Mix

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The Use of High Specific Activity Tritiated Thymidine and Autoradiography for Studying Molluscan Cells

Since the first successful attempts to incorporate ^{3}H into the thymidine molecule in 1957, tritiated thymidine (^{3}H-TdR) and autoradiography have been used to study normal cell renewal systems and cell kinetics in homoiothermic organisms. Unfortunately, there have been few successful attempts in using this valuable technique in studies of molluscan cells. In preliminary studies, D. P. Cheney (Ph D thesis, Univ. of Washington, 1969) obtained virtually no cell labeling in *Crassostrea gigas* or *Mytilus edulis* although he subsequently utilized autoradiography to study the morphology, morphogenesis, and reactive responses of Manila clam, *Tapes semidecussata*, blood cells. The reason for this failure in *C. gigas* and *M. edulis* is not entirely clear since it is possible to observe numerous mitoses in several tissues of these animals. Cheney (1969) and M. C. Mix (*Radiat. Res.* 49, 176–189, 1972) have speculated that *C. gigas* may have a relatively long period of DNA synthesis and slow cell turnover times.

Pulse labeling with ^{3}H-TdR is depressed when a large amount, e.g., 100×, of unlabeled TdR is added to dilute the labeled precursor, i.e., the specific activity is low (L. E. Feinendegen, 1967, "Tritium-Labeled Molecules in Biology and Medicine," Academic Press). Therefore, it seemed theoretically possible that if precursors could be used in which the proportion of ^{3}H-TdR molecules was much greater than the unlabeled form (high specific activity), labeling would occur even though cell turnover times may be long.

Recently, a high specific activity ^{3}H-TdR (40–60 Ci/millimole) has become available (New England Nuclear; Cat. No. NET 027Z). It is the purpose of this paper to describe our techniques and results using this high specific activity ^{3}H-TdR and the freshwater mussel (*Margaritifera margaritifera*) as our experimental animal.

Six clams weighing approximately 30 g each (excluding shell weight) were injected with 30 μCi of ^{3}H-TdR (1.0 μCi/g; specific activity, 50.3 Ci/millimole; conen, 30μCi/ml). Three clams were injected in the pericardial sinus after removal of the left valve, wrapped in moist towels, and subsequently sacrificed at 1, 2, and 4 hr postinjection. The other three were injected in the visceral mass (shell not removed) by inserting a needle between the valves; they were sacrificed at 10, 24, and 48 hr. All animals were fixed in cold, neutral, buffered 10% formalin, routinely processed, sectioned at 6μm and the paraffin was removed prior to dipping in emulsion (Ilford K5). After dipping, the slides were placed in light-tight black slide boxes and stored in a refrigerator at 6°C until development. Test slides were developed every other day for 10 days.

The significant findings of this preliminary work are summarized below: (1) Labeling was observed in all tissues studied—gut, stomach, digestive tubules, gills and blood cells (Figs. 1, 2). (2) Labeled cells were first found 4 hr after injection although it is not yet definite that labeled cells do not appear prior to this time—in this study, 1 and 2 hr postinjection. (3) Injection into the pericardial sinus or visceral mass were both effective means of introducing ^{3}H-TdR. (4) It was necessary to expose the slides for only 3–5 days

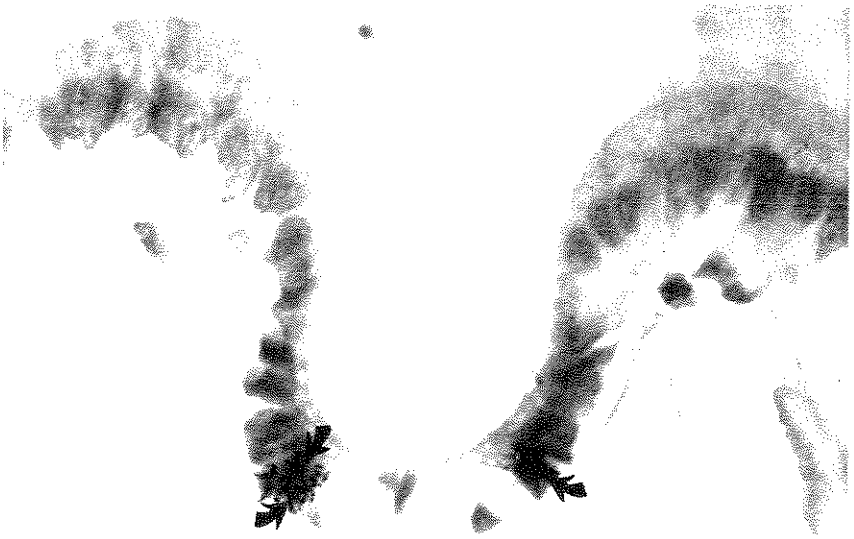

FIG. 1. Two labeled epithelial cell nuclei (arrows) in the basal groove between two ordinary gill filaments of *M. margaritifera* sacrificed 10 hr after injection with ^{3}H-TdR. The slide was exposed for 5 days. 800×.

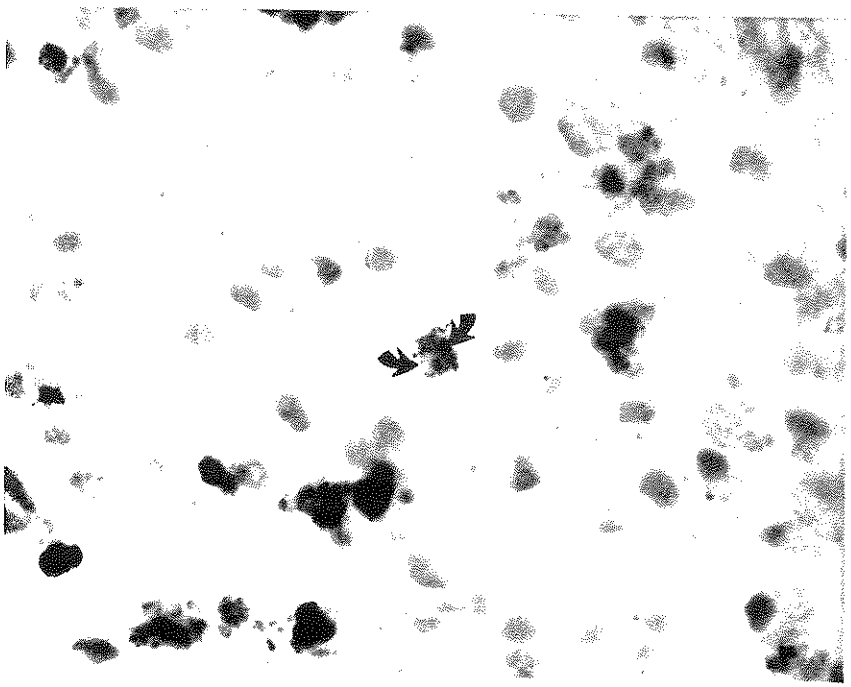

FIG. 2. A labeled cell nucleus (arrows)—thought to be a granular leukocyte—in the connective tissue of *M. margaritifera* sacrificed 48 hr after injection with ^{3}H-TdR. The slide was exposed for 5 days. 800×.

compared to exposure times of 15 days to 6 mo in other studies of cell renewal in mollusks using low specific activity ^{3}H-TdR.

Thus, it appears that the use of high specific activity ^{3}H-TdR has significant potential as a nuclear label for cells of living mollusks and perhaps other invertebrates. However, it must be pointed out that there may be some potential problems. For example, high specific activity and high concentrations necessarily mean an increased radiation dose to cells and tissues; it may be possible that such doses will adversely affect cell renewal systems and disturb normal cell kinetics. It is also possible that, because of relatively large radiation doses, there will be increased production of secondary products that will result in extraneous labeling.

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