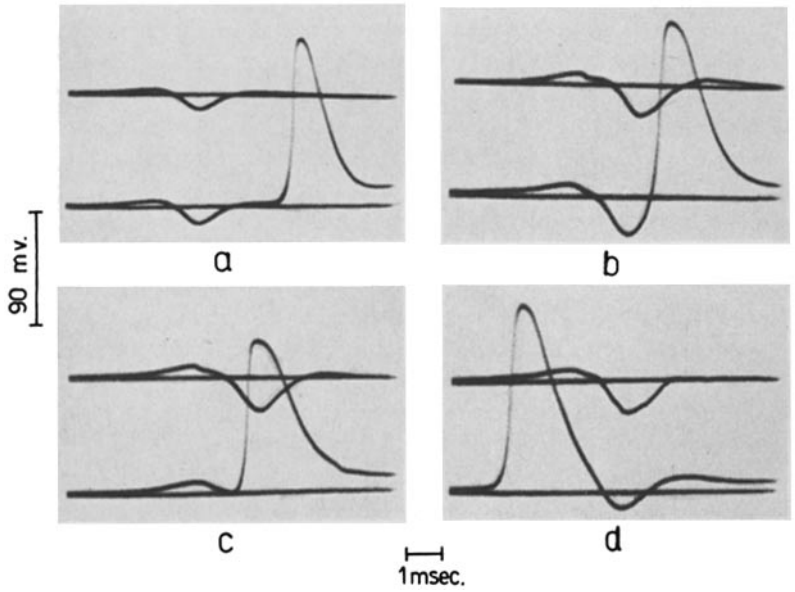

FIG. 3. Examples of extrafibrillary potentials. *a* shows resting potential, action potential, and extrafibrillary potential superimposed in one photograph. *b*, *c*, and *d* are further examples from another muscle, in which the extrafibrillary potentials occur at different time periods and lead to distortion of the action potential.

tential (A.P.) and would sum with it. Our procedure therefore was as follows: the resting potential of the cell was measured, the muscle was then stimulated directly, and the action potential recorded, the electrode was then withdrawn a slight distance from the membrane and the muscle restimulated. The four photographs were taken on the one frame, and the overshoot was measured from the peak of the action potential to the corresponding point, vertically below, on the extrafibrillary potential (see Fig. 3). The nature of the extrafibrillary potential has been investigated more throughly than has been described above, and the justification for the summation carried out here will be the subject of another paper (12). Briefly, the size of the extrafibrillary potential was not reduced if the toad were cura-

rized, but was lessened to one half and sometimes was absent altogether if, as sometimes happened, the muscle were covered with a deep layer (2 mm.) of extracellular fluid, or Ringer solution were placed in the skin flap "reservoir" to about the same depth. When these corrections for the extrafibrillary potentials were made the large scatter of the values of the overshoots was reduced; *i.e.*, the low values (10 to 20 mv.) were not obtained but high values (about 45 mv.) were found to be unaccompanied by extra-

TABLE II

Na Content and Overshoot in Isolated and in Vivo Sartorii

In vivo		Isolated	
Intracellular Na, *m.eq./kg. wet weight*	Overshoot, ±S.E. *mv.*	Intracellular Na, *m.eq./kg. wet weight*	Overshoot, ±S.E. *mv.*
10.1	29.0 ± 1.8 (21)	41.9	34.8 ± 1.2 (18)
11.3	42.0 ± 1.2 (24)	42.0	41.0 ± 0.9 (29)
14.7	38.9 ± 2.1 (17)	36.8	33.2 ± 1.6 (13)
18.5	42.8 ± 1.4 (26)	42.1	37.1 ± 1.6 (19)
18.8	41.5 ± 0.9 (67)	35.7	39.8 ± 0.9 (39)
23.8	37.7 ± 1.9 (15)	32.1	36.6 ± 1.4 (12)
24.1	35.6 ± 1.9 (24)	44.6	33.9 ± 1.4 (28)
27.7	32.8 ± 1.3 (29)	54.0	35.8 ± 0.8 (34)
29.8	35.7 ± 2.1 (18)	52.8	33.4 ± 2.0 (22)
30.4	34.9 ± 1.1 (31)	39.2	38.7 ± 1.1 (18)
32.6	37.8 ± 2.9 (20)	18.5	30.8 ± 1.2 (17)
35.2	44.0 ± 0.5 (14)	32.7	36.8 ± 1.8 (16)
35.7	33.2 ± 1.4 (17)	32.5	35.7 ± 3.3 (11)
36.2	34.9 ± 1.2 (22)	58.0	40.2 ± 1.1 (22)
48.9	37.2 ± 2.6 (12)	41.0	33.7 ± 1.3 (16)
53.4	33.5 ± 2.2 (16)	38.0	40.0 ± 0.8 (10)
57.8	33.8 ± 1.9 (19)	37.8	42.8 ± 1.1 (22)
68.0	39.0 ± 1.4 (17)	36.1	33.2 ± 1.3 (16)

fibrillary potentials. The fact that the individual variation was decreased may also be taken as a justification of our procedure.

Extrafibrillary potentials were sometimes obtained in isolated preparations, but when present were considerably smaller (approximately 4 mv.). The isolated muscles were always covered with a layer of Ringer solution several millimeters deep; if this were replaced by paraffin oil the extrafibrillary potentials were markedly increased. This is in conformity with the results obtained *in vivo*.

It should be stressed that there is no difficulty associated with the measurement of the resting potential in the *in vivo* preparations.

The results obtained for the values of the resting potential and overshoot

in vivo and in isolated preparations have been compared in Tables II and III. Figs. 4 and 5 show the plot of resting potential and overshoot against

TABLE III

K Content and Resting Potential in Isolated and in Vivo Sartorii

In vivo		Isolated	
Intracellular K content, *m.eq./kg. wet weight*	R.P., *mv.* S.E.	Intracellular K content, *m.eq./kg. wet weight*	R.P., *mv.* S.E.
83.5	88.4 ± 1.0 (18)	75.7	90.8 ± 1.1 (22)
85.6	76.8 ± 1.2 (11)	73.8	82.5 ± 1.1 (11)
91.5	87.2 ± 1.0 (26)	73.5	85.3 ± 0.8 (19)
94.3	88.5 ± 1.2 (24)	76.5	87.8 ± 1.1 (29)
96.2	90.0 ± 1.6 (19)	83.0	99.1 ± 1.3 (7)
97.0	91.0 ± 1.7 (21)	90.0	93.6 ± 1.2 (14)
98.5	87.8 ± 1.9 (6)	89.2	84.1 ± 2.2 (10)
99.3	81.2 ± 1.5 (14)	93.8	88.7 ± 1.5 (8)
101	88.8 ± 1.4 (24)	88.0	88.0 ± 1.2 (28)
101	89.1 ± 1.2 (29)	76.0	80.2 ± 0.7 (34)
101	90.7 ± 0.5 (20)	97.5	85.4 ± 0.9 (17)
101	89.6 ± 1.2 (20)	93.2	99.0 ± 2.8 (18)
102	87.0 ± 1.2 (22)	94.0	85.2 ± 1.7 (22)
103	86.6 ± 3.3 (8)	72.0	92.7 ± 1.2 (16)
105	85.3 ± 2.2 (16)	89.8	87.0 ± 0.7 (16)
109	89.2 ± 1.6 (17)	93	90.3 ± 1.1 (13)
109	86.6 ± 0.6 (68)	88.4	85.4 ± 0.8 (39)
110	90.5 ± 0.9 (14)	86.5	82.7 ± 1.0 (19)
111	88.6 ± 1.3 (12)	90	89.2 ± 1.7 (10)
112	89.0 ± 1.3 (16)	109	83.0 ± 3.9 (22)
112	90.4 ± 1.6 (15)	103	90.2 ± 1.0 (12)
113	83.0 ± 2.4 (8)	78.0	79.5 ± 1.5 (6)
114	87.2 ± 0.5 (16)	100	86.4 ± 0.4 (16)
114	91.0 ± 1.3 (19)	100	87.5 ± 1.1 (22)
115	88.7 ± 1.2 (11)	90.5	84.4 ± 1.2 (9)
117	84.0 ± 1.0 (21)	94.6	84.6 ± 1.2 (18)
122	89.7 ± 1.4 (15)	87.3	99.4 ± 5.1 (13)
124	84.0 ± 1.2 (16)	88.0	90.8 ± 0.8 (10)
125	87.3 ± 1.0 (13)	102	90.6 ± 1.0 (16)
126	86.6 ± 3.2 (8)	92.3	84.7 ± 1.3 (14)
126	88.4 ± 2.7 (16)	98.8	84.5 ± 1.9 (8)
128	84.0 ± 0.9 (19)	88.1	87.4 ± 1.2 (12)
132	88.4 ± 1.3 (31)	104	85.2 ± 1.2 (18)

log K^+_{in} and log Na^+_{in} respectively. It will be seen that while the intracellular Na^+ varies from 10.1 to 58.0 m.eq./kg., the K^+ only varies from 72.0 to 132 m.eq./kg. The overshoot does not show any variation such as is shown by the intracellular Na^+, but remains comparatively constant throughout the series of muscles examined (37 mv. ± 0.48, 362 readings).

The resting potential has also remained comparatively constant, although the intracellular K has varied from 72.0 to 132 m.eq./kg. (87.1 mv. ± 0.26, 748 readings).

The best fitting line for fig. 4 was found to be

$$y = 83.35 + 4.04 \log x$$

The standard error of the regression coefficient b is 11.92, showing that

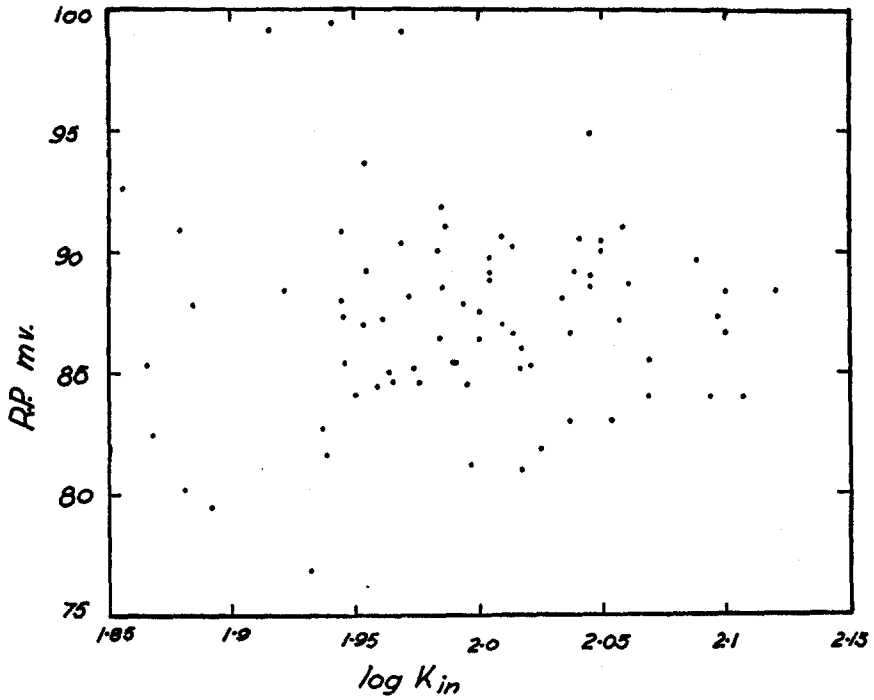

FIG. 4. The relation between log K^+_{in} and resting potential.

there is no evidence of correlation between y and log x. Moveover, the value $b = 4.04$ differs significantly from 58, the theoretical value. This shows that the lack of agreement between theory and experiment is not simply due to variability in the recorded measurements of the resting potential, assuming always that the experimental technique is adequate. The intercept $a = 83.35$ has standard error 9.26 and is undoubtedly different from the postulated value. One concludes that it is virtually impossible for a phenomenon governed by the Nernst law to throw up data such as have been obtained.

The linear regression in Fig. 5 is given by

$$y = 37.67 - 0.91 \log x,$$

with the regression coefficient $b = -0.91$ not significantly different from zero but significantly different from the postulated value -58. The standard error of b here is 5.09. The intercept $a = 37.67$ has a standard error 2.94.

In Tables II and III the potentials found *in vivo* in one muscle have been compared with the potentials obtained for the muscle taken from the other leg, which had been soaked in Ringer for a period of at least 2 hours.

The results have been listed in order of increasing intracellular Na^+ concentration (*in vivo*). When the companion muscle was soaked in Ringer it gained Na^+ if the *in vivo* Na^+ concentration was below about 50 m.eq./kg. Above this concentration the muscle extruded Na^+ into the Ringer against a concentration gradient. This is another example of the "pumping" of Na^+

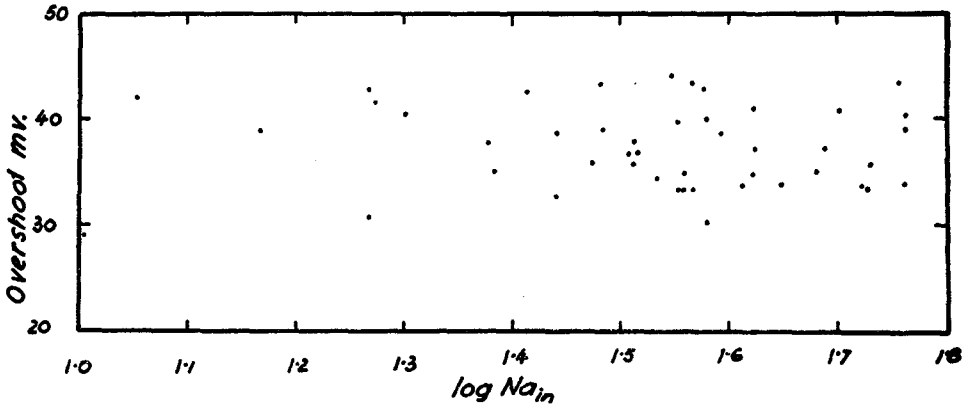

FIG. 5. The relation between log Na^+_{in} and the overshoot.

from the cell, against a concentration gradient, which is not associated with a concomitant K^+ uptake. A similar phenomenon has been demonstrated by the authors when muscles previously equilibrated in Ringer are transferred to Ringer with half the Na^+ replaced by sucrose (13).

When the muscles showed a gain of Na^+ on soaking there should, of course, have been a decrease in the value of the overshoot. Our results show that there was no significant difference in the measured potentials. Those muscles which extruded Na^+ should have shown an increase in the value of the overshoot. This could not be demonstrated.

Implicit in these remarks is the notion that gain of Na^+ means transfer to an aqueous medium inside the region across which the potential difference occurs. Obviously, available methods do not give this spatial information. From this one may take the extreme viewpoint that analytical data for total ionic content are not adequate for proving or disproving an ionic theory for the transmembrane potential. There may be binding of ions intra-

cellularly and, less likely, extracellularly. Evidence to be presented later (10) would indicate that binding of Na^+ at least, is unlikely.

All the muscles in this series showed a very significant decrease in K^+ content when soaked in Ringer. The average loss in K^+ was 17.4 m.eq./kg. per pair. This would correspond to a lowering of the resting potential by 4.5 mv. (calculated from the Nernst equation). Over the whole series there was no statistically significant difference between the resting potential of either preparation. The resting potential *in vivo* was 87.1 mv. $\pm$ 0.26 (748 observations), and the resting potential in the isolated preparation was 87.4 $\pm$ 0.38 mv. (491 observations), thus it should have been possible to detect the predicted drop in resting potential on soaking the muscle.

The rate of rise of the action potential was remarkably constant in all the *in vivo* preparations (320 volts per second). The rate of rise of the action potential in isolated preparations was, in two cases out of three, the same as that found *in vivo*. At other times it was slower (240 volts per second). The duration of the action potential was always increased in the isolated preparation (*in vivo* 2 msec.; isolated 3 msec.).

DISCUSSION

Our results clearly show, by means of two criteria, that there is no correlation such as would be expected from the Nernst equation, between the ratio $\frac{K^+_{in}}{K^+_{out}}$ and $\frac{Na^+_{out}}{Na^+_{in}}$ and the resting potential and action potential respectively. First, as will be seen from Figs. 4 and 5, a comparison of the results of the analyses for K^+ and Na^+ of individual muscles with the bioelectric potentials obtained shows an absence of correlation. Second, the alteration in ionic content of muscles which takes place on soaking is not reflected by a change in the bioelectric potentials which remain unaltered (see Tables II and III).

In no respect are our measurements at variance with those which have been obtained by other workers in the past. It will be noticed that over the small range of intracellular Na^+ and K^+ which is most frequently found in Ringer soaked muscles there is good agreement between the experimental and theoretical values for the resting potential and overshoot. It is only when the potentials are measured in cells whose ionic content varies markedly from the average that the non-correlation becomes apparent. In particular, it should be noted from Fig. 1 that the distribution of internal Na in the intact animal is such that only 17 per cent of muscles should give, from the Nernst equation the observed overshoot of 37 mv. 76 per cent of muscles should give a higher overshoot (more than 55 mv.) and 7 per cent of muscles of the series should give an overshoot less than 26 mv. It would appear that in the past other workers in this field have been content to use average values

of ionic content for their calculations of potential. These average values were obtained at different times and in different parts of the world.

It now remains to deal with the possible errors in our results. The major possible source of error in the measurement of the action potential *in vivo* is the extrafibrillary potential. We must leave a justification of our procedure to another paper (12). This error does not apply to recordings made with isolated preparations nor to measurements of the resting potential. We also know that there is an error associated with the method of recording by means of microtips, but this should not, in such a large series, obscure any definite trends in the observed potentials.

There could possibly be a variation in the ionic content of the extracellular fluid. This we have checked by making frequent analyses of the plasma, and occasional analyses of the tissue fluid whenever a sufficient quantity could be obtained. The analyses showed that the variations were not great, being usually of the order of a few per cent, and never greater than 15 per cent. This objection would not apply to isolated muscles, in which we observed a similar lack of correlation.

The potential readings were, of course, made on cells contained in the first few layers of the upper surface of the muscle, while the analysis would give the mean ionic content of all fibers. There is, however, no evidence that the ionic composition of the surface cells is different from that of cells more centrally situated. There was no deterioration of the surface fibers with time, as constant results were obtained for up to 2 hours from the commencement of the experiment.

The values calculated for the intracellular Na^+ and K^+ will depend upon the value assumed for the extracellular volume. The difficulties associated with this estimation have been discussed earlier in the paper. We have not calculated the concentrations on the basis of concentration in the fiber water because we do not consider it possible to make adequate allowance for the extracellular solids in the calculation of the fiber water. This will, of course, affect our absolute values, but it will not influence the results in which we have compared isolated and *in vivo* preparations, as each result will be altered by a similar amount.

The fact that the low theoretical values of the overshoot are not obtained experimentally when we have a high intracellular Na^+ could result from a binding of part of the Na^+, or a considerable reduction of the activity of this ion in these muscles. This explanation cannot be used to account for normal values of the overshoot which are found in muscles which contain little intracellular Na^+.

Desmedt (7) is the only other worker who has determined bioelectric potentials and ionic contents on the same preparations. The plot of Na^+_{in} against overshoot published by this author shows a lack of correlation at low Na^+_{in} levels. This he ascribes to the effect of K^+ and Cl^- permeability on the height

of the overshoot. We have also obtained results with low Na^+ muscles which do not agree with theory, but in addition we found non-correlation in high Na^+ muscles. The invoking of a permeability change is less likely to be applicable here. The reduction of the overshoot at high Na^+_{in} levels was found by Desmedt on muscles which had been held at low temperature in reduced K^+ Ringer for several days. This treatment admittedly leads to deterioration of surface fibers; and potentials obtained from deeper fibers are always subject to distortion by extrafibrillary potentials. Further, the increases in overshoot reported by Desmedt when the muscles are returned to room temperature in high K^+ Ringer, in only two of the four experiments reported, are proportionally as great as the Na^+ extrusion, and may in fact parallel the improvement in the metabolic status of the tissue rather than the restoration of ionic gradient. In this respect it is noteworthy that the depression in the rate of rise of the action potential after cooling is not reversed when the tissue is warmed in high K^+ Ringer.

It has also been reported by Desmedt (7) that there is a lack of correlation between K^+_{in} and resting potential, similar to that found by ourselves. The discrepancies are quite large. For instance, he found that muscles soaked in Ringer containing 0.2 m.eq./liter K^+ had a resting potential of 100 mv., whereas the calculated value would be 156 mv. More important still, this value did not change during 70 hours' soaking, although the K^+_{in} had dropped by 25 per cent, which should have resulted in a fall of 34 mv.

Throughout this work potentials have been calculated by means of the Nernst equation. We have not used the Goldman (4) equation as the constants have not been measured for toad muscle. However, it is possible to discuss some aspects of our results in the light of this equation. For instance, our constancy of the overshoot in the presence of varying amounts of internal Na^+ could be explained by (*a*) a variation in the permeability of Na^+ in high and low Na^+ muscles, but such variation would be reflected in the resting potential, which however, remains constant; (*b*) if the Na^+ permeability remains constant there would have to be an alteration in the permeability of K^+, *i.e.* because the overshoot and resting potential are constant in the presence of large variations in concentration of Na^+ and K^+, the permeabilities of both ions would have to be variable, and dependent on the concentrations. The problem is further complicated because the variations in Na^+ and K^+ concentrations are not related. It is particularly noteworthy that the great variation in the Na^+ ratio (from 1:2 to infinity) between the intracellular Na^+ and the plasma Na^+ has not produced any concomitant variation in the resting potential. If the Goldman equation were correct in predicting the resting potential then as the Na ratio increased there should be a falling off in the resting potential unless the chloride ratio increased at the same time. We have not investigated changes in Cl^- level, but this work is in progress.

The experiment complementary to ours has been carried out by Grundfest

et al. (8), who altered the internal environment of squid axons by microinjection. We have come to conclusions similar to theirs.

If calculations based on the Nernst (or Goldman) equations are unable to account for the potentials observed over a wide range of ionic content in individual muscles, it is necessary to offer some alternative hypothesis to account for bioelectric potentials. We lean to the view of Grundfest *et al.* (8) that one is really concerned with a charged membrane of unknown nature, and the degree of the charged is influenced by the ionic environment. This view has been further strengthened by experiments involving alteration of the external ionic environment, which will be published in a later paper (10).

SUMMARY

The sartorius muscles of 320 toads have been analyzed for Na^+ and K^+. There is a wide variation in the Na^+ content which when calculated intracellularly varied from 0 m.eq./kg. to 58 m.eq./kg. In particular it was found that the distribution of internal Na^+ in the intact animal was such that only 17 per cent of the muscles should give from the Nernst equation the observed overshoot of 37 mv.

In contrast to this wide variation the K^+ content is comparatively constant, the range being 71 to 112 m.eq./kg. The mean observed resting potential of 87 mv. agreed well with the potential calculated from the mean intracellular K^+ by the Nernst equation.

Analyses of plasma show that the Na^+ content is constant at 130 m.eq./liter, and the K^+ is 3.0 m.eq./liter.

The resting and action potentials of 77 muscles have been recorded and then the muscles have been analyzed. The results have shown that there is no correlation between the level of intracellular Na^+ and the overshoot. Furthermore the apparent correlation between the average K^+ content and the *average* resting potential has been shown to be fortuitous, when the correlation in *individual* muscles is considered.

When a muscle is soaked in Ringer solution for several hours there is a gain of Na^+ and a loss of K^+. These shifts should result in changes in the respective potentials, but such changes were not found.

The above findings have been discussed in the light of the present theories that the resting potential and the action potential are directly related to the ionic ratio across the membrane. Our results very definitely do not support the theory that the overshoot is related to the Na^+ gradient, and this also applies with respect to the K^+ gradient and the resting potential.

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REFERENCES

1. Hodgkin, A. L., *Biol. Rev.*, 1951, **26,** 929.
2. Dean, R., *Biol. Symp.*, 1941, **3,** 331.
3. Hodgkin, A. L., and Katz, B., *J. Physiol.*, 1949, **108,** 37.
4. Goldman, D. E., *J. Gen. Physiol.*, 1943, **27,** 37.
5. Jenerick, H. P., *J. Cell. and Comp. Physiol.*, 1953, **42,** 427.
6. Ling, G. W., and Gerard, R. W., *J. Cell. and Comp. Physiol.*, 1949, **34,** 383.
7. Desmedt, J. E., *J. Physiol.*, 1953, **121,** 191.
8. Grundfest, H., Kao, C. Y., and Altamirano, M., *J. Gen. Physiol.*, 1954, **38,** 245.
9. Shaw, F. H., and Simon, S. E., *Australian J. Exp. Biol. and Med. Sc.*, 1935, **33,** 153.
10. Shaw, F. H., Simon, S. E., Johnstone, B. M., and Holman, M. E., data to be published.
11. Easton, D. M., *J. Neurophysiol.*, 1955, **18,** 375.
12. Johnstone, C. G., and Johnstone, B. M., data to be published.
13. Shaw, F. H., and Simon S. E., *Nature*, 1955, **176,** 1031.
14. Boyle, P. J., and Conway, E. J., *J. Physiol.*, 1941, **100,** L.