BRIEF COMMUNICATIONS

CATION CONCENTRATIONS IN THE HEMOLYMPH OF *LOLIGO PEALEI*

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ABSTRACT Hemolymph and protein-free hemolymph obtained from *Loligo pealei* were analyzed for cation concentration by the method of atomic absorption spectroscopy, for the following ions: Na^+, K^+, Ca^{++}, and Mg^{++}. No significant differences were seen in ion concentration between hemolymph and protein-free hemolymph. Of particular neurophysiological significance is that K^+ ion concentration is much closer to that of seawater than previously reported.

INTRODUCTION

A recent survey of the literature concerning the ionic composition of squid hemolymph revealed an unexpected finding. J. D. Robertson (1965) reported that the plasma of *Loligo forbesi* contains potassium ions in a concentration of approximately 20 mM (mean for three samples is 20.57 mM). This value is twice the concentration routinely used for making artificial seawater (ASW) for voltage clamp studies on *L. pealei* giant axons (Adelman and Palti, 1969; Adelman et al., 1973) and is twice the value used in the original voltage clamp studies upon the axons of *L. forbesi* (Hodgkin et al., 1952). The ion determinations performed by Robertson employed gravimetric and titrimetric techniques. In particular, Robertson and Webb (1939) noted that of the ion determination methods employed, potassium determination was the most subject to error. Manery (1939) analyzed hemolymph from *Loligo pealei* for sodium and potassium ions and reported a mean value of 16.6 mM for potassium concentration. This determination also employed a gravimetric technique.

To verify the magnitude of external potassium concentration, analysis of cation content was performed upon samples of both hemolymph and protein-free hemolymph from *L. pealei*. An accurate knowledge of ion concentration in squid hemolymph is of considerable significance in terms of the *in situ* electrophysiological behavior of the axon.

METHODS

Specimens of *Loligo pealei,* obtained from the Marine Biological Laboratory supply department, were decapitated, and the mantle cavity was exposed and pinned open. The central region over the hearts and gills was blotted as dry as possible. Hemolymph samples were obtained by inserting a fine syringe needle into either the vena cava or branchial arteries and withdrawing the hemolymph into the syringe with suction. Hemolymph upon withdrawal appeared dark blue. Care was taken to avoid contamination of the sample with interstitial fluid or seawater, and any samples suspected of contamination were discarded. A total volume of 0.204 ml was collected from two animals.

The hemolymph samples were diluted 1:1,000 (vol/vol) with distilled water and divided into two aliquots for further dilution: one aliquot for whole hemolymph, and a second for preparing a protein-free solution. The second aliquot was treated with sufficient trichloroacetic acid (TCA) to correspond to a 10% TCA solution of the original sample. The TCA-protein precipitate was cleared by centrifugation and the supernatant was used for the ion determinations. The determination of concentrations of Na^+, K^+, Mg^{++}, and Ca^{++} ions was performed on an Instrumentation Laboratory atomic absorption spectrophotometer, Model 251 (Instrumentation Laboratory, Inc., Lexington, Mass.). To have a dilution of each ion appropriate to the most sensitive range of the method, the concentrations of the above ions in seawater were used as an initial choice of the most probable concentrations. Thus, further dilutions of 5×10^{-4}, 1.25×10^{-4}, and 1×10^{-4} (vol/vol) were made.

A standard curve obtained by appropriate dilution of commercial ion standards (Instrumentation Laboratory) to bracket the estimated concentration for each ion determination was made. The unknown concentration was then obtained for the absorbance value and the regression line generated from the standard values. In addition, samples of the appropriate dilution of 10% TCA solution alone and the distilled water were analyzed for ionic content. Values obtained for protein-free hemolymph were corrected for any increase in ion concentration due to TCA or the water. Six separate determinations were made for Na, seven for K, four for Mg, and five for Ca.

RESULTS

Values for ion concentrations determined in hemolymph and protein-free hemolymph are presented in Table I.

TABLE I

ION CONCENTRATIONS IN SQUID HEMOLYMPH

	Sodium	Potassium	Magnesium	Calcium
		mM		
Hemolymph	365.3	11.5	45.2	11.0
	378.3	11.3	42.8	10.5
	387.0	12.3		12.0
	390.5	11.3		
	427.5			
Protein-free	374.0	10.7	47.3	10.5
hemolymph		12.5	48.1	8.2
		12.4		
Mean values	392.9	11.7	45.9	10.4
SD	±25.1	±0.7	±2.4	±1.4

DISCUSSION

The mean values reported by Robertson (1965) for the above ions in *L. forbesi* are Na^+, 419 mM; K^+, 20.6 mM; Mg^{++}, 51.6 mM; Ca^{++}, 11.3 mM. The discrepancy between K values is particularly interesting. An increase in external K from 10 to 20 mM results in a decrease in the Nernst potential for potassium of 17 mV at 5°C. If one uses the Goldman (1943) relation to solve for the resting membrane potential at 5°C and assumes an internal concentration for Na and K of 40 and 300 mM, respectively, and a P_{Na}/P_K of 0.043, the twofold increase of external potassium results in a depolarization of the membrane of approximately 8 mV. From the data of Hodgkin and Huxley (1952), this 8-mV depolarization would effect a reduction of the steady-state value of the sodium inactivation parameter, h, from approximately 0.59 to 0.29. Further, Adelman and Palti (1969) have shown that such an increase in external K results in an approximate decrease in the steady-state value of h 30% beyond that expected from the Hodgkin-Huxley (1952) voltage dependence alone. It appears that if an axon were actually bathed in 20 mM K^+, it would be in an extremely refractory condition and thus greatly reduced in its ability to conduct action potentials if capable at all, especially given the range of conductance parameters from one axon to the next. In addition, Moore and Cole (1960) measured resting and action potentials of giant axons in vivo in *L. pealei* and found resting potential values very difficult to explain if the extracellular potassium concentration were 20 mM.

To verify this analysis, simulated action potentials elicited by 0.5-ms current pulses were calculated with a program developed by Dr. Richard FitzHugh (Adelman and FitzHugh, 1975) for axon parameters dependent upon external ion concentrations. When external K was increased twofold, the axon threshold was increased by about 15 mV and the action potential amplitude reduced by a third. It should be noted that our mean value for hemolymph sodium concentration effects a reduction in E_{Na} of about 2 mV compared to the value of Robertson (1965), but this reflects less than a 4% reduction in the driving force for I_{Na}. The differences for divalent cation values between hemolymph and ASW are not expected to have any noticeable physiological significance. Therefore, the use of ASW as an inorganic substitute for hemolymph is well founded.

There are several possibilities for the difference between the potassium concentration in *L. forbesi* and *L. pealei* hemolymph. First, the atomic absorption method is relatively insensitive to the presence of other ions or substances, while, as Robertson and Webb (1939) note in their original report of their methods, "Most animal body fluids, however, contain quantities of protein which are sufficient to interfere with these methods and cause serious errors." It is possible in Robertson's (1965) determinations for *L. forbesi*, since the hemolymph was subject only to centrifugation before analysis, that contaminating serum proteins remained and thus the potassium determinations were affected. Alternatively, surrounding tissue may have been injured, allowing the leakage of high intracellular K into the sample.

It is also possible that *L. forbesi* hemolymph does in fact contain a higher level of K ions, but in view of the general correspondence between the electrophysiological be-

havior of axons from this species and *L. pealei,* this seems unlikely. Manery's (1939) mean value for potassium in *L. pealei* is considerably closer to the value we have determined. In this case the particular gravimetric method used is known to give high estimates if theoretical conversion factors are employed (Korenman, 1969), which may be sufficient in itself to account for the discrepancy.

It should be noted here that the value of hemolymph potassium for a related genera, *Octopus dofleine* is reported (Potts and Todd, 1965) as 10.3 mM, again closer to the value expected for physiological activity.

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