BBA 75120

THE DISTRIBUTION OF INORGANIC IONS IN LOBSTER MUSCLE

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(Received October 20th, 1967)

SUMMARY

Compartmentation of intracellular chloride (Cl_i^-) in muscle of lobster walking leg was shown by two independent methods: (1) after equilibration in low-Cl^- medium, Cl_i^- was reduced from 85 to 30 mM/kg cells; (2) after equilibration in medium containing ^{36}Cl, a similar concentration of Cl_i^-, 32.5 mM/kg cells, remained unexchanged with the isotope. The Cl^- remaining in muscles in low-Cl^- medium was essentially nonexchangeable, showing that the immobile compartment of Cl_i^- is independent of changes in the mobile compartment of Cl_i^- and of the external concentration of Cl^-. The mobile compartment varied linearly with external Cl^-, with a constant ratio of about 10:1.

Sodium appeared to be distributed similarly to chloride, both qualitatively and quantitatively. From experiments on the relation between external K^+ concentration and the resting membrane potential, and on the exchangeability of intracellular K^+, the possibility of compartmentation of K_i^+ is discussed.

INTRODUCTION

The classical view of the distribution of inorganic ions across cell membranes, based primarily on the study of frog muscle, holds that ions are distributed according to the Donnan relation[1–4]. In this view, the membrane is freely permeable to K^+ and Cl^-, but not to Na^+, and the distribution of K^+ and Cl^- is governed by the constant product relation according to the Donnan ratio: $K_i^+/K_o^+ = Cl_o^-/Cl_i^-$***. The K^+ gradient is regarded as the determining factor and the distribution of Cl^- is considered to be passive. There is evidence, however, that the Donnan equilibrium is not the sole determinant of the distribution of ions[5,6]. For example, the role of active transport of Cl^- as well as transport of Na^+ and K^+ must be taken into account. In addition to the functional complexity introduced by active transport of ions, the intracellular material, particularly in cells as structurally complex as muscle fibers, offers many possible degrees of heterogeneity of the state and distribution of ions. A number of workers hold that the cell membrane is not the principal determinant of ionic distribution and bioelectrical phenomena (see refs. 7, 8).

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*** The subscripts i and o refer to intracellular and extracellular, respectively.

In the present study, compartmentation of Cl^- and Na^+ in skeletal muscle of the lobster is shown by measurements of intracellular concentrations, net fluxes, and exchangeability with isotopic tracers of the ions. Some experiments on the distribution of K^+ were also performed. A preliminary report of some of the results has been published[9].

EXPERIMENTAL

Materials

The bender muscles of the walking legs of *Homarus americanus* were exposed by dissecting away a small piece of the exoskeleton. Care was taken to sever as few as possible of the connections of the fibers to the exoskeleton. Each leg with its muscle thus exposed was soaked in 25 ml of medium of a particular ionic composition at 5–7°. At various intervals, a leg was removed from the bathing medium. The muscle was dissected out, blotted on filter paper, and weighed on a torsion balance. The weights of the muscles ranged between 100 and 200 mg.

The tendon was found to account for less than 6 % of the weight of the muscle. Tendons were analyzed for K^+, Na^+, and Cl^-, and the concentrations were intermediate between those for muscle and plasma. The corrections of the concentrations in muscle for the ions in the tendon would be negligible.

The standard medium used in this work contained 15 mM KCl, 455 mM NaCl, 24 mM $CaCl_2$, 8 mM $MgCl_2$, and 6 mM H_3BO_3. The final pH was 7.5. This medium differs somewhat from the composition of lobster blood, principally by the elevated K^+. COLE[10] found 8.9 mM K^+, 459 mM Na^+, and 488 mM Cl^- in blood of *H. americanus*. Measurements made in conjunction with the present study gave somewhat different values: 5.6 mM K^+, 400 mM Na^+, and 490 mM Cl^-.

Chemical analyses

Electrolytes were extracted for analysis from the muscle in dilute acetic acid (2 drops of glacial acetic acid in 10 ml distilled water) by heating briefly near boiling and allowing the muscles to stand for 2 h. This extraction procedure gave results identical with those for muscles digested in nitric acid. Na^+ and K^+ concentrations were determined with a Coleman flame photometer. Standards with Na:K ratios near those of the tissue extracts were used, thus making unnecessary corrections for interference error. Cl^- concentrations were determined electrometrically with an Aminco-Cotlove titrator.

Tracer analyses

The rate and extent of exchange of intracellular ions with ions in the medium were determined by adding traces of the appropriate radioisotopes to the medium. Radioactivity of ^{42}K and ^{24}Na in samples of tissue extracts and media were determined using a crystal well scintillation detector. ^{36}Cl was counted from dried samples using a thin window low background gas flow detector. $AgNO_3$ was added to the samples before drying to precipitate Cl^-, thus preventing loss of ^{36}Cl by evolution of HCl during the drying.

Membrane potentials

Membrane potentials were determined with 3 M KCl-filled glass microelectrodes coupled to a high-input impedance amplifier and a cathode ray oscilloscope. Only microelectrodes with tip potentials less than 5 mV and resistances between 10 and 20 MΩ were used.

Extracellular space

In order to express cellular ion concentrations in mM/kg cells, extracellular spaces were determined using [^{14}C]inulin. The muscles were soaked for at least 12 h in media containing labeled inulin at concentrations less than 0.01 %. The muscles were then extracted for chemical analysis as described above. Radioactivities of dried samples of tissue extracts and of media were determined using a thin window gas flow detector. Counting of digests of extracted residues of tissue showed that the inulin is completely extracted in the dilute acid.

The rate of entry of [^{14}C]inulin into lobster muscles is shown in Fig. 1. These data show that 10–12 h are sufficient for equilibration. The mean value for extracellular space was 9 %. ROBERTSON[11] reported an extracellular space of 12 % for the Norway lobster.

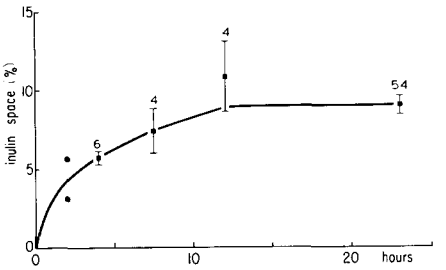

Fig. 1. Rate of equilibration of [^{14}C]inulin with lobster muscle at 5–7°. Points at 2 h represent single determinations. The square symbols represent means. S.E. and numbers of determinations for each mean are indicated.

This mean value of 9 % was used only in those experiments in which other radioisotopes were included. Otherwise corrections for extracellular space were made from each individual determination. All tissue ion concentrations in this paper are expressed in mM/kg cells.

The presence of inulin did not affect the resting potential of the muscle fibers. In 20 cells of 2 muscles which had been soaked in the standard medium containing 0.01 % inulin, the mean resting potential was 69.3 mV (± 0.37, S.E., 20 determinations). In two control muscles, the mean potential was 68.8 mV (± 0.35, S.E., 20 determinations).

Dry weights of muscles were determined by drying to constant weight at 100°. The dry/wet wt. ratio averaged 0.18.

Extracellular space is the largest uncertainty in measurements of intracellular ion concentrations. Even with inulin, there is the possibility of penetration into the cell, or binding at the cell surface. However, inulin remains the substance most likely to give an accurate estimate of extracellular space[12,13].

RESULTS

Intracellular concentrations of K^+, Na^+, and Cl^-

Muscles were soaked for 24 h at 5–7° in media containing, respectively, 0, 15, and 45 mM potassium (K_o^+). The intracellular concentrations of K^+, Na^+, and Cl^- are given in Table I, expressed as mM/kg cells, after correction for extracellular spaces. Data from a few determinations on fresh muscle are included, presented both in mM/kg cells and in mM/kg cell water, the latter after correction for the dry/wet weight ratio. Also included in Table I are measurements of ion concentrations in lobster muscle taken from the literature.

TABLE I

CONCENTRATIONS OF K^+, Na^+, Cl^- IN LOBSTER SKELETAL MUSCLE

A. Muscles of *H. americanus* soaked for 24 h in media containing various concentrations of K^+. Results are given in mM/kg cells as means ± 1 S.E. and the number of determinations.

Medium	K_i^+	Na_i^+	Cl_i^-	*Number of determinations*
0 mM K_o^+	106 ± 7.1	106 ± 4.5	93.6 ± 7.3	4
15 mM K_o^+	115 ± 1.3	89.2 ± 2.5	84.8 ± 2.3	15
45 mM K_o^+	142 ± 3.7	71.2 ± 6.1	84.9 ± 3.3	4

B. Ion concentrations in fresh muscle of *H. americanus* and of other species of lobster, the data taken from the literature. Values for *H. americanus* are given both in mM/kg cells and in mM/kg cell water. Other values are in mM/kg cell water.

Species	*Units*	K_i^+	Na_i^+	Cl_i^-	*Reference*
Homarus americanus	mM/kg cells	124	83	71	Present report
Homarus americanus	mM/kg cell water	155	104	89	Present report
Homarus vulgaris	mM/kg cell water	153	55	—	14
Nephrops norvegicus	mM/kg cell water	167	83	110	15
Nephrops norvegicus	mM/kg cell water	188	24.5	53	11

In the present work, the changes in intracellular K^+, Na^+, and Cl^-, after soaking for 24 h in standard medium were less than 10%. Only slightly more K^+ was lost after soaking in zero K_o^+ than was lost in standard medium. Lobster muscle has a much greater capacity to retain K^+ in the absence of external K^+ than does frog muscle[16]. The muscles in K^+-free medium gained more Na^+ than they lost K^+, and gained less Cl^- than Na^+.

The muscles soaked in 45 mM K_o^+ gained K^+ and lost a small amount of Na^+. The change in Cl_i^- was negligible. Although the Donnan product $K_o^+ \times Cl_o^-$ was varied over a wide range by varying K_o^+ from zero to 45 mM, the internal product of $K_i^+ \times Cl_i^-$ was nearly constant. The theory of distribution of ions in muscle based upon the Donnan equilibrium predicts that Cl_i^- in zero K_o^+ would be much less than in 45 mM K_o^+, when actually Cl_i^- was slightly higher in zero K_o^+ than in 45 mM K_o^+. In no way can the data obtained here be fit to a Donnan distribution of ions.

Distribution of chloride

The steady state relationship between Cl_i^- and Cl_o^- was determined by soaking muscle preparations for 22–24 h in media of different concentrations of Cl^-. In one experiment K_o^+ was kept at 15 mM and in another at 45 mM. Cl_o^- was varied between the control level (534 mM) and 15 mM by substituting propionate for Cl^-. The concentrations of Cl^- in muscles so treated are shown in Fig. 2. At all concentrations of Cl_o^-, Cl_i^- was the same at both 15 and 45 mM K_o^+. The value for Cl_i^- extrapolated to zero Cl_o^- was 28 mM/kg cells. The data suggest two compartments of Cl_i^-, one with 28 mM/kg cells which is constant despite changes in Cl_o^-, and might be called bound or immobile. The other compartment is mobile and varies directly with Cl_o^-, with a constant ratio of about 10:1. In the experiments shown in Fig. 2, in both 15 and 45 mM K_o^+, K_i^+ and Na_i^+ were changed less than 10% by soaking in reduced Cl_o^-, despite the marked reduction in Cl_i^-.

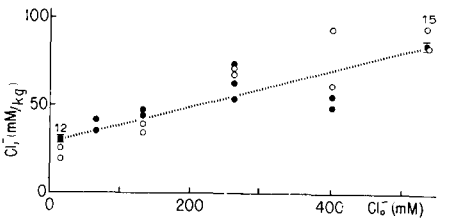

Fig. 2. Intracellular chloride concentrations (Cl_i^-, in mM/kg cells) in lobster muscles equilibrated for 22–24 h at 5–7° in media containing various concentrations of chloride (Cl_o^-) between 15 and 534 mM. Cl_o^- was reduced by replacement with propionate. The media contained potassium concentrations of either 15 mM (solid symbols) or 45 mM (open symbols). The circles represent single determinations, the squares, means, with S.E. and the numbers of determinations indicated. In both 15 and 45 mM K_o^+, intracellular K^+ and Na^+ were altered less than 10% by equilibration in reduced Cl_o^-.

To show that the muscles represented in Fig. 2 were in steady state, measurements were made of the rate of efflux of Cl_i^- into the low-Cl^- medium (15 mM Cl_o^-). The results are shown in Fig. 3a. Cl_i^- declined to about 30 mM/kg cells in 12 h and remained constant through the remainder of the experiment, another 12 h. Again in this experiment, K_i^+ and Na_i^+ remained constant within 10%. Thus the muscles in the experiment shown in Fig. 2 had been in steady state for 12 h.

The mobile and immobile compartments of Cl_i^- were also shown by experiments involving equilibration of muscles with media containing ^{36}Cl. The data from two experiments are shown in Fig. 4a. The data are presented as the amount of non-exchanged Cl^- (in mM/kg cells) remaining in the cells after equilibration for various times in media containing trace amounts of ^{36}Cl. These values were obtained from the following expression:

$$Cl_i^-\,(\text{nonexch.}) = \left[1 - \frac{\text{Spec. act.}_i}{\text{Spec. act.}_o}\right] \times Cl_i^-$$

where Spec. act.$_i$ and Spec. act.$_o$ are the specific activities of the cells and the medium, respectively. This manner of expressing the results is used to emphasize the non-exchangeable or immobile portion of intracellular Cl^-. The experiment in Fig. 4a represented by the solid symbols shows the exchange of Cl_i^- with ^{36}Cl in muscles

equilibrated with standard medium. 32.5 mM/kg cells (± 4.6, S.E., 6 determinations) of Cl_i^- remained unexchanged in 24 h. This is nearly the same as the concentration of Cl^- remaining in cells equilibrated in low-Cl^- medium (Fig. 3a).

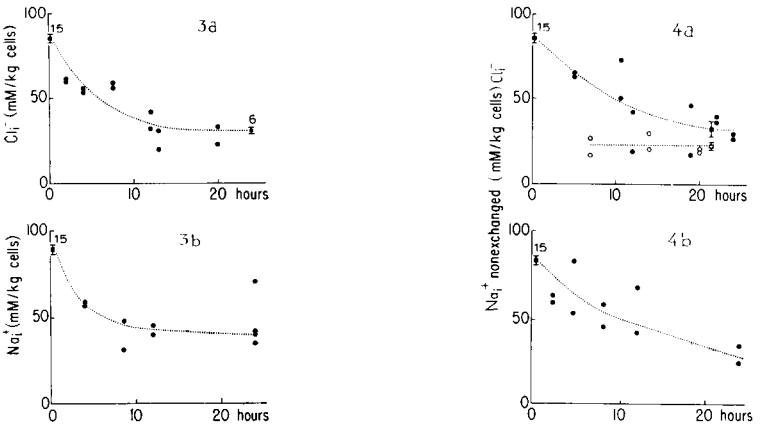

Fig. 3. Rate of net efflux of Cl^- (a) and Na^+ (b) from lobster muscles equilibrated, beginning at time zero, in a low-Cl^- medium (15 mM Cl^-) (a), the Cl^- replaced with propionate, and in Na^+-free medium (b), the Na^+ replaced with lithium. Na_i^+ and Cl_i^- are given in mM/kg cells. The squares represent means with S.E. and numbers of determinations indicated. The data at zero time, taken from Table I, are for muscles equilibrated 24 h in the standard medium. The circles represent single determinations.

Fig. 4. Equilibration of lobster muscles with media containing ^{36}Cl (a) or ^{24}Na (b). The data are given as the concentration of nonexchanged Cl^- or Na^+ remaining in the cells at various times after addition of isotopes to the medium (see text for method of calculation). a. Exchange of Cl_i^- with ^{36}Cl. The solid symbols represent the concentration of nonexchanged Cl_i^- in muscles in the standard medium. In the experiment represented by the open symbols, the preparations were soaked in a low-Cl^- medium (15 mM Cl_o^-) for 6 h before addition of ^{36}Cl to the medium. The mean, standard error of the mean, and number of determinations at zero time, taken from Table I, are for muscles equilibrated 24 h in the standard medium. The solid square symbol at 21.5 h represents the mean of the 6 determinations on muscles in standard medium from 19 to 24 h. The open square symbol represents the mean of all 6 determinations in the low-Cl^- medium. Standard errors for both means are shown by the brackets. b. Exchange of Na_i^+ with ^{24}Na. Concentrations of Na^+ in the cells not exchanged with the isotopes are indicated. The meanings of the symbols are the same as in 4a.

In another experiment, shown in Fig. 4a by the open symbols, muscles were first soaked in low-Cl^- medium for 6 h, at which time Cl_i^- had declined to about 50 mM/kg cells (see Fig. 3a). At this time (time zero in Fig. 4a), ^{36}Cl was added to the medium. By the time the first sample was taken, after 7-h exposure to ^{36}Cl, the muscles had been in low-Cl^- medium for 13 h, and the mobile compartment of Cl_i^- was assumed to be virtually depleted, with total Cl_i^- about 30 mM/kg cells. In these preparations only a small fraction of Cl_i^- exchanged with ^{36}Cl after 24 h. The concentration nonexchangeable Cl_i^-, 22.3 mM/kg cells (± 2.2, S.E., 6 determinations), was only slightly less than in preparations in the standard medium. This similarity was observed despite the large difference in total Cl_i^- in cells in the two kinds of media. The degree of exchangeability of Cl_i^- in cells in low-Cl^- medium provides further evidence for an immobile compartment of Cl_i^-, which is independent of changes in Cl_o^- and the mobile compartment of Cl_i^-.

Distribution of sodium

The rate and extent of net efflux of Na_i^+ were determined on cells soaked in Na^+-free medium, with lithium replacing the Na^+. The results are shown in Fig. 3b. Both the rate and extent of the efflux of Na_i^+ were similar to those of Cl_i^- into low-Cl^- medium.

The exchange of Na_i^+ with ^{24}Na in muscles in the standard medium shown in Fig. 4b, was also similar to the exchange of Cl_i^- with ^{36}Cl. The concentration of immobile, nonexchangeable Na_i^+ was about 35 mM/kg cells.

Potassium exchange

Fig. 5 shows the rate of exchange of K_i^+ with ^{42}K added to the medium. The muscles had been soaked in the standard medium (15 mM K_o^+) and therefore were in steady state. The rate of exchange was slow compared to the rates for mobile Cl_i^- and Na_i^+. Since the fibers begin to deteriorate after 24–30 h, it was not possible to determine the total extent of exchangeability of intracellular K^+.

Because of the relatively large size of the pieces of tissue used in this work, interfibrillar diffusion was of considerable importance in determining flux rates. Therefore, calculations of absolute transmembrane fluxes and permeabilities from the data presented here would be of little value.

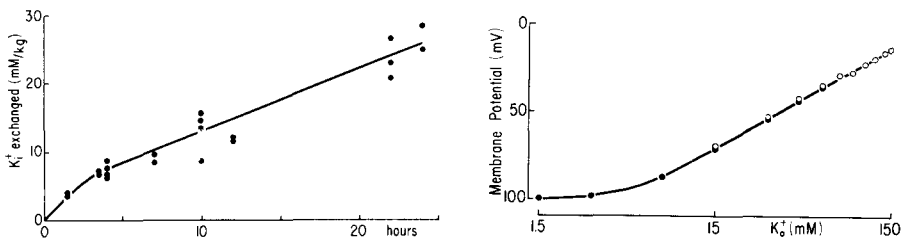

Fig. 5. Exchange of potassium in lobster muscles with ^{42}K. Muscles were in the standard medium, containing 15 mM K^+, at 5–7°. The data are in mM/kg cells exchanged after various exposure times. Each symbol represents a single determination.

Fig. 6. The resting membrane potential of lobster muscle plotted against the $\log_{10}$ of the concentration of K^+ in the medium (K_o^+, in mM/l). Open symbols: muscles first equilibrated in a medium containing 15 mM K^+. Solid symbols: muscles first equilibrated in a medium containing 1.5 mM K^+. The slope of the curve above 7.5 mM K_o^+ is 56 mV/10-fold change in K_o^+.

Resting membrane potentials

The relationship between the resting membrane potential and the concentration of K^+ in the medium was determined for muscle fibers previously equilibrated in the standard medium (15 mM K_o^+) and in a medium containing a low K^+ concentration (1.5 mM K_o^+). The increases in K_o^+ were made by adding an appropriate amount of 1.5 M KCl to the medium, so that the increase of K_o^+ also produced a hyperosmotic condition, and swelling of the fibers was reduced. Measurements of potential were begun after a 15-min equilibration period at each level of K_o^+, although the new potentials were more rapidly established. The results are shown in Fig. 6. Each point represents a mean of 10 determinations from 10 superficial fibers of the muscle. The slope of the curve relating potential and $\log_{10}$ K_o^+ was 56 mV per 10-fold increase in K_o^+ above 7.5 mM. Below 7.5 mM K_o^+, the slope of the curve was considerably

reduced. This near-ideal slope for the potential/$\log_{10} K_o^+$ curve was also observed for lobster muscle by WERMAN AND GRUNDFEST[17]. Although the data in Table I show that K_i^+ increases in lobster muscle after soaking in media containing elevated K_o^+, the equilibration times used in the experiments in which potentials were measured were probably not sufficient to allow the expected increases in K_i^+.

DISCUSSION

The results show that chloride in lobster muscle fibers is distributed in two distinct compartments: (1) a mobile compartment, proportional to Cl_o^- and readily exchangeable with ^{36}Cl in the medium; and (2) an immobile compartment, unexchangeable and constant despite changes in the mobile compartment and Cl_o^-. Intracellular Na^+ is apparently distributed similarly to Cl^-. No direct evidence was obtained indicating compartmentation of K_i^+, but the rapidly established relationship between the membrane potential and K_i^-, in contrast with the slow equilibration of K_i^+ with ^{42}K, may indicate some compartmentation of K^+.

Intracellular chloride

Two independent series of experiments provided the evidence for the immobile Cl^- compartment. A substantial fraction of Cl_i^- (28 mM/kg cells) remained after long equilibration in the low-Cl^- medium. An essentially similar fraction (32.5 mM/kg cells) was not exchangeable with ^{36}Cl. The immobile Cl^- compartment is constant despite changes in Cl_o^- and mobile Cl_i^- (Fig. 4a). There is no indication of the mechanism by which the Cl^- is immobilized, whether by tight chemical binding or by inclusion in a membrane-bounded structural compartment which is impermeable to Cl^-. These two alternatives have also been mentioned with regard to Na^+ compartmentation in frog skin[18]. Since binding and exchangeability are not necessarily mutually exclusive, inclusion in an impermeable organelle may be more likely. Neither the location of the immobile Cl^- nor its function can be determined from the present work.

An immobile compartment of intracellular Cl^- has also been suggested for the cortex of guinea-pig kidney[19], taenia coli muscle of guinea pig[20] and crayfish skeletal muscle[21]. Compartmentalized Cl^- has been suggested for frog muscle[22,23], but there is no immobile Cl^- in frog muscle[4],*.

ROBERTSON[11] investigated intracellular binding of ions in muscle of the Norway lobster *Nephrops norvegicus* by comparing concentrations of ions in intact muscle with concentrations in a fluid expressed from the muscle in a tissue-press. He obtained evidence for binding of cations (see below), but not for binding of Cl^-. The difference between ROBERTSON's data and those presented here for *H. americanus* may be due to the generic difference. However, it might also be due to the difference in techniques used to demonstrate immobile Cl^-. Preliminary evidence from studies on Cl^- binding in crayfish muscle have indicated that the binding depends upon the integrity of the cell. Whereas immobile Cl^- in crayfish has qualitative and quantitative characteristics similar to those of immobile Cl^- in lobster muscle, the immobile Cl^- in crayfish muscle is readily released from injured crayfish muscle fibers*. Immobile Cl^- in Nephrops muscle may have been released upon destruction of the cells in the tissue-press.

* P. B. DUNHAM, unpublished results.

In the present study, the fraction of Cl_i^- which exchanged with ^{36}Cl, and which was subject to efflux in low-Cl^- medium, constitutes the mobile compartment of Cl_i^-. The mobile compartment was about 65 % of total Cl_i^- in cells equilibrated in the standard medium, or 55 mM/kg cells. If the mobile Cl^- is restricted to some fraction of the volume of the cell, then the actual concentration of the mobile compartment will be greater than 55 mM. The minimum concentration of mobile Cl^- is greater than the concentration predicted on electrochemical grounds, assuming that mobile Cl^- is passively distributed across the cell membrane. The reversal potential of the inhibitory postsynaptic potential appears to be the equilibrium potential for chloride (E_{Cl^-}) (ref. 24). The reversal potential of the inhibitory postsynaptic potential in lobster muscle after 24 h in standard medium was about 70 mV. Taking this potential difference as E_{Cl^-}, Cl_i^- would be 33 mM, calculated from the Nernst equation, much lower than the minimum concentration of mobile Cl^- found in the present study. Furthermore, after 24 h in K^+-free medium, the resting potential of lobster muscle was 110–115 mV (ref. 25, 26). The reversal potential of the inhibitory postsynaptic potential was still negative to the resting potential, in this case by 10–15 mV. For E_{Cl^-} to be so negative in the fibers in K^+-free medium, the fibers should have lost Cl^-, when actually a small gain was observed (Table I). Thus the mobile Cl^- compartment can be correlated neither quantitatively nor qualitatively with the electrophysiological data.

Several other systems have a higher Cl_i^- concentration than would be predicted from a passive distribution of Cl^-. These include cells that are permeable to Cl^- such as crayfish giant axons[27] and mammalian cardiac muscle[28,29], as well as cells that are known or presumed to be effectively impermeable to Cl^-, such as the squid giant axon[30]. Taenia coli muscle of the guinea pig does not gain water in an isosmotic KCl medium, but it loses water in a hyperosmotic KCl medium[31]. These findings indicated[32] that this muscle is effectively impermeable to Cl^-, but as in the case of squid axon the impermeability is not absolute. A slow net flux of Cl^- was observed for taenia coli muscle[31]; Cl^- in squid axons exchanged slowly with ^{36}Cl (ref. 33).

Lobster muscle, like lobster axon[30], is readily permeable to Cl^-. Net efflux of Cl^- from lobster muscle was observed in the present work. Permeability to Cl^- was also indicated by the transient depolarization of the membrane potential when cells were transferred to a low-Cl^- medium (H. GAINER AND H. GRUNDFEST, unpublished results). Upon return to standard medium, a transient hyperpolarization was observed. These transient changes are expected for a system permeable to KCl (ref. 3). The net efflux of the mobile Cl_i^- in lobster muscle is no doubt associated with the repolarization of the membrane potential in the low-Cl^- medium.

The operation of a Cl^- pump in lobster muscle, such as has been postulated for the giant axons of squid[33] and of crayfish[27], is not precluded by the present data. Active Cl^- transport may be indicated by the fact that the reversal potential of the inhibitory postsynaptic potential (E_{Cl^-}) is consistently negative to the resting potential. However the structure of crustacean muscle is complicated by the existence of the transverse tubular system[34–36]. It is thus conceivable that the high concentration of mobile Cl^- in lobster muscle might be due to the presence of a portion of this in an extracellular compartment that is not penetrated by inulin. Such a situation has been suggested by experiments on crayfish muscle[21]. This fraction of mobile Cl^- would play no role in determining E_{Cl^-}.

Intracellular sodium

Intracellular Na^+ in lobster muscle is apparently distributed similarly to Cl^-. The immobile and mobile compartments of Na^+ were of about the same size as those of Cl^-. The rates of exchange and net efflux of the mobile compartments of Cl^- and Na^+ were also about the same.

Heterogeneous distribution of intracellular Na^+ has been suggested for frog muscle[37,38], smooth muscle[39,20], and turtle bladder[40]. From data obtained using cation-selective glass microelectrodes, LEV[41] calculated activity coefficients in frog muscle of about 0.78 for K^+ and 0.19 for Na^+, and suggested that some 70 % of Na_i^+ is somehow excluded from solution in the myoplasm. HINKE[14], using a similar technique, found activity coefficients for Na^+ of about 0.26 in muscle of both Carcinus and *H. vulgaris*. The activity coefficient for K^+ in Homarus muscle was 0.55. These data indicate binding of a significant fraction of Na_i^+, and possibly a small fraction of K_i^+.

HINKE[42] also measured intracellular activities and concentrations in squid axons. Assuming K^+ in the axoplasm 100 % free, the data showed 24 % of Na_i^+ bound. If 10 % of K_i^+ were bound[43] HINKE's data showed 31.5 % of Na_i^+ bound.

The same technique applied to muscle fibers of the giant barnacle by MCLAUGHLIN AND HINKE[44] indicated at least 84 % of Na_i^+, or 57 mM/kg cell water, was bound or compartmentalized. Unlike the immobile Cl^- compartment shown in the present work, the bound Na^+ in barnacle muscle was reduced in low-Na^+ medium so that the ratio of bound to mobile Na^+ was constant. MCLAUGHLIN AND HINKE[44] also analyzed Cl^- in barnacle muscle fibers, and found an average concentration of 97 mM/kg cell water. They assumed no bound Cl^- (because of the net anionic charge of myoplasm), but performed no relevant experiments.

ROBERTSON[11] obtained evidence for binding of intracellular K^+ and Na^+ in muscle of Nephrops. His approach, mentioned above, was to compare concentrations of ions in intact muscle and in fluid expressed from the muscle in a tissue-press. He calculated bound compartments of 82 % of Na_i^+ and 26 % of K_i^+. Total Na_i^+ was only 26.5 mM/kg cell water, so the concentration of immobile Na^+ in Nephrops was considerably less than that proposed here for *H. americanus*.

Recently COPE[45] suggested binding of 70 % of Na^+ in frog muscle, based on studies involving measurement of nuclear magnetic resonance of excised tissue.

Intracellular potassium

Intracellular K^+ found by chemical analysis in the present work was considerably lower than the concentration predicted from the relation between resting potential (E_m) and K_o^+. Assuming the membrane to be a K^+ electrode, and assuming the intracellular and external activity coefficients for K^+ to be the same, K_i^+ should be 240 mM in cells in standard medium. The highest value found in this study was for fresh muscle, 155 mM/kg cell water.

The slope of the $E_m/\log_{10} K_o^+$ curve was very near that predicted from the Nernst equation for a K^+-diffusion potential, suggesting a high transport number for K^+. However, the rate of exchange of K_i^+ with ^{42}K was slower than the rates of exchange of mobile Na_i^+ and Cl_i^- with tracer. This apparent discrepancy may indicate that the mobile compartments of Na^+ and Cl^- are superficially situated, and the compartment of K^+ which exchanges with tracer is distributed throughout the cell.

The predicted relationship between resting potential and K_o^+ has been reported for many systems, including frog muscle[46], squid axon[47], and lobster muscle[17], although at K^+ concentrations in the physiological range and lower, the relationship often fails. This has been explained in terms of a contribution of other ionic gradients to the potential difference at low K_o^+ (ref. 48), or by a decrease in the relative permeability to K^+ (ref. 49).

Many attempts have been made to show the nature of the relationship between K_i^+ and the membrane potential by changing K_i^+ by various means and measuring the resultant potential difference. The preponderance of the results have shown an equivocal relationship between K_i^+ and the membrane potential[50–59].

The lack of correlation between K_i^+ and the membrane potential found in the present work and elsewhere may be due to compartmentation of intracellular K^+ in one or both of two forms. The membrane potential may be determined by a K^+ compartment occupying only a fraction of the volume of the cell. This compartment would be in contact with the cell membrane and could be altered rapidly relative to the K^+ in the bulk of the cell. Furthermore, if a sizeable portion of intracellular water is bound, as has been suggested numerous times, K^+ may be excluded from the bound water, and the effective K^+ concentration would be higher than that determined by chemical analysis. Exclusion of alkali metal ions from water of hydration around DNA has been shown[60]. MCLAUGHLIN AND HINKE[44] measured an intracellular activity for K^+ which was higher than the concentration of K_i^+ determined by chemical analysis, a finding which is consistent with compartmentation of K^+ by exclusion from a portion of cell water.

ACKNOWLEDGEMENTS

We thank Dr. HARRY GRUNDFEST for his guidance in this study. The investigation was supported by National Science Foundation grant GB-1615 and National Institutes of Health grant GM-11441 to Dr. P. B. DUNHAM; National Institutes of Health grant NB-05043 to Dr. H. GAINER; National Institutes of Health grants NB-03728, NB-03270, and 5T NB-5328, National Science Foundation grant GB- 2940, and a grant from the Muscular Dystrophy Association of America, all to Dr. H. GRUNDFEST. Dr. H. GAINER was a Special Research Fellow of the National Institute of Neurological Diseases and Blindness.

REFERENCES

1 P. J. BOYLE AND E. J. CONWAY, *J. Physiol.*, 100 (1949) 1.
2 A. L. HODGKIN, *Biol. Rev.*, 26 (1951) 339.
3 A. L. HODGKIN AND P. HOROWICZ, *J. Physiol.*, 148 (1959) 127.
4 R. H. ADRIAN, *J. Physiol.*, 151 (1960) 154.
5 H. GRUNDFEST, in T. SHEDLOVSKY, *Electrochemistry in Biology and Medicine*, Wiley, New York, 1955, p. 141.
6 D. C. TOSTESON, in J. F. HOFFMAN, *The Cellular Functions of Membrane Transport*, Prentice-Hall, Englewood Cliffs, N. J., 1964, p. 3.
7 G. N. LING, *A Physical Theory of the Living State: The Association–Induction Hypothesis*, Blaisdell, New York, 1962, p. 680.
8 G. N. LING, *Perspectives Biol. Med.*, 9 (1965) 87.
9 P. B. DUNHAM AND H. GAINER, *Biol. Bull.*, 123 (1962) 494.
10 W. H. COLE, *J. Gen. Physiol.*, 23 (1940) 575.
11 J. D. ROBERTSON, *J. Exptl. Biol.*, 38 (1961) 707.

12 G. N. LING AND M. H. KROMASH, *J. Gen. Physiol.*, 50 (1967) 677.
13 E. BOZLER, *J. Gen. Physiol.*, 50 (1967) 1459.
14 J. A. M. HINKE, *Nature*, 184 (1959) 1257.
15 J. D. ROBERTSON, in B. SCHEER, *Recent Advances in Invertebrate Physiology*, University of Oregon Publ., Eugene, Oreg., 1957, p. 229.
16 H. B. STEINBACH, *J. Gen. Physiol.*, 44 (1961) 1131.
17 R. WERMAN AND H. GRUNDFEST, *J. Gen. Physiol.*, 44 (1961) 997.
18 C. A. ROTUNNO, V. KOWALEWSKI AND M. CEREIJIDO, *Biochim. Biophys. Acta*, 135 (1967) 170.
19 R. WHITTAM, *J. Physiol.*, 131 (1956) 542.
20 B. BUCK AND P. J. GOODFORD, *J. Physiol.*, 183 (1966) 551.
21 P. B. DUNHAM, J. P. REUBEN AND P. W. BRANDT, *Biol. Bull.*, 127 (1964) 364.
22 E. J. HARRIS, *J. Physiol.*, 141 (1958) 351.
23 E. J. HARRIS, *J. Physiol.*, 166 (1963) 87.
24 H. GRUNDFEST, J. P. REUBEN AND W. H. RICKLES, *J. Gen. Physiol.*, 42 (1959) 1301.
25 H. GRUNDFEST AND J. P. REUBEN, in E. FLOREY, *Nervous Inhibition*, Pergamon Press, London, 1961, p. 92.
26 M. MOTOKIZAWA, J. P. REUBEN AND H. GRUNDFEST, *J. Gen. Physiol.*, 50 (1967) 2491.
27 A. STRICKHOLM AND B. G. WALLIN, *Nature*, 208 (1965) 790.
28 O. F. HUTTER AND D. NOBLE, *J. Physiol.*, 147 (1959) 16P.
29 J. F. LAMB, *J. Physiol.*, 157 (1961) 415.
30 A. R. FREEMAN, J. P. REUBEN, P. W. BRANDT AND H. GRUNDFEST, *J. Gen. Physiol.*, 50 (1966) 423.
31 R. CASTEELS AND H. KURIYAMA, *J. Physiol.*, 184 (1966) 120.
32 H. GRUNDFEST, *Federation Proc.*, 26 (1967) 1613.
33 R. D. KEYNES, *J. Physiol.*, 169 (1963) 690.
34 L. GIRARDIER, J. P. REUBEN, P. W. BRANDT AND H. GRUNDFEST, *J. Gen. Physiol.*, 47 (1963) 189.
35 P. W. BRANDT, J. P. REUBEN, L. GIRARDIER AND H. GRUNDFEST, *J. Cell Biol.*, 25 (1965) 233.
36 A. SELVERSTON, P. W. BRANDT AND J. P. REUBEN, *Biol. Bull.*, 131 (1966) 407.
37 E. J. CONWAY AND M. CAREY, *Nature*, 175 (1955) 773.
38 C. EDWARDS AND E. J. HARRIS, *J. Physiol.*, 135 (1957) 567.
39 G. BURNSTOCK, D. J. DEWHURST AND S. E. SIMON, *J. Physiol.*, 167 (1963) 210.
40 C. M. PAINE AND E. C. FOULKES, *Biochim. Biophys. Acta*, 78 (1963) 767.
41 A. A. LEV, *Nature*, 201 (1964) 1132.
42 J. A. M. HINKE, *J. Physiol.*, 156 (1961) 314.
43 A. L. HODGKIN AND R. D. KEYNES, *J. Physiol.*, 119 (1953) 513.
44 S. G. A. MCLAUGHLIN AND J. A. M. HINKE, *Can. J. Physiol. Pharmacol.*, 44 (1966) 837.
45 F. W. COPE, *J. Gen. Physiol.*, 50 (1967) 1353.
46 G. LING AND R. W. GERARD, *J. Cellular Comp. Physiol.*, 34 (1949) 383.
47 A. L. HODGKIN AND R. D. KEYNES, *J. Physiol.*, 128 (1955) 61.
48 A. L. HODGKIN AND B. KATZ, *J. Physiol.*, 108 (1949) 37.
49 H. GRUNDFEST, *Ann. N. Y. Acad. Sci.*, 94 (1961) 405.
50 H. GRUNDFEST, C. Y. KAO AND M. ALTAMIRANO, *J. Gen. Physiol.*, 28 (1954) 245.
51 R. H. ADRIAN, *J. Physiol.*, 133 (1956) 631.
52 W. K. STEPHENSON, *J. Cellular Comp. Physiol.*, 50 (1957) 105.
53 K. KOKETSU AND Y. KIMURA, *J. Cellular Comp. Physiol.*, 55 (1960) 239.
54 P. F. BAKER, A. L. HODGKIN AND T. I. SHAW, *J. Physiol.*, 164 (1962) 355.
55 P. F. BAKER, A. L. HODGKIN AND H. MEVES, *J. Physiol.*, 170 (1964) 541.
56 T. NARAHASHI, *J. Physiol.*, 169 (1963) 91.
57 I. TASAKI AND T. TAKENAKA, *Proc. Natl. Acad. Sci. U.S.*, 50 (1963) 619.
58 I. TASAKI AND M. LUXORO, *Science*, 145 (1964) 1313.
59 S. HAGIWARA, S. CHICHIBU AND K.-I. NAKA, *J. Gen. Physiol.*, 48 (1964) 163.
60 J. E. HEARST AND J. VINOGRAD, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 1005.