INCORPORATION OF TRITIATED THYMIDINE BY ARTEMIA SALINA

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INTRODUCTION

Tritiated thymidine (thymidine-H^3) is a specific precursor for DNA and is incorporated into cells which are actively synthesizing DNA. Autoradiography following exposure of various stages of *Artemia* to thymidine-H^3 makes it possible to find sites of DNA synthesis and cell division during development. Details of development and description of the stages of *Artemia* referred to in this paper are given by Weisz (1).

MATERIALS AND METHODS

Artemia (General Biological Supply House, Chicago) were hatched and grown in a salt solution described by Michael et al. (2). Thymidine-H^3 (Nuclear-Chicago) was diluted to 2.5 μc/ml. in this salt solution. Two or three animals at different stages of growth were placed in each ml. of the thymidine-H^3 solution for one day. After removal from the solution, the animals were placed for one day in a salt solution which contained no thymidine-H^3. This procedure eliminated any of the tracer which was not incorporated into newly synthesized DNA. Other animals were fixed immediately after removal from the thymidine-H^3 solution to determine the distribution of unincorporated tracer. The animals were fixed in Bouin's, imbedded in paraffin, and sectioned at 10μ. The sections were pre-stained with iron-hematoxylin and covered with Kodak AR.10 stripping film. The film was developed in Kodak D-19 developer for 5 minutes after a one week exposure in a refrigerated dessicator.

RESULTS

UPTAKE AND INCORPORATION OF THYMIDINE-H^3 BY METANAUPLII

Autoradiographic sections of animals which were fixed immediately after removal from the thymidine-H^3 solution show the distribution of unincorporated tracer in the tissues. There seems to be diffusion of labeled material from the gut through the gut wall and into the tissues.

Some of the animals which were fixed one day after removal from the thymidine-H^3 solution show incorporation of the tracer.

[1] This study was part of a Master of Arts thesis done while the author was a participant of the 1962-63 Academic Year Institute of the National Science Foundation at the University of South Dakota.

Figure 1 is an autoradiographic longitudinal section of a stage 10-11 metanauplius which shows the pattern of incorporation of thymidine-H^3.

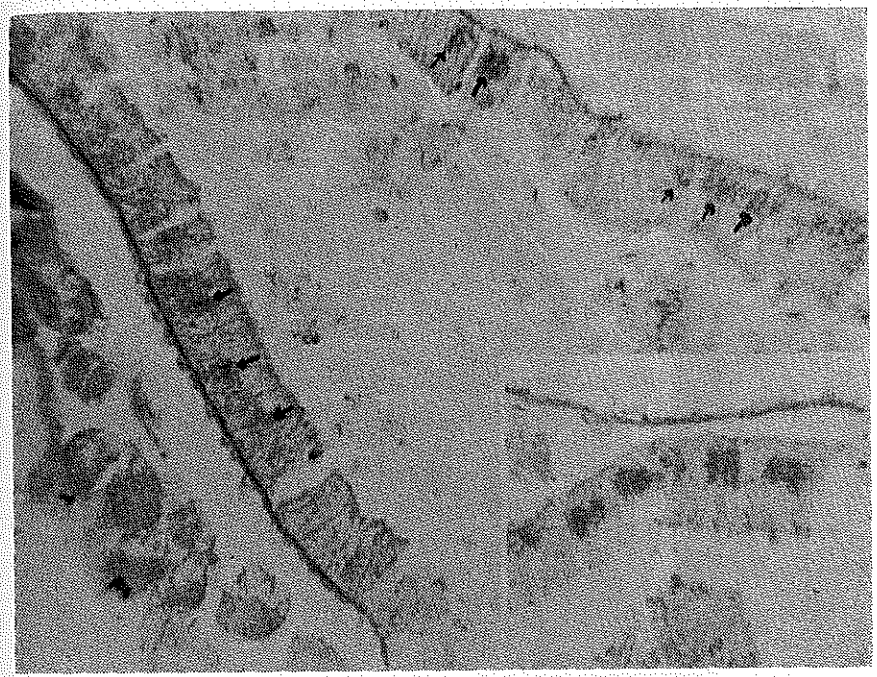

Figure 1. Autoradiographic longitudinal section of posterior gut region of *Artemia* metanauplius (stage 10-11) showing tritiated thymidine labeling (arrows) over nuclei of gut epithelial cells (X1600). Inset is slightly enlarged region which shows more clearly the apparent simultaneous division of labeled cells.

The most intense labeling is over the nuclei of epithelial cells which are located in the posterior region of the gut. Pairs of cells seem to have simultaneously divided in such a manner as to make the gut expand in length (Figure 1, inset). The developing appendage buds in the posterior region of the same animal contain labeled mesoderm cells (Figure 2). This labeling is less intense than that of the gut cells, and fewer cells are labeled.

INCORPORATION OF THYMIDINE-H^3 BY OTHER STAGES

1. *Nauplii.* There is no observed incorporation of thymidine-H^3 by nauplii which are exposed to the tracer during the first few days after excystment.

2. *Adult females.* Autoradiographs of adult *Artemia* females contain labeled cells in the oviducts (figure 3). These cells are in association with yolk masses.

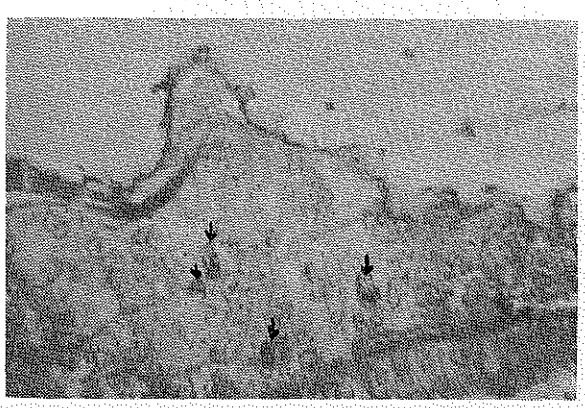

Figure 2. Autoradiographic longitudinal section of developing appendage in posterior region of the same animal as shown in figure 1. Tritiated thymidine labeling (arrows) is over the nuclei of a few mesoderm cells (X1200).

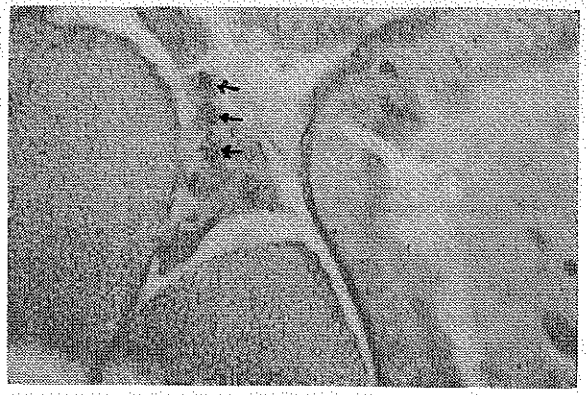

Figure 3. Autoradiographic cross-section through oviduct of adult *Artemia* female showing labeled cells (arrows) in association with yolk masses (X1200).

DISCUSSION

Artemia is able to incorporate thymidine-H^3 which is dissolved in water. This incorporation may be directly from the media, or as suggested by Clegg (3), the initial incorporation of the tracer might be by the gut flora, followed by their degeneration and release of labelled nucleotides which can be utilized by *Artemia.* It has previously been shown that radioactive materials may be taken up by *Artemia* through ingestion of food (4, 5) and other materials may be taken directly from the media (6).

Artemia nauplii apparently do not incorporate thymidine-H^3 during the first few days after excystment, even though vital stains can enter during the same period (unpublished observations). During this time, the nauplii develop for several days independent of an external source of food. They molt once to form the first metanauplius. The lack of incorporation indicates that there is sufficient DNA or its precursors stored within the encysted embryo to allow development through the first metanauplius stage. This is comparable to observations that cytoplasm of fertilized frog eggs contain DNA (7). It has also been suggested that chromosomal nucleic acids may be synthesized from simple materials contained in the cells of developing sea urchin embryos (8). Feulgen stained sections of encysted *Artemia* embryos show DNA to be concentrated in nuclei (unpublished observations) so it seems likely that any extranuclear material in *Artemia* embryos is in the form of DNA precursors rather than DNA.

The cells of encysted *Artemia* embryos do not incorporate thymidine-H^3 (9), probably because no cell division occurs during excystment (9, 10). However, it seems possible that embryos can be labeled while undergoing development within the adult female (figure 3).

The labeling pattern on the cells in the posterior region of the gut (figure 1) indicates that these cells are actively synthesizing DNA, and that cell division appears to be almost simultaneous in this region. This region is where new segments are forming (1). The less intense labeling on the adjoining appendage buds (Figure 2) may be interpreted as follows: The gut cells synthesize DNA and divide to cause some gut elongation. This event is followed very quickly by the synthesis of DNA and the division of mesoderm cells in the appendage buds. This interpretation agrees with that of Weisz (1) who suggested a similar sequence based on histological studies.

The almost simultaneous division of cells in the gut may be important during molting cycles, and probably contributes significantly to elongation of the animal. It should be noted that molting among crustaceans involves a series of physiological events (11). Actual DNA synthesis and cell division is only one of these events, and occurs only during a part of a molting cycle. Therefore, only those animals which are actually synthesizing DNA while exposed to thymidine-H^3 become labeled. It would be desirable to do similar labeling studies on larger crustaceans, with an attempt to corre-

late other physiological events with cell division during a molting cycle.

SUMMARY

Sites of cell division and DNA synthesis during the development of *Artemia salina,* the brine shrimp, were studied by autoradiography using tritiated thymidine as a tracer.

There is no apparent DNA synthesis by nauplius larvae during the first few days after excystment. This observation indicates that there is sufficient DNA or its precursors stored within the developing nauplius to allow development through the first metanauplius stage.

The most intense labeling on later metanauplius stages is on posterior gut cells. The gut seems to increase in length by the almost simultaneous division of gut epithelial cells near the region of segment formation. Appendages in the region of labeled gut cells contain fewer and less intensely labeled cells. The sequence of growth appears to be that the gut cells divide shortly before mitosis of the mesoderm cells in developing appendage buds.

The oviducts of adult females contain labeled cells in association with yolk masses.

REFERENCES CITED

1. Weisz, P. B., The Histological Pattern of Metameric Development in *Artemia salina,* J. Morph., *81,* 45 (1947).
2. Michael, A. S., Thompson, G. S., and Abranovitz, M., *Artemia salina* as a Test Organism for Bioassay, Science, *123,* 464 (1956).
3. Clegg, James S., personal communication (1964).
4. Boroughs, H., Townsley, S. J., and Ego, W., The Accumulation of Sr^{90}-Y^{90} From an Equilibrium Mixture of Sr^{90}-Y^{90} by *Artemia salina* L., Limnl. and Oceanogr., *3,* 413 (1958).
5. Grosch, D. S., and Plumb, M. E., A Sex Different in Radioactivity of *Artemia* cultured in Sea Water Containing Phosphorus 32, Nature, *183,* 122 (1959).
6. Provasoli, L., and Shiraiski, K., Anexic Cultivation of the Brine Shrimp, *Artemia salina,* Biol. Bull., *117,* 347 (1959).
7. Sze, L. C., Changes in the Amount of Desoxyribonucleic Acid in the Development of *Rana pipiens,* J. Exp. Zool., *122,* 577 (1953).
8. Abrams, R., Synthesis of Nucleic Acid Purines in the Sea Urchin Embryo, Exp. Cell Res., *2,* 235 (1951).
9. Emerson, D. N., The Metabolism of Hatching Embryos of the Brine Shrimp, *Artemia salina,* Proc. S. D. Acad. Sci. *42,* 131 (1963).

10. Nakanishi, Y. H., Iwasaki, T., Okigaki, T., and Kato, H., Cytological Studies of *Artemia salina.* I. Embryonic Development Without Cell Multiplication After the Blastula Stage in Encysted Dry Eggs, Annot. Zool. Japon., *35,* 223 (1962). (Paper not seen.)
11. Passano, L. M., Molting and Its Control, in, "The Physiology of Crustacea, vol. 1," 473 (Academic Press, New York, 1960).