Physiological Saline Suitable for the Marine Isopod Crustacean Bathynomus doederleini

Authors: F.-Tsukamoto, Yoko, Kuwasawa, Kiyoaki, Takeuchi, Shigeo, and Mano, Motoko

Source: Zoological Science, 17(4) : 425-430

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/0289-0003(2000)17[425:PSSFTM]2.0.CO;2

ZOOLOGICAL SCIENCE **17**: 425–430 (2000)

Physiological Saline Suitable for the Marine Isopod Crustacean *Bathynomus doederleini*

Yoko F.-Tsukamoto[1], Kiyoaki Kuwasawa[1]*, Shigeo Takeuchi[2]**
and Motoko Mano[2]

[1]*Department of Biological Sciences, Tokyo Metropolitan University, Minami-ohsawa 1-1, Hachioji-shi, Tokyo 192-0397, Japan*
[2]*Department of Chemistry, Nihon University School of Medicine, Ohyaguchi-Kamimachi 30-1, Itabashi-ku Tokyo 173-8610, Japan*

ABSTRACT—In order to establish the physiological saline suitable for the giant marine isopod *Bathynomus doederleini*, we analyzed ionic compositions, pH and osmotic pressure of the hemolymph. We made three test salines on the basis of ionic compositions of the hemolymph. Saline A contains surplus Cl^- (89.5 mM over in comparison with the serum) and no SO_4^{2-}. Saline B contains surplus Cl^- (71.4 mM over) and normal SO_4^{2-}. Saline C contains normal Cl^- and surplus SO_4^{2-} (35.7 mM over). We examined the effects of the test solutions and other salines on activities of isolated hearts. The heart in the salines continued to beat regularly more than 20 hr at 20°C. Heart rate in the test solutions was significantly higher (saline A: 66.5±5.1/min, n=8, saline B: 60.4±7.0/min, n=9, saline C: 67.9±8.4/min, n=8) than that in sea water (SW: 50.2±4.8/min, n=10) and was significantly lower than that in the saline for decapod which was originally designed by Pantin (Pantin's saline : 77.6 ± 8.9 /min, n=8). Among the test salines, there were no significant differences in heart rate, systolic force and membrane potential of cardiac ganglionic neuron and myocardial cell. The standard physiological saline for *Bathynomus* was determined as follows (mM) ; Na^+, 479.4; K^+, 15.7; Ca^{2+}, 14.6; Mg^{2+}, 60.7; Cl^-, 627.6; SO_4^{2-}, 9.1; HEPES, 5; pH 7.9.

INTRODUCTION

Although many physiological salines have been designed for crustaceans, only a few kinds for isopods have been reported. There are salines for *Porcellio* (Holley and Régondaud, 1963), *Asellus* (Kawaguti, 1933; Needham, 1954) and *Saduria* (Bogucki, 1931) cited in Midsukami (1991). Holley and Delaleu (1972) and Delaleu and Holley (1976) designed physiological salines for *Porcellio* and *Oniscus*, respectively. Yamagishi and Ebara (1985) composed a *Ligia* saline based on the data in Parry (1953). There are only a few references available for ionic composition of marine isopod hemolymph.

For physiological study of the giant marine isopod *Bathynomus*, sea water (SW) has been used as saline (Kihara and Kuwasawa, 1984; Tanaka *et al*. (1992); Fujiwara-Tsukamoto *et al*., 1992; Okada and Kuwasawa, 1995). In natural SW, the isolated *Bathynomus* heart continued regular heartbeats for more than twelve hours, stimulation of cardiac nerves was effective and neuromuscular junctional potentials could be recorded intracellularly from the cardioarterial valves (Kihara and Kuwasawa, 1984). Tanaka *et al*. (1992) and Okada *et al*. (1997) used the saline for marine decapod crustacean originally designed by Pantin (1934) and modified by Matsui (1957) and then Yazawa and Kuwasawa (1984). The ionic composition of the saline of Pantin (1934) was based on concentration for *Carcinus* hemolymph (Bethe, 1929, cited in Pantin, 1934). In their physiological studies using *Bathynomus*, they succeeded in obtaining pharmacological responses in some *Bathynomus* tissues. However, the candidate neurohormones such as serotonin, octopamine, norepinephrine and proctolin contracted valve muscles but they never changed the membrane potential in SW (F.-Tsukamoto and Kuwasawa, 1997). Normal ionic composition and physiological conditions were required to examine the peculiar phenomena.

Ionic compositions and physiological conditions of *Bathynomus* hemolymph were analyzed biochemically and physiologically. Based on the analyzed parameters, we designed the suitable physiological saline. Furthermore, we tested some variations derived from the saline using the isolated *Bathynomus* heart.

MATERIALS AND METHODS

Animals

The giant marine isopod *Bathynomus doederleini* (9–15 cm in body length) was used. Animals were collected at Sagami Bay and off Tokyo Bay in fishing traps, and kept at 15°C in a laboratory

* Corresponding author: Tel. +81-426-77-2578;
FAX. +81-426-77-2559.
E-mail: kuwasawa-kiyoaki@c.metro-u.ac.jp

** deceased

aquarium. About seventy animals were used in this study.

Samples

Before dissection, an animal was immersed in ice-cold SW (2°C). After SW on the body surface was completely wiped off, the basipodites of thoracic legs were cut. The cut ends of the legs were introduced into glass centrifuge tubes to collect hemolymph. The coagulated hemolymph was ground with a glass rod and centrifuged under refrigeration at 700×g for 20 min. Separated supernatants were kept in vial at 4°C as test serum samples. The clots were collected to use for measurement of elementary concentrations of blood cells. All glassware used for an ionic analysis were immersed in 1N nitric acid solution for deionization, rinsed with deionized water and dried before use.

Measurements of ion and element concentrations

We used ion-selective electrode equipped with an apparatus (Hitachi, H-736) to measure Na^+, K^+ and Cl^- concentrations, and ion chromatography (DIONEX QIC, collumn: DIONEX AS4A-SC) for SO_4^{2-}.

Inductively coupled plasma (ICP) spectrometry (Hitachi ICP emission analyzer system, sequential type) was employed for the qualitative analysis of serum elements and a quantitative analysis for measurement of the concentration of Ca, Mg and other elements (Takeuchi *et al.*, 1995). The samples were diluted with internal standard solution (ISS: 0.2 ppm Be /0.05N HCl in DW). For the measurement of the elementary concentrations in blood cells, the clot containing blood cells was dried at 120°C and then ashed in order to remove organic compounds. The ashed samples were diluted with ISS. For convenience of calculation, the specific gravity of hemolymph was regarded as 1.

Measurements of pH and osmotic pressure

Fresh and non-coagulated hemolymph exuded when a piece of carapace just above the heart was cut off and was used to measure pH by test paper (BTB and CR, TOYO; pH-Fix 7.5–9.5, MACHEREY-NAGEL). The osmotic pressure of test serum samples was measured with an osmometer (Kyoto Daiichi Kagaku, Auto & Stat™ OM-6030).

Electrophysiology

Before dissection, an isotonic 10–20 ml of $MgCl_2$ (0.36 M) solution was injected into the body to anesthetize an animal. The cephalon, thoracic appendages, sternum, digestive organs and reproductive organs were removed carefully. For recording of the mechanogram of the heartbeat, the cardiovascular system with the carapace was pinned ventral side up to the Sylgard (Dow corning)-lined bottom of the experimental chamber. For the intracellular recording from cardiac ganglionic neurons and the myocardial cells, the heart was isolated, and cut open along the midline of the ventral wall to expose cardiac ganglion on the dorsal wall of the heart.

Heartbeat was monitored with a strain gauge mechanotransducer (Nihon Kohden, TB611T). Intracellular recording was carried out with a grass microelectrode filled with 3M KCl (tip resistance, 8–20 MΩ). The intracellular microelectrode, connected to an Ag-AgCl wire, was coupled to a high input-impedance preamplifier (Nihon Kohden, MEZ-8300). Signals were displayed on a pen-writing chart recorder (Nihon Kohden, WI-641G) and on a CRO (Nihon Kohden, VC-11). Signals on the CRO display were photographed by a CRO camera (Nihon Kohden, RLG-6201).

Specimens were perfused with each of three test solutions, filtered SW and modified Pantin's saline (Yazawa and Kuwasawa, 1984). The five perfusates were applied in irregular order. In case of mechanogram recording, the heart was perfused with the solutions through a polyethylene cannula (tip diameter, 250 μm) inserted into the heart through a small opening on the ventral side of the heart. The electrophysiological data were taken 30 min after switching for one to another perfusate since it took about 20 min for the previous perfusate to be replaced with next perfusate in the perfusing system.

Analyses of data

All results are expressed as mean±SD. One-way analysis of variance (ANOVA) and Scheffe's multiple comparisons test were used for statistical analyses. Significance was determined at $P<0.05$.

RESULTS

Among the 50 measurable elements by ICP spectrometry, Na, K, Ca, Mg, Be, Si, P, V, Cr, Mn, Fe, Cu, Zn, Sr, Cd, Ba, and W were detected in *Bathynomus* serum. The concentration of Na^+, K^+ and Cl^- measured by ion-selective electrodes (n=6) are shown in Table 1. Table 2 shows the results of a quantitative analysis by ICP spectrometry about Ca, Mg, Cu, Fe, P, Sr and Zn (n=5).

Since it was impossible to measure the concentration of S in this ICP spectrometry system, the concentration of SO_4^{2-} was measured to be 9.12±2.16 mM (n=6) by ion chromatography. BTB pH test paper showed the tone of color of 7.2~7.6, CR showed 7.8~8.2 and pH-Fix 7.5–9.5 showed 7.9~8.2. Osmotic pressure of serum was 1140±66 mOsm (n=6). From these results, ionic compositions of *Bathynomus* serum revealed as follows (mM); Na^+, 479.4; K^+, 15.7; Ca^{2+}, 14.6; Mg^{2+}, 60.7; Cl^-, 556.2; SO_4^{2-}, 9.1; osmotic pressure, 1140 mOsm; pH 7.9.

To eliminate the intracellular components of blood cells from the test serum samples, we measured the volume rate of blood cells to the total hemolymph volume. Most of the blood cells were oval. The blood cell was 17.5±0.6 μm (n=6) in length of major axis whereas the minor axis was 12.5±0.4 μm (n=6). The volume of the blood cell was calculated approximately to be 1766.5 μm^3. The number of blood cell counted with the erythrocytometer was 1180±444 /mm^3 (n=6). The volume rate of blood cells was calculated to be 0.21% of the total hemolymph. Results from a quantitative analysis by ICP spectrometry are shown in Table 3.

We prepared three test salines (saline A, B and C in Table 4) which had different Cl^- and SO_4^{2-} concentrations. Saline A contains surplus Cl^- (89.5 mM over in comparison with the serum) and no SO_4^{2-}. Saline B contains surplus Cl^- (71.4 mM over) and normal SO_4^{2-}. Saline C contains normal Cl^- and surplus SO_4^{2-} (35.7 mM over). In Table 4, the ionic composi-

Table 1. Concentrations of Na^+, K^+ and Cl^- in the serum measured by ion-selective electrodes (mM). n=6.

	Na^+	K^+	Cl^-
mean	479.42	15.72	556.17
SD	32.59	2.69	41.19

Table 2. Concentrations of elements in the serum measured by ICP spectrometry (mM). n=5.

	Ca	Mg	Cu	Fe	P	Sr	Zn
mean	14.59	60.69	1.90	0.01	14.03	0.10	0.05
SD	1.69	5.22	0.79	0.01	7.31	0.01	0.03

Table 3. Concentrations of elements in blood cells measured by ICP spectrometry (mM). n=6.

	Na	K	Ca	Mg	Cu	Fe	P	Sr	Zn
mean	253.05	8.78	7.10	28.77	0.61	0.01	4.13	0.05	0.02
SD	46.84	2.09	1.31	4.04	0.12	0.01	1.01	0.01	0.01

Table 4. Ionic composition of perfusates (mM).

	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}	HEPES	mOsm	pH
SW	457.9	9.8	10.1	52.5	534.2	27.7	—	1093.5	7.9
saline A	479.4	15.7	14.6	60.7	645.7	0	5	1221.1	7.9
saline B	479.4	15.7	14.6	60.7	627.6	9.1	5	1212.1	7.9
saline C	479.4	15.7	14.6	60.7	556.2	44.8	5	1176.3	7.9
Pantin	526.0	11.0	18.0	23.5	620.0	0	5*	1203.5	7.9

*Tris-HCl was used in Yazawa and Kuwasawa (1984).

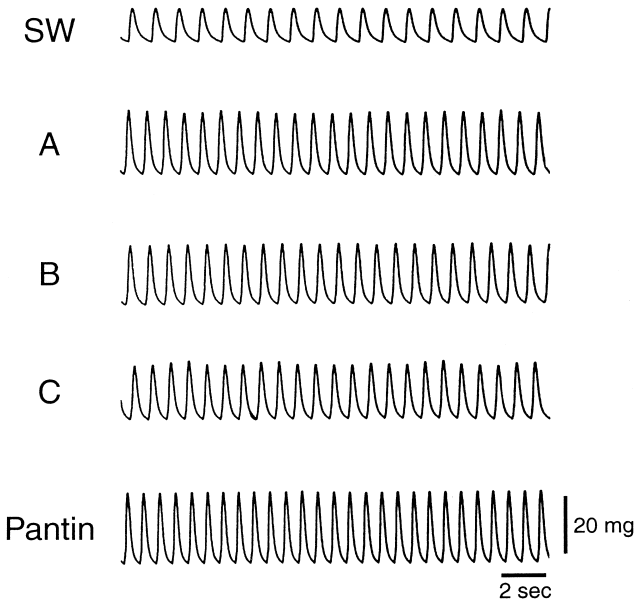

Fig. 1. Effects of ionic compositions of perfusates on the intact heart. The mechanograms of heartbeat in five kinds of perfusates were recorded from the same preparation. Top and bottom of each trace are systole and diastole of heartbeat respectively. SW; sea water, A; saline A, B; saline B, C; saline C, Pantin; Pantin's saline.

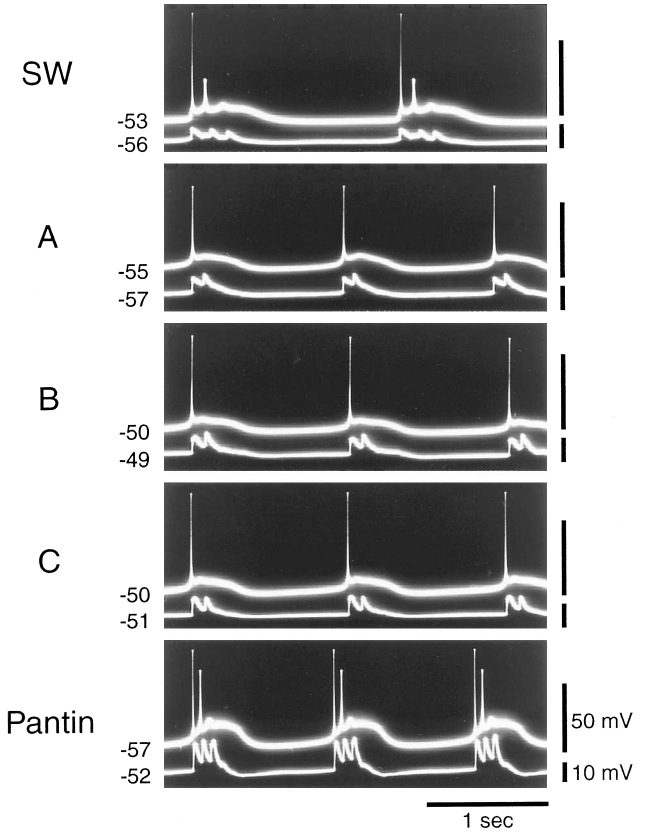

Fig. 2. Effects of ionic compositions of five kinds of perfusates on the membrane potential of cardiac ganglionic neuron (upper trace) and myocardial cell (lower trace) simultaneously recorded. Numerals at the beginning of traces are the most hyperpolarized membrane potential for cardiac ganglionic neuron and the resting membrane potential for myocardial cell (mV).

tions of SW (Cavanaugh, 1964) and Pantin's saline are simultaneously shown for comparison. NaOH was used for pH adjustment in A, B, C and Pantin's salines.

Fig. 1 shows the mechanograms of heartbeat of intact heart preparation immersed in five kinds of perfusates. The heart rate was highest in Pantin's saline and lowest in SW. Fig. 2 shows simultaneously recorded membrane potentials of the cardiac ganglionic neuron and the myocardial cell in the perfusates. The cardiac cycle in this dissected preparation was shortest in Pantin's saline and longest in SW. The histogram of the heart rate in intact heart preparations (n=8–10) is shown in Fig. 3A. The heart rate was significantly lower in SW than in saline A, C, and Pantin's saline. In Pantin's saline, the heart rate was significantly higher than in saline B. There is no significant difference among the heart rate in three test solutions (saline A, B and C). The heart rate in SW and that in Pantin's saline are significantly different from that in the test solution group.

Although the differences in the systolic force were seen in the preparation of Fig. 1, statistical analysis showed no significant difference among five perfusates (Fig. 3B). The level of membrane potential of cardiac ganglionic neurons affects

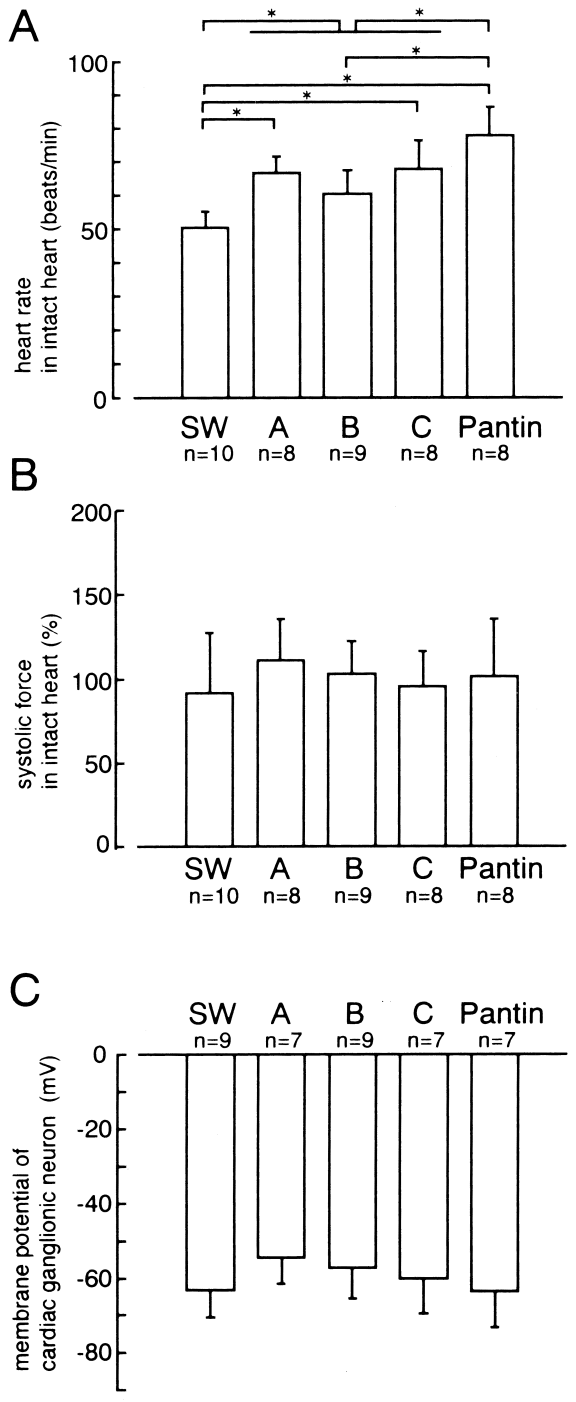

Fig. 3. Histograms of the heart rate (**A**), the systolic force (**B**) and the most hyperpolarized membrane potential of cardiac ganglionic neurons (**C**) in five perfusates. The data in **A** and **B** were obtained from intact hearts, and the data in **C** were from dissected hearts for intracellular recordings. In **B**, the systolic force expressed by the ratio to mean values of each preparation (100%). Bars, standard deviations. *, P<0.05

the bursting frequency of the cardiac ganglion (Matsui *et al.*, 1977). In this experiment, no significant difference in the effects on the most hyperpolarized membrane potential of cardiac ganglionic neurons was observed (Fig. 3C). The resting membrane potential of myocardial cells and the number of spikes in one cycle of ganglionic burst activity showed no significant difference among five perfusates. In saline B as well as in SW, heartbeat of intact heart continued regularly more than 20 hr at 20°C.

DISCUSSION

Unlike the heart of Lepidopterous insects (Ai *et al.*, 1995), the heart of *Bathynomus* is durable in maintaining long term beating in a wide range of ionic concentrations. SW or saline for decapods has been used in previous physiological studies. In this study, we made a physiological saline suitable for marine isopod *Bathynomus doederleini* for the first time in order to perform the pharmacological experiments with perfusates having various ionic compositions.

During the process of sampling hemolymph specimens, artificial contamination of elements was carefully avoided as mentioned in methods. The mixture of intracellular ionic components of blood cells in the sample certainly occurred since hemolysis was observed in the precipitation of centrifuge. However, the volume rate of blood cells was about 0.21%, so that the influence of the contamination of intracellular blood cells' components could be negligible.

ICP spectrometry employed in this study used Be as an internal standard substance. The simultaneous measurements of the concentration of 0.2 ppm Be in 0.05 N HCl (ISS) and of the concentration of elements in sample diluted with ISS were usually performed. The measurement of a Be concentration in ISS is for correction of measured values since the spectrometry system always has some instability. The simultaneous measurement of a Be concentration in sample solutions is for correction of measured values which may be affected by proteins in sample. This correction method was developed for measurement of the concentration of minor elements in human serum in which Be was not detected. Since a quantity of Be was revealed to be far too small in the *Bathynomus* serum, the influence of this correction method on the measured values was thought to be negligible.

ICP spectrometry measures concentrations of bound and ionic elements. Therefore, the values measured by ICP spectrometry may be higher than the concentrations of the ionic elements. The ionic concentration of K^+ was measured as 15.72±2.69 mM (n=6) (Table 1) by ion selective electrode, but the element concentration of K was measured as 18.04±1.32 mM (n=5) by ICP spectrometry. The higher value by ICP spectrometry may be attributable to over estimation because of the bound potassium to the substrates such as cation-binding proteins in the serum.

In human serum, it is known that 47.5% and 80% are free Ca and Mg ions respectively. Numanoi (1937) reported that a quarter of total Ca in hemolymph was bound to proteins in the terrestrial isopod *Ligia*. However, there is no available data for the ratio of free Ca and Mg in *Bathynomus* serum. Concentrations of Ca^{2+} and Mg^{2+} shown in this study are possibly over estimated for those in normal hemolymph.

P and Cu showed relatively large amounts among the elements detected by ICP spectrometry except for Ca and Mg. Cu may be largely attributed to hemocyanin of a respiratory pigment. P may exist as phosphoric acids either HPO_4^{2-} or $H_2PO_4^-$.

The main buffers in hemolymph are certain weakly acidic groups of proteins. In decapod, hemocyanin and CO_2-carbonate systems are known to work as buffers (Truchot, 1983). In the test solutions, HEPES 5 mM is used for a buffer. HEPES is used most widely for cell culture, and is known to be less toxic than Tris (Gillespie and McKnight, 1976). In saline B as well as in SW, the heartbeat continued normally more than 20 hr at 20°C.

In intact whole animals equipped with implanted electrode for ECG, heart rate in resting phase was from 40 to 90 beats/min (Tanaka and Kuwasawa, 1991). The range of heart rate in five perfusates measured in this study was within this range. Significant differences in heart rate appeared among the effects of SW, test solutions and Pantin's saline (Fig. 3A). The heart rate of *Bathynomus* is determined by the pacemaker rhythm of the cardiac ganglion consisting of 12 similar cells in size which are located on the inner surface of the heart (Kihara and Kuwasawa, 1984). It seemed to correlate with concentrations of Na^+ or Ca^{2+} because heart rate was highest in Pantin's saline (77.6±8.9 beats/min) which contains the highest concentration of Na^+ and Ca^{2+} ions and was lowest in SW (50.2±4.8 beats/min) which contains the lowest concentration of Na^+ and Ca^{2+} (Fig. 3A). The effects of the ionic concentrations on the systolic force of heartbeat and membrane potential of cardiac ganglionic neurons and myocardial cells were not detectable.

Saline A contains surplus Cl^- (89.5 mM over in comparison with that in the serum) and no SO_4^{2-}. Saline B contains surplus Cl^- (71.4 mM over) and normal SO_4^{2-}. Saline C contains normal Cl^- and surplus SO_4^{2-} (35.7 mM over). However, there was no significant difference among the three test solutions (saline A, B and C) in heart rate, systolic force and membrane potential of cardiac ganglionic neurons and myocardial cells. Therefore, we may use any one of the three test solutions as physiological saline for *Bathynomus*. The saline B has a concentration of SO_4^{2-} which is same as the average value of the serum. We propose the saline B as a standard physiological saline for *Bathynomus*.

ACKNOWLEDGMENTS

This work was supported by the grants from the Ministry of Education, Science, Sports and Culture of Japan No. 08454271 (KK), and was partly supported by the Sasakawa Scientific Research Grant from the Japan Science Society (YF-T). We thank Mr. J. Suzuki for kindly collecting animals. We would thank also Prof. Dr. S. Takii, Tokyo Metropolitan University for his help in an analysis of hemolymph ionic compositions. We are very grateful to Prof. Dr. R. B. Hill, University of Rhode Island, for revising this manuscript and giving helpful comments. Contribution from the Shimoda Marine Research Center, University of Tsukuba, No. 645.

REFERENCES

Ai H, Kuwasawa K, Yazawa T, Kurokawa M, Shimoda M, Kiguchi K (1995) A physiological saline for Lepidopterous insects: Effects of ionic composition on heart beat and neuromuscular transmission. J Insect Physiol 41: 571–580

Bethe A (1929) Ionendruchlässigkeit der Körperoberfläche von wirbellosen Tieren des Meeres als Ursache der Giftigkeit von Seewasser abnormer Zusammensetzung. Pflügers Arch 221: 344–362

Bogucki M (1931) O regulowaniu cisnienia osmotycznego hemolimfy równonogów morskich (*Mesidotea entomon* [L.]). Acta Biol Exp 7: 61–78

Cavanaugh GM (1964) Formulae and Methods V., the Marine Biological Laboratory, Woods Hole

Delaleu JC, Holley A (1976) Contribution of an electrogenic pump to the resting membrane polarization in a crustacean heart. J Exp Biol 64: 59–74

Fujiwara-Tsukamoto Y, Kuwasawa K, Okada J (1992) Anatomy and physiology of neural regulation of haemolymph flow in the lateral arteries of the isopod crustacean, *Bathynomus doederleini*. In "Phylogenetic Models in Functional Coupling of the CNS and Cardiovascular System." Comp Physiol Vol 11, Ed by RB Hill, K Kuwasawa, Karger, Basel, pp 70–85

F-Tsukamoto Y, Kuwasawa K (1997) A neurotransmitter for valve excitatory nerves in the isopod crustacean, *Bathynomus doederleini*. Zool Sci 14 Suppl: 120

Gillespie JS, McKnight AT (1976) Adverse effects of Tris hydrochloride, a commonly used buffer in physiological media. J Physiol 259: 561–573

Holley A, Régondaud P (1963) Composition ionique et pression osmotique moyennes du plasma de l'Oniscoïde *Porcellio dilatatus* (Brandt). Mise au point d'un liquide physiologique adapté à la survie du coeur. C r Séanc Soc Biol 157: 1100–1104

Holley A, Delaleu JC (1972) Electrophysiology of the heart of an isopod crustacean: *Porcellio dilatatus*. I. General properties. J Exp Biol 57: 589–608

Kawaguti S (1933) On the effects of chlorides of sodium, magnesium and calcium on the respiratory quotient of *Asellus nipponensis*. J Fac Sci Imp Univ Tokyo, Ser.4, 3: 189–204

Kihara A, Kuwasawa K (1984) A neuroanatomical and electrophysiological analysis of nervous regulation in the heart of an isopod crustacean, *Bathynomus doederleini.* J Comp Physiol A 154: 883–894

Matsui K (1957) Spontaneous discharges of the isolated gabglionic trunk of the lobster heart (*Panulirus japonicus*). II. Effect of some ions. Sci Rep Tokyo Kyoiku Daigaku, Sect B, 8: 148–173

Matsui K, Kuwasawa K, Kuramoto T (1977) Periodic bursts in large cell preparations of the lobster cardiac ganglion (*Panulirus japonicus*). Comp Biochem Physiol 56A: 313–324

Midsukami M (1991) Physiological salines. The formulae for cell and tissue research. Maruzen Company Ltd., Kanazawa

Needham AE (1954) Physiology of the heart of *Asellus aquaticus* L. Nature 173: 272

Numanoi H (1937) Migration of calcium through blood in *Ligia exotica* during its moulting. Jap J Zool 7: 241–249

Okada J, Kuwasawa K (1995) Neural mechanisms governing distribution of cardiac output in an isopod crustacean, *Bathynomus doederleini*: reflexes controlling the cardioarterial valves. J Comp Physiol A 176: 479–489

Okada J, Kuwasawa K, Kihara K, F.Tsukamoto Y, Yazawa T (1997) Cholinergic inhibitory innervation of the cardioarterial valves in the isopod *Bathynomus doederleini*. Zool Sci 14: 571–579

Pantin CPA (1934) On the excitation of crustacean muscle. I. J Exp Biol 11: 11–27

Parry G (1953) Osmotic and ionic regulation in the isopod crustacean *Ligia oceanica*. J Exp Biol 30: 567–574

Takeuchi S, Kimoto I, Mano M, Arakawa Y, Sasaki T, Sudo S, Sudo Y (1995) Relation between latent ascorbic acid deficiency and renal osteodystrophy in hemodialyzed patients. Biomed Res Trace Elements 6: 109–116

Tanaka K, Kuwasawa K (1991) Central outputs for extrinsic neural control of the heart in an isopod crustacean, *Bathynomus doederleini*: neuroanatomy and electrophysiology. Comp Biochem Physiol 98C: 79–86

Tanaka K, Yazawa T, Kuwasawa K (1992) Cholinergic and GABAergic control of the heart in the isopod crustacean, *Bathynomus doederleini*. In "Phylogenetic Models in Functional Coupling of the CNS and Cardiovascular System" Comp Physiol Vol 11, Ed by RB Hill, K Kuwasawa, Karger, Basel, pp 132–140

Truchot JP (1983) Regulation of acid base balance. In "The Biology of Crustacea Vol 5 " Ed by LH Mantel, Academic Press, New York, pp 431–457

Yamagishi H, Ebara A (1985) Spontaneous activity and pacemaker property of neurons in the cardiac ganglion of an isopod, *Ligia exotica*. Comp Biochem Physiol 81A: 55–62

Yazawa T, Kuwasawa K (1984) The cardio-regulator nerves of the hermit crabs: anatomical and electrophysiological identification of their distribution inside the heart. J Comp Physiol A 154: 871–881

(Received August 10, 1999 / Accepted November 12, 1999)