Effects of Certain Physiological Solutions on the Embryo of a Terrestrial Isopod, *Porcellio scaber*[1)]

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(*With 1 Text-figure*)

With regard to the adaptation of the terrestrial isopods from the aquatic life, much importance must be attached to the medium of their larval stage. There is, however, only Ban's work (1950) on the constitution of culture media for the eggs of *Armadillidium*. It has been shown in the author's previous study on *Porcellio* (1956) that the mucous substance surrounding the embryos is secreted from the thoracic processes in the brood chamber. Before investigating the functional significance of the mucus, the present study was undertaken to ascertain the optimum concentration of culture medium and its pH during the course of embryonic development.

It is the writer's great pleasure to dedicate this paper to Professor Tohru Uchida, under whose direction the study has been carried on, in the honor of his sixtieth birthday.

Material and method

The eggs of a terrestrial isopod, *Porcellio scaber*, were removed from the brood chamber by a smooth glass needle, and were washed in distilled water for a few seconds before being immersed into test solutions. At the beginning of the experiment, in the most of eggs there occurred the formation of the blastodisc or the appearance of segments (early or late naupliar stage). Two hours after the treatment, abnormal embryos (for example one with broken chorion) were eliminated and the remaining ones were kept at room temperature (18-20°C) ; the solutions were renewed each day.

Experimental results

At first, the embryos were kept in variously concentrated NaCl or Ringer's solution named A in Table 2. The pH values of these test solutions were adjusted to 7.2 by $NaHCO_3$. Three tests were repeated with each solution and results obtained are summarized in Table 1. To compare the results, χ-square tests were applied for the mortalities at the 24th hour in NaCl solution or at 72nd hour in Ringer's solution; this was done because in 1/2 M solution the longest duration

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of life was indicated and at the periods mentioned above the mortality rose to nearly fifty per cent. There were significant differences between 1 M and 2/3 M, 1/2 M and 1/3 M but difference of mortalities was not indicated between 2/3 M and 1/2 M both in NaCl and Ringer's solutions ($\chi^2=26.265$ and 16.072, 31.803 and 5.341, 0.623 and 1.717 respectively). Therefore, it is assumed that the optimum concentration for development lies between 2/3 M and 1/2 M. In the concentration higher or lower than this optimal zone, the mortalities increased

Table 1. Percentages of mortalities in variously concentrated NaCl or Ringer's solutions

Osmotic conc. in Mols	Test solution	Life Duration (hrs.)	% of mortality at 24 hrs. in NaCl sol.	% of mortality at 72 hrs. in Ringer's sol.	Number of eggs tested
1	NaCl Ringer	18 24	100	100	42 46
2/3	NaCl Ringer	62 244	52.4	48.9	42 47
1/2	NaCl Ringer	70 240	43.9	44.7	41 47
1/3	NaCl Ringer	32 140	85.7	68.8	42 45
1/4	NaCl Ringer	24 96	100	83.0	42 47
1/8	NaCl Ringer	20 96	100	86.6	42 45

Table 2. Percentages of mortalities in Ringer's solutions with different salts contents

Test solution	Salts cont. in volume basis*			Life Duration (hrs.)	% of mortality at 72 hrs. after treatment	Number of eggs tested
	1/2 M NaCl	1/2 M KCl	2/3 M $CaCl_2$			
A	100	2	7.5	240	44.7	47
B	100	2	5	246	45.7	35
C	100	2	2	182	56.2	32
D	100	8	8	128	72.7	33
E	100	8	2	154	81.8	33

* Salts were dissolved in distilled water with 7.2 pH-value adjusted by $NaHCO_3$.

rapidly, though the causes of death were different as follows. In 1 M solution, the eggs were normal both in size and shape, but the blastodisc more or less distinctly shrank and became irregularly coagulated at the one pole of the egg. The shrunken blastodisc was considerably transparent, the small oil particles were clotted in the yolk, and finally the development was arrested. In the low concentrations, on the other hand, the chorion broke, then the embryo irregularly swelled up and became completely translucent.

A second series of experiments was carried out in various constitutions of Ringer's solution in the same concentration, 1/2 M; results observed in two repetitions are given in Table 2. There is no statistically significant difference in adjacent test solutions, but the values of χ-square between A and D, B and D indicate a high significance (6.322, 5. 133). From these examinations it is difficult to demonstrate that the ionic constitution of A test solution is much more suitable for the development than the others, although the least mortality was observed in A test solution. There seems, however, to be indicated a general tendency that the larger the ratio of $CaCl_2$ by KCl becomes, the more favourable the solution is for development. For this reason A test solution was chosen for next experiments in which the optimum pH was sought.

The effects of pH in 1/2 M A test solution were observed at 4.0, 6.0, 7.2, 7.8, and 8.8 adjusted by $NaHCO_3$ or HCl. As is shown in Fig. 1, the axis of ordinate is the percentage of mortality and that of abscissa indicates the duration of life in hours graduated in logarithmic scale. The transitions of mortalities show

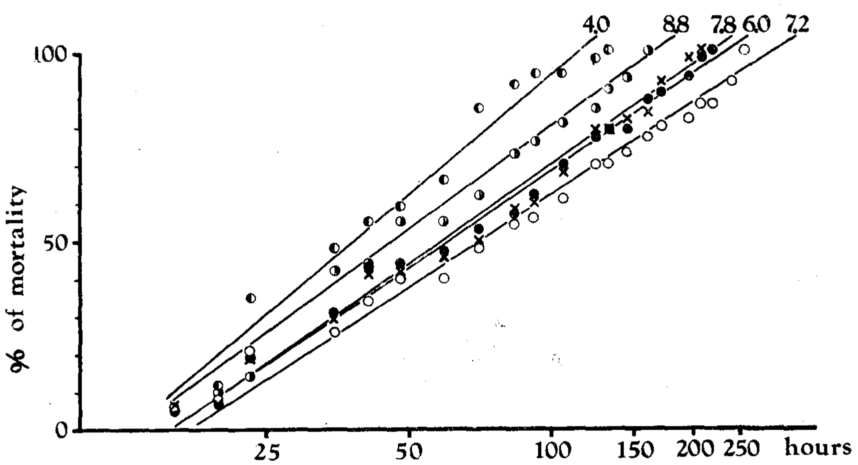

Fig. 1. Effect of pH on the eggs of *Porcellio*. The axis of ordinate is % of mortality and that of abscissa is the duration of life in hours graduated in logarithmic scale.

linearly in general, but these lines are still beyond explanation. There were significant differences among the mortalities according to the statistical tests mentioned above, except in the case between 6.0 and 7.8; thus the optimum pH for development is near 7.2. In the alkalized solution, especially at pH 8.8, the breaks in chorion were often observed in a similar way as in low concentrations, but the duration of life out of the chorion was considerably longer; the mean time was 43 hours and the maximum 72 hours, while in low concentration 6 and 10 hours, respectively. Since the chorion was not shrunken in high concentration, the break of chorion in low concentration appears to be caused by an osmotic unbalance due to the pressure of a colloidal substance in the perivitelline space. In alkalized solutions, however, the chorion became somewhat weakened and a break in it was probably caused by a mechanical shock.

Discussion

Ban reported in the eggs of *Armadillidium* that the range of the osmotic pressure suitable for the development was relatively wide, that is, from 0.36 M to 0.72 M, and the optimum concentration was 0.48 M. Results obtained in the present study essentially accord with them, although the optimum concentration for the embryos of *Porcellio* is anticipated to be somewhat higher, namely between 0.50 M and 0.67 M. It is noteworthy that the osmotic pressure of sea water is about 0.62 M, hence approximately equal to that of the medium mentioned. According to the investigations on adult isopods, it was recognized that certain physiological differences exist between *Porcellio* and *Armadillidium*, such as in the internal temperature (Edney, 1953) and the osmotic pressure of blood (Parry, 1953). According to Parry's determination of freezing-point depression of blood, the mean value of Δ 1.30°C in *Porcellio* is greater than that of Δ 1.18°C in *Armadillidium*. The osmotic pressure of blood in *Porcellio* and *Armadillidium* is, after the conversion of the present author, isotonic with 0.388 M and 0.353 M NaCl solution respectively. Thus there is a relatively large difference of about 0.13 M between the osmotic concentration of the adult blood and the optimum concentration for the development of embryo. Contrary to this consideration, a marked increase in osmotic concentration is stated by Robertson (1937) to occur in *Carcinus* before the molt.

Parry also stated in *Ligia* that the blood of individuals kept for 2,3 and 4 days after the molt still showed no subsequent drop in the osmotic pressure, namely Δ 2.15°C of blood in 'intermolt' stage (=0.650 M NaCl, by the author's conversion) rose to Δ 2.55°C in 'post-molt' stage (=0.761 M NaCl). Hence the fact that the oviposition in *Porcellio* occurs just after the molting seems probably to be concerned with the findings that the embryos require a relatively high osmotic concentration of the physiological solution in the course of development.

Summary

The embroys of *Porcellio* outside the brood chamber developed well in physiological solution prepared from 0.50 M to 0.67 M of osmotic concentration, and the ionic composition is as follows; NaCl 100 parts +KCl 2 parts + $CaCl_2$ 5 parts in the same Mols. The optimum pH for the development is estimated near 7.2, in the case of adjustment by $NaHCO_3$.

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