IONIC COMPOSITION OF THE HAEMOLYMPH OF THE WHIP SCORPION, *THELYPHONUS INDICUS* STOLICZKA, AND ITS SALINE FORMULA

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Synopsis

GEETHABALI and K.P. RAJASHEKHAR (Neurophysiology Laboratory, Department of Zoology, Bangalore University, Bangalore-560 056, India): Ionic composition of the haemolymph of the whip scorpion *Thelyphonus indicus* STOLICZKA, and its saline formula. *Acta arachnol.*, 36: 87-92 (1988).

The concentration of major ions (mM/l) in haemolymph of the whip scorpion, *Thelyphonus indicus* STOLICZKA, were found to be $Na^+=222.6$, $K^+=14.24$, $Ca^{2+}=12.50$, $Mg^{2+}=7.52$ and $Cl^-=235.16$. The pH of haemolymph was found to be 7.60. Based on these values the saline formula was established as follows: NaCl =13.00 g/l, KCl=1.06 g/l, $CaCl_2$=1.38 g/l, $MgCl_2$=1.52 g/l. The pH was adjusted to 7.60 with 0.2 M, Tris-HCl buffer. The suitability of the prepared saline for neurophysiological studies has been tested.

Introduction

Neurophysiological studies often need maintenance of dissected preparations using physiological saline which provides ions in proper concentration. While initiating such studies on the central nervous system of the whip scorpion, *Thelyphonus indicus* STOLICZKA, there was a need to newly prepare a suitable saline. The present paper describes the ionic composition of the haemolymph and provides a recipe for the preparation of the whip scorpion Ringer.

Materials and Methods

Specimens of the whip scorpion, *Thelyphonus indicus* STOLICZKA, were collected in the vicinities of Bangalore, south India, during Monsoon months from

June to September. Specimens were reared in glass terraria filled with soil and fed on a diet of cockroaches. Water was provided in a petri dish and the soil was kept moist with periodical water sprinkling.

Animals were starved for five days prior to the collection of haemolymph. Haemolymph samples (0.1 ml) were collected by inserting the needle of a hypodermic syringe through the joint of the pedipalp. Samples were centrifuged and cell-free haemolymph was analysed for Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- concentration. Na^+ and K^+ were estimated by the flame photometry but Ca^{2+} and Mg^{2+} using the Perkin-Elmer 403 atomic absorption spectrophotometer after a requisite dilution. Chloride content was estimated by the method of VAN SLYKE (1923). The pH of the haemolymph was measured directly from the droplet of haemolymph extruded on puncturing the arthropodal joint using the Beckman micro pH electrode.

Results

The concentration of major ions in the haemolymph is given in Table 1. The pH was found to be 7.60±0.08. The total cation concentration was higher than that of chloride.

The physiological saline was prepared using chloride salts in concentrations corresponding to the mM values of major cations estimated in the haemolymph. These values are given in Table 2. The saline was buffered at pH 7.60 with 0.2 M Tris-HCl buffer. Attempts to maintain the pH using sodium bicarbonate as one of the constituents did not prove satisfactory since it yielded precipitate with chloride of calcium.

Table 1. Concentration of inorganic ions in the haemolymph of *Thelyphonus indicus* STOLICZKA.

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
mM/l±SD	222.6±12.8	14.24±1.54	12.50±1.28	7.52±0.82	235.16±10.59

Table 2. Physiological saline formula based on the ionic concentration of haemolymph in *T. indicus*.

	NaCl	KCl	$CaCl_2$	$MgCl_2$	pH
g/l	13.00	1.06	1.38	1.52	7.60

The suitability of the saline was tested by studying the spontaneous electrical activity in certain central neurons as well as their responses to mechanical and electrical stimuli before and after washing with the prepared saline. The animals were dissected dorsal side up, to expose the ventral nerve cord. Recordings were made using platinum hook electrodes and conventional electrophysiological setup. Responses from the central neurons at the region corresponding to the seventh abdominal segment are shown in Figures 1 and 2. Reproducible results were obtained for about eight hours after which the preparation showed signs of deterioration.

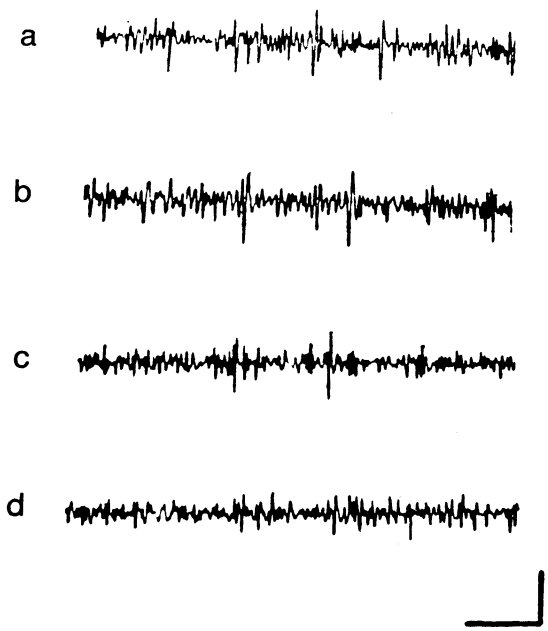

Fig. 1. Spontaneous electrical activity recorded from the ventral nerve cord of the whip scorpion immediately after dissection (a), after washing with the prepared saline (b), six hours after dissection (c), and after eight hours showing signs of degeneration (d).
Calibration: 0.2 mV, 20 m sec.

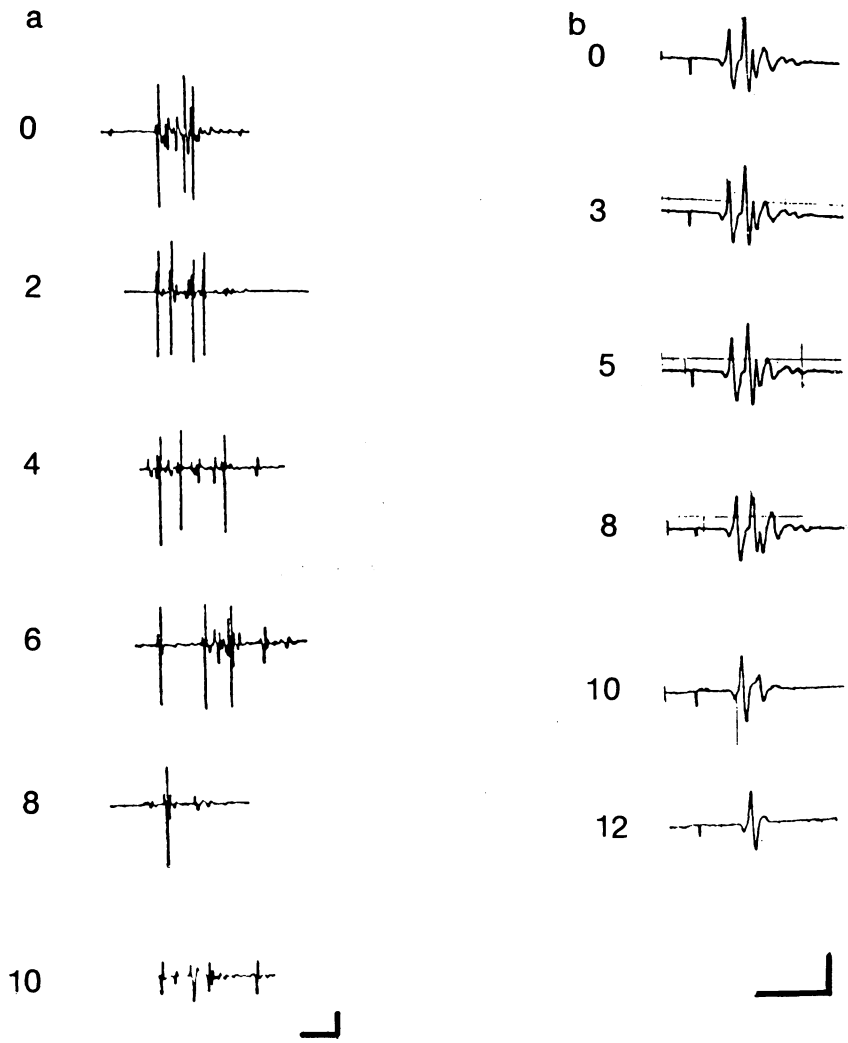

Fig. 2. Oscillographic recordings of the electrical activity of the central nervous system of the whip scorpion. (a) Response from units in the ventral nerve cord to mechanical stimulus of the flagellum brought about by an electrically driven solenoid. Identical responses were obtained for more than six hours. (b) Response to electrical stimulation from an isolated preparation. Square wave stimulus of fixed strength and duration elicited unremittent responses for about eight hours. Numericals at left indicate time (in hours) after dissection.
Calibration: (a) 0.5 mV, 40 m sec.
(b) 0.25 mV, 20 m sec.

Discussion

BURTON (1984) analysed the existing data on the major cations in the haemolymph of scorpions and spiders, and established the regression equation* which provides the quantitative interrelation between inorganic ions. Application of this regression equation to Ca^{2+} on other cations to the values obtained in the present study gives a value 10.05 mM and the observed concentration of 12.50 mM is comparable though slightly higher. This indicates the similarity of the ionic composition of whip scorpion to that of other arachnids.

The haemolymph of whip scorpion is slightly alkaline with pH of 7.60. This falls within the range of haemolymph values observed in other arachnids (6.3-8.3). Such a wide variation in the arachnids is not observed in other arthropod groups. It would be of interest to study the ecological and physiological factors responsible for such a variation.

The fact that the saline formulated for whip scorpion is suitable for neurophysiological studies was evident from our electrophysiological experiments. Consistent responses were obtained from identifiable units of the ventral nerve cord maintained in the prepared saline for more than six hours. These responses were essentially similar to the responses observed before washing with saline showing that the physiological properties are not altered on application of the saline. The chloride concentration of the saline exceeds that of the haemolymph but this does not seem to have any deleterious effect. Since the saline is buffered it can be used as a medium to study the effect of drugs and other chemicals on the nervous system without altering the pH. Tris-HCl buffer was made use of to maintain the pH as does not cause undesirable effects and the same has been used by PADMANABHANAIDU (1967) and BOWERMAN (1976) while formulating the salines for scorpions. The use of inorganic salts for maintaining the pH may affect in two ways; firstly by increasing the cation concentration and secondly by altering the osmotic concentration.

References

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* $[Ca^{2+}]=0.428[K^+]+0.0215[Na^+]+0.276[Mg^{2+}]-2.9(mM)$.

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