Cell Division During the Development of *Artemia salina*

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**Summary.** Cell division during embryonic development of the brine shrimp, *Artemia salina* has been studied by counting nuclei and mitotic figures. No cell division was observed during development of the encysted gastrula until about an hour before emergence of the embryo (a pre-nauplius) from the cyst, and even then only a few mitotic figures were observed. Following emergence, and during further development up to the stage II nauplius larva an increase of about 25% in the number of cells occurs. However, when the newly hatched larva is exposed to FUdR (10 μg/ml) cell division is largely inhibited, but observable development nevertheless proceeds normally. Evidently all processes involved with the development of the gastrula into a stage II nauplius larva can occur with far fewer cells than normally are present.

**Key words:** Cell division — Development — Cryptobiosis — Nauplius larva.

**Introduction**

Dormant cysts of the brine shrimp, *Artemia salina*, have attracted considerable attention in recent years as an interesting object for the study of various aspects of development and physiology (Clegg, 1974; Hentschel and Tata, 1976). The large amount of literature on *Artemia* has recently been compiled in bibliographic form by Sorgeloos (1976). Although the developmental stage at which dormancy occurs has previously been described as a blastula (Nakanishi et al., 1962) the definitive work of Benesch (1969) clearly shows that gastrulation has occurred prior to dormancy. The dormant embryo is released from the mother into the environment (brine) where desiccation occurs. Following rehydration the cysts eventually break open in an event called “emergence” that usually occurs after 12 to 24 hours of incubation, depending on experimental conditions and the geographic origin of the cysts. The embryo at emergence is a partially formed larva and will be referred to as a prenauplius. Upon its emergence the prenau-
plius, still enclosed by a membranous envelope, is just visible through a crack in the shell and this form is called the E-1 stage (Nakanishi et al., 1962). From 1 to 2 h later the prenauplius pushes out of the shell completely, but is still enclosed by its "hatching" membrane which in turn is tenuously attached to the empty shell (stage E-2, Nakanishi et al., 1962). After an additional 4 h the prenauplius begins to twitch intermittently, and within another hour it breaks free of its membrane in a process called "hatching". The developmental stage is now referred to as a stage I nauplius larva. The next moult produces a stage II nauplius, requiring about 18 h of development, and further moult takes place at regular intervals (Olson, 1970).

It has been shown by Nakanishi et al. (1962) that development of the encysted gastrula into the prenauplius (stage E-1) occurs in the absence of cell division, and the extensive studies of Benesch (1969) indicate that considerable cellular differentiation and morphogenesis occur during this period. Finamore and Clegg (1969) suggested on the basis of indirect evidence that *Artemia* nauplii appeared to continue development and undergo moult even when DNA synthesis was 90% inhibited. These results therefore suggested that morphogenesis and cell differentiation might occur in the absence of cell division and DNA synthesis, from the gastrula stage up to the stage-I nauplius. In this paper we will reinvestigate these contentions and determine the extent to which development can proceed in the absence of cell division.

**Materials and Methods**

*Materials.* Brine shrimp cysts from salterns near San Francisco, California, were obtained from Brine Shrimp Sales Co., Hayward, California, and cysts from The Great Salt Lake were from Long Fish Food Products, Harrison, N.J. "Antiformin" (7.8 g NaOH, 3.2 g Na₂CO₃, 100 m 5% NaHClO) was used to partially dechorionate the cysts (Nakanishi et al., 1962). FUdR (5-fluorodeoxyuridine) was a gift from Hoffmann-La Roche Inc., lot 002106, which is gratefully acknowledged. [Methyl-³H] thymidine was purchased from New England Nuclear (16.1 C/µ mol).

*Treatment of Cysts.* Cysts were washed and rendered aseptic by immersion in a 7% (v/v) solution of antiformin in a 0.25 M NaCl for 15 min (Finamore and Clegg, 1969).

*Incubation.* Washed, aseptic cysts were left at 0-4° C in 0.25 M NaCl (an hour to overnight) until time for incubation. Under these conditions no metabolism is detectable (Muramatsu, 1960) and, consequently, no development. Incubation was carried out in sea water in 500 ml Erlenmeyer flasks at 30±1° C. The exact "age" of the nauplii used for incubation will be defined in the appropriate experiments.

In experiments employing FUdR the cysts were washed as described above and then placed in either the control medium (20 µg/ml uridine in sea water) or the experimental medium (20 µg/ml uridine and 10 µg/ml FUdR in seawater) for incubation at 30° C. Variations in this procedure will be specified with the results. Although the shell is impermeable to these compounds, the emerged embryos and nauplii are permeable. Thus, the prenauplius is exposed to the drug immediately upon emergence. Uridine is used to block the hydrolysis of FUdR to 5-Fluorouracil (Volkin and Ruffilli, 1962) which would disrupt RNA synthesis (Heidelberger et al., 1957).

*Nuclear Counts and Mitotic Figures.* Naupliii, emerged embryos and cysts were either squashed directly in acetic orcein, or first fixed in ethanol-acetic acid (3:1) run through the Feulgen procedure, and then squashed. The slides were sealed with vaseline and left as wet mounts. Counting was