The Osmotic Behavior of the Blastula of the Sea Urchin[1]

By

Jutaro Shinozaki

(Biological Section, Institute of Low Temperature Science, Hokkaido University)

(*With 2 Text-figures*)

Concerning the osmotic phenomena of sea urchin eggs, a number of investigations have been made by many authors, but comparatively little attention, as far as the writer is aware, has been given to the osmotic behavior of blastula.

According to the immersion and the injection experiments of Chambers and Pollack ('27), the blastula remains freely permeable to the salt constituents of sea water and to such substances as phenol red and other sulphophthaleins dissolved in sea water. Further, Moore and Burt ('39) demonstrated that sea urchin blastula, up to about tenth cleavage, are freely permeable to sucrose, and after the tenth segmentation the blastular wall becomes relatively impermeable to sucrose in both directions.

It is the object of this paper, in sea urchin blastula, to describe the behavior of the blastomeres in heterotonic sea water and the passage of water and solutes between the blastocoel and surrounding sea water.

Material and Methods

The material used in this study is the blastula of sea urchin, *Strongylocentrotus intermedius*. The lots of good fertilizable eggs were selected ; the eggs were artificially inseminated by ordinary method and then reared for 6 to 7 hours until the blastular stage (20°–25°C). About 10 hours after the insemination, the larva begins to hatch. For convenience of observation the unhatched larva was employed in this experiment. In one series of experiments, the outside and the inside diameters of the blastula which had been equilibrated with variously diluted sea

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water were measured both on the polar and the equatorial axes, using the ocular micrometer previously calibrated. The volume of the larva proper was calculated from the mean of the polar and the equatorial diameters. In the other series the time courses of volume change of the blastulae in 60% sea water and further of the blastulae in 100% sea water, which had been completely swollen in 60%, were estimated, by measuring, at intervals of one minute, the outside and the inside diameters along the axis which was chosen at random, for the blastula is practically regarded as a spherical shell, as shall be mentioned later. The drawing of the larva in various strengths of sea water was made by means of the camera lucida. Each experiment was executed at room temperature, varying within 1°C during one series of the experiments.

Results

The volume of blastula, when placed in hypotonic sea water, increases by swelling of each blastomere, enlarging its outside diameter and narrowing the blastocoel (Fig. 1 and Tab. 1). These facts show that the volume of the blastula varies inversely with lowering of the concentration of sea water.

The outside diameters at the polar and the equatorial axes are equal to each other, therefore the shape of the blastula, after swelling, can still be regarded as spherical and also the mean values of the inside diameters at these two axes are nearly equal to each other. However, the shape of the blastocoel is hardly considered as spherical from the facts that the standard deviation on the inside diameters is relatively great and that the shape of the blastocoel is irregular. The

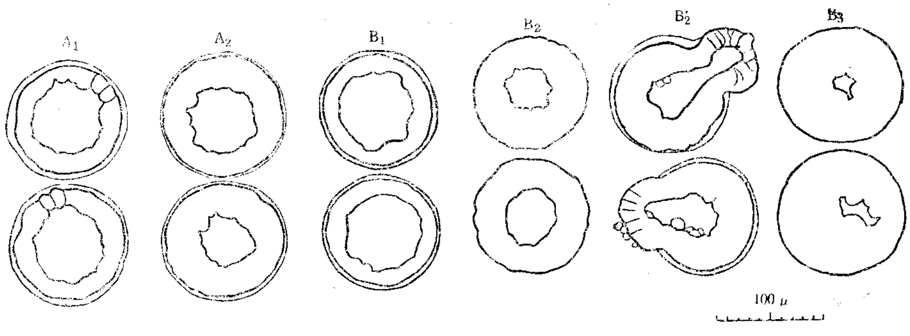

Fig. 1. The optical sections of the blastula larvae in different strengths of sea water. The drawings were made, using a camera lucida. In lot A, A_1 is the larva in 100% sea water and A_2 those in 60%. In lot B, B_1 and B_3 are the larvae in 100% sea water and in 40% respectively and B_2 and B_2' represent those in 60% sea water. These drawings show that the dimension of the larva becomes greater as the concentration of sea water decreases. As regards B'_2, see text (p.105).

reaction of blastular wall to heterotonic sea water is considerably rapid. The following fact shows this clearly that both the outside and the inside diameters immediately begin to change, when the blastula is bathed in heterotonic sea water (Fig. 2).

Table 1. The diameters (in μ) of the blastulae in various strengths of sea water. a and b are outside and inside diameters on polar axis and c and d those on equatorial axis respectively. Temp. 20°-25°C.

Concentration of sea water		lot A	lot B	lot C
100%	a	109.92±1.87	102.72±2.30	101.44±1.86
	b	74.31±0.92	61.70±5.06	56.48±5.22
	c	110.24±0.88	102.56±2.56	100.54±1.54
	d	77.34±1.66	61.34±6.88	56.26±2.75
60%	a	119.38±2.15	112.16±1.66	110.50±1.57
	b	59.55±5.68	50.62±6.66	45.28±7.23
	c	120.84±2.58	111.52±1.82	109.76±1.63
	d	64.35±5.51	51.10±5.98	45.44±7.14
40%	a		118.14±1.28	
	b		22.40±7.81	
	c		117.60±1.38	
	d		20.93±6.56	
60%→100% (1)	a			100.38±2.27
	b			58.88±5.25
	c			99.62±1.89
	d			57.70±6.46
40%→100% (1)	a		101.28±2.50	
	b		63.04±5.41	
	c		102.72±1.18 *	
	d		65.15±4.32	
Mean of		11	20	20

(1) The dimensions of the blastulae returned from 60% or 40% sea water to 100% sea water.

* The value given in the case of 40%→100% are mean of 10 measurements.

As is clear from Table 1, the blastula, when returned from 40% or 60% sea water to natural sea water, shrinks again to the original volume, indicating that the volume change is entirely reversible.

The applicability of Boyle-van't Hoff's law to the blastular wall, i.e. a layer of blastomeres, was examined on the volume calculated from the mean of the polar and the equatorial diameters, assuming the blastocoel to be spherical (Table 2). Taking into consideration the facts that the diameters have not been accurately observed because of the irregularity of the shape of the blastocoel and that the volume of intercellular space which might be changed by swelling has been neglected, it may be safely said that this law is applicable to the blastular wall as in the case of an egg cell, showing that each blastomere behaves as the osmometer. The value of b regarded as the volume of the osmotically inactive fraction in the blastomeres attains to about 30% or more, with exception in the lot A ; comparing with that of unfertilized egg this value is very high. In this case, however, it must be considered that the b includes, besides the volume of the osmotically inactive

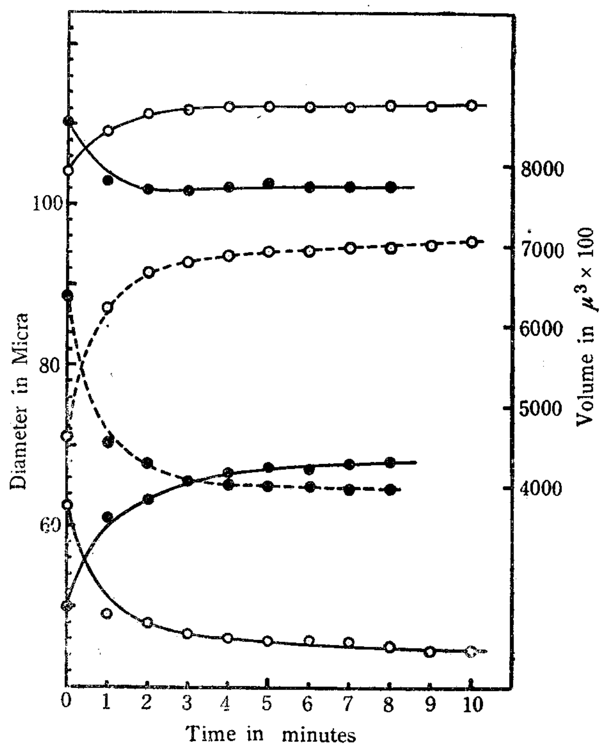

Fig. 2. Time courses of the diameter changes of the blastula larva placed in 60% sea water and of the larva returned from 60% to 100% (solid line), and time courses of the volume changes calculated from the diameter changes (broken line). Open circles represent the observed diameters and the calculated volumes in swelling, and solid circles in shrinking. Upper two solid curves express the change of the outside diameter and lower two the change of the inside. All the points indicate the mean of 7 measurements. Temp. about 25.5°C.

fraction in the blastomeres, also the volume of the intercellular space.

The diameters of the fertilization membrane both in 100% and in 60% sea water are almost equal to each other. However, when immersed in 40% sea water, the diameter becomes somewhat gerater, in this case expansion of the blastula may be prevented by the elasticity of the fertilization membrane (Fig. 1).

Table 2. Application of Boyle-van't Hoff's law to the blastula equilibrated with the different concentrations of sea water. The volume was calculated from the diameters in Table 1. The actual volume in cubic micra is obtained by multiplying the observed volume in the table by 100. The relative pressure is expressed as a fraction of the pressure of natural sea water which is taken as unity.

Lot	Relative Pressure	Volume observed	PV	P(V-b)	Volume calculated from	
					$V_e = \frac{P_o V_o}{P_e}$	$V_e = \frac{P_o(V_o - b)}{P_e} + b$
A	1.0	4,702	4,702	4,602*	4,702	4,702
	0.6	7,770	4,662	4,602*	7,837	7,770
B	1.0	4,443	4,443	2,828	4,443	4,443
	0.6	6,585	3,951	2,979	7,405	6,325
	0.4	8,582	3,433	2,785	12,108	8,678
C	1.0	4,456	4,456	3,098*	4,456	4,456
	0.6	6,522	3,913	3,098*	7,427	6,521

A .. b=100, B .. b=1,620, C .. b=1,358.

* Mathematically, it is a natural consequence that P(V-b) is constant with b estimated from two sets of measured values.

In some cases some blastulae in 60% sea water took form like the exogastrula (Fig. 1, B'_2). This phenomenon may be explained as follows: each blastomere swells up and in addition increase of the volume of the blastocoel, caused by relatively high impermeability of the intercellular substance to salts, takes place, so that the expansion force exceeds the limit of the elasticity of the fertilization membrane, resulting in its rupture. However, this case is exceptional in this experiment. During the course of experiment, the change of the dimension of the blastula owing to its development, if it occurs, is negligible, since the larva returned from 40%, or 60% sea water to 100% completely regains the original volume (Tab. 1).

The fact that active ciliary movement was observed later on the blastula, the volume of which returns again to the original level in natural sea water, proves that the diluted sea water affects no impairment of the blastula.

Discussion

From the above descriptions it may be presumed that each blastomere is encased with a semipermeable membrane and behaves as the osmometer in the same manner as the egg cell. And it may be also deducible that the intercellular substance is freely permeable to water and to salts in sea water, because if not so, when the blastula is placed in hypotonic sea water, by osmotic swelling of each blastomere its protoplasm should become hypotonic to the blastocoel fluid and in consequence the volume of blastocoel should be enlarged by absorbing water both from the blastomeres and from the surrounding medium through the intercellular substance, but in fact immediately after immersing in hypotonic sea water the blastular wall begins to swell both outwards and inwards.

The writer is grateful to Prof. K. Aoki at whose suggestion this work was begun.

Summary

1. The permeability of the blastula of sea urchin, *Strongylocentrotus intermedius*, to water and to salts was examined from the volume change of the blastula in heterotonic sea water.

2. As soon as the blastula is placed in hypotonic sea water, its volume clearly increases by swelling of blastomeres, enlarging the outside diameter and narrowing the blastocoel, and when this swollen blastula is returned into natural sea water it regains the original volume.

3. The swelling of the blastula increases as the concentration of sea water becomes more dilute.

4. Considering the inaccuracy of the observed diameters and the neglecting of the volume of the intercellular space, Boyle-van't Hoff's law might be as applicable to the blastular wall as to the egg cell.

5. The intercellular substance of the blastula is freely permeable to water and to the salts dissolved in sea water.

Literature Cited

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