

SOME PROBLEMS IN THE EMBRYOGENESIS OF *HABROTROCHA ROSA* DONNER 1949

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Abstract

Several parameters connected with the biology of *H. rosa* were investigated under laboratory conditions: average life span (20 days) divided into three characteristic stages, mean number of eggs laid (30 eggs) and average time of egg development (31.5 hours). Ontogenesis was studied (until the stage of early organogenesis) and a spiral type of cleavage and epibolic gastrulation were observed. The paper also presents data on the origin of the digestive system and sex cells.

Introduction

Problems connected with early ontogenesis – which is the subject of part of this study – have been studied in only few rotifer species mostly of the Monogonont group (Tannreuther, 1920; Beauchamp, 1965). In these forms a spiral type of cleavage, and gastrulation of typically epibolic type (*Ploesoma* sp.) or resembling this type (*Asplanchna* sp.) with some reservations (Beauchamp, 1965), were observed. However, data on the development of the Bdelloidea are lacking.

Material and methods

A culture of *H. rosa* was maintained in laboratory conditions in 500 ml flasks in a synthetic medium composed of: NH_4Cl -60 mg, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -2 mg, KH_2PO_4 anh. -20 mg, K_2HPO_4 anh. -50 mg, NaCl -1000 mg, H_2O dist. -1000 ml, pH 7.0, supplemented with crystalline penicillin (1000 u/ml). The rotifers were fed with bacteria (*Micrococcus* sp.). Every three weeks the culture was renewed by introducing 30 ml rotifer inoculum into 300 ml fresh medium. Studies on single specimens were conducted at

room temperature in July in small watch-glasses into which single eggs were transferred. Observations on single rotifers were made continuously and eggs laid were removed with a micropipette. To avoid aging of the medium, part of the fluid was removed daily and replaced with a suspension of bacteria in fresh medium. In studies on time of egg development and the early stages of cleavage the specimens were maintained in a hanging drop. The development stages were examined with the use of the following techniques: 1. direct observations of unstained material (sometimes in phase contrast) or similar observations of thermally fixed (+ 60°C) material; 2. observations on material fixed with alcohol and made translucent with glycerol; 3. preparations (lightly squashed) fixed in Bouin's fluid and stained with iron-hematoxylin or stained without fixing by the RVD method (R. V. Dipfel, 1955; Plasota & Plasota, this volume).

Results and discussion

A. Observations on the biology of reproduction of *H. rosa*. By examining several specimens in microcultures (Table 1) we found that the average life span under laboratory conditions was approximately 20 days. However, some of the rotifers died earlier, probably due to disorders in the egg laying process since the eggs were not laid one after the other but accumulated in the body of the specimen (2-3 eggs). Such specimens are rather frequent in cultures and the development of the eggs within the dead body is quite normal.

The life of an individual consists of three stages (Table 1): a period before maturation (2-3 days), egg laying period (6-9 days) and a period of full biological activity unaccompanied by egg laying (10-14 days). The number of

Table 1. Data on life span and reproduction of *H. rosa*.

specimen	number of days of life			life span	number of eggs laid
	before egg laying stage	in egg laying stage	after egg laying stage		
1	3	6	10	19	24
2	2	6	12	20	22
3	3	9	10	22	35
4	2	5	—	7°	20
5	2	9	14	25	33
6	2	8	2?	12?	34

° Dead rotifer, filled with three unlaidd eggs.

eggs laid during the life of a single specimen averages 30 and may reach 6 eggs per day. This observation is compatible with cytological observations which have shown that the highest number of oocytes in the ovaries of rotifers entering the stage of sexual activity is also about 30. Eggs are very large compared to the dimensions of a rotifer—approximately $\frac{1}{4}$ of its body length. At the moment of laying, their shell is still soft. When the egg passes through the cloaca it becomes constricted (Fig. 1). After several minutes it hardens and acquires its characteristic surface structure.

Under laboratory conditions the average time of egg development from laying till hatching is 31.5 hours and is fairly constant (± 1 h). The process of hatching takes only 2-2.5 minutes. We also found that some of the eggs—particularly those laid by a rotifer shortly after its maturation—may become arrested at an early stage of development and die. Moreover, completely formed embryos

may also be unable to hatch if they overstay (by several hours) in the egg shell.

B. Selected problems in the ontogenesis of *H. rosa*. Our observations extended until the stage of early organogenesis. The course of early ontogenesis in the examined representative of the Digonont—that is the first two divisions resulting in the four blastomere stage, indicates the occurrence of a cleavage pattern of the spiral type (Fig. 1).

Two further divisions lead after about two hours to the 16 blastomere stage in which after the separation of the micromeres four macromeres can be distinguished. In the

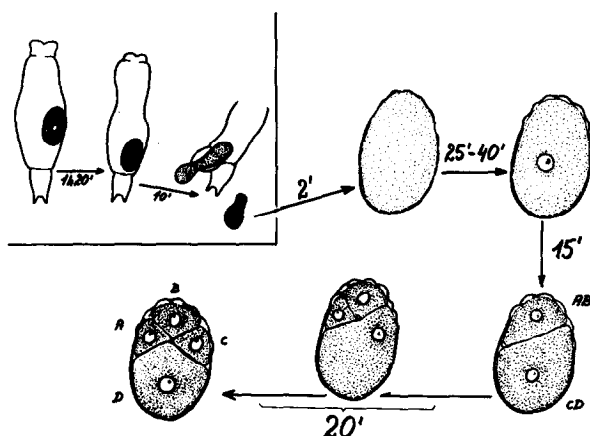


Fig. 1. Early ontogenesis in *H. rosa* from egg-laying stage to 4 blastomere stage. The times of the individual stages are given.

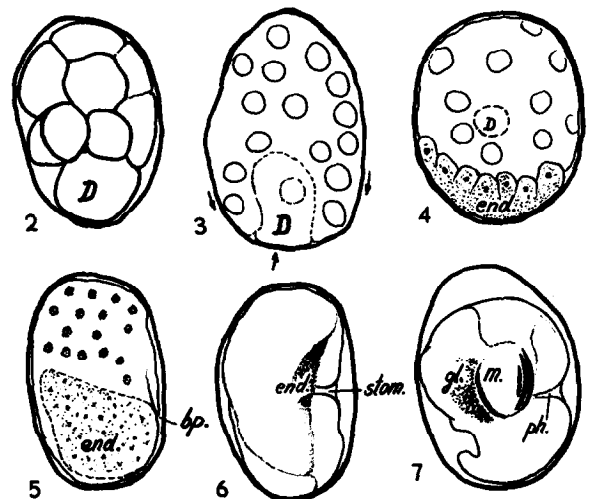


Fig. 2. Further two divisions leading to 16 blastomere stage; visible macromere D.

Fig. 3. Beginning of genital line; visible macromere D.

Fig. 4. Differentiation of endoderm (end.).

Fig. 5. Gastrulation (bp. - blastopore).

Fig. 6. Cavitation of stomodeum (stom.).

Fig. 7. Development of digestive system (ph. - pharynx, gl. - stomach glands, m. - mastax).

course of these divisions the largest macromere D becomes evident (Fig. 2). It moves inwards (Fig. 3) and gives rise to the genital line (vitellocytes and oocytes). The cells formed from divisions, of the other macromeres (Fig. 4) – which seem to be the presumptive endoderm proper – are subject to epiboly and move under and are covered by the micromeres; a remnant of this process is a residual blastopore (Fig. 5). Soon afterwards, at the cost of the ectodermal micromere material, a cavity of the stomodeum is formed (Fig. 6) from which the pharynx and mastax originate. The endodermal cells form the inner gut and its glands (Fig. 7).

Summary

The average life span of *H. rosa* under laboratory conditions is approximately 20 days and divides into three stages. In the period of full biological activity (6-9 days) the specimen lays an average of 30 eggs. The development of the egg from the moment of laying until hatching of a young rotifer lasts 31.5 ± 1 hour. The cleavage is of the spiral type. The macromeres give rise to the sex cells and endoderm. Gastrulation is of the epibolic type. The anterior part of the alimentary tract (pharynx and mastax) forms from the ectodermal micromere material and the inner gut and the stomach glands from the endoderm.

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