The Application of Autoradiography to Developmental Studies in the Hydrozoa

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SYNOPSIS. The uptake of isotopically labeled nucleotides and amino acids has been studied in five species of hydrozoans. In three species the label was introduced both through immersion in a medium containing the labeled compound and by injecting the labeled compound into the gastrovascular cavity. In the remaining two species the label was introduced by immersion only. The comparison of soaked and injected specimens clearly indicates that injection is the method of choice whenever the injection of compounds into the gastrovascular cavity is possible. The relative ease with which labeled compounds were absorbed can be correlated with the ultrastructure of the epidermal and gastrodermal cell surfaces and their associated extracellular coats. The use of these autoradiographic techniques is illustrated by the use of injected tritiated thymidine and tritiated uridine to follow the replacement cycle of the zymogenic secretory cell in Hydra, and the use of immersion to introduce tritiated thymidine into the planula larva of Pennaria.

METHOD OF ADMINISTRATION

Introduction

Early investigations on the incorporation of isotopically labeled compounds by hydrozoans used soaking in a medium containing the isotope as a means of introducing the label. These studies met with variable results. With some marine hydrozoans this produced successful labeling (Braverman, 1968); in others it resulted in only slight labeling of cells (Tweedell, 1970). In Hydra, soaking the intact animal met with failure to incorporate any label, while soaking regenerating animals produced successful labeling (Burnett et al., 1962). It was not until Campbell (1965) injected label into the gastrovascular cavities of mature animals that a reliable method for labeling non-regenerating Hydra cells was found. In contrast with the results of Burnett et al. (1962) and Campbell (1965), Clarkson (1969), using liquid scintillation counting, detected the incorporation of both nucleotides and amino acids when intact Hydra were soaked in a medium containing these compounds. Because of the differential sensitivities of the assay techniques (autoradiography and scintillation counting), it is difficult to compare the two types of results. However, since qualitatively different results have been obtained it is clear that the method of introduction of the label causes variations which cannot be attributed to the differential sensitivity of the assays.

Because of the differential ability of Hydra and to a degree other hydrozoans to incorporate label from their medium and because of the different results reported for the soaking of Hydra by different investigators, we initiated a study of the abilities of five species of hydrozoans to incorporate labeled thymidine, uridine, and amino acids. In the species in which the individuals are large enough to permit the injection of label, the incorporation of injected compounds was compared with the incorporation of label applied by soaking.

Our most complete data are for Hydra and we will present these in greatest detail.
We will then compare these with the data on the other species which are still under investigation.

Because our observations of label incorporation have yielded recognizable differences in the ability of the different organisms to absorb labeled compounds, we have begun an ultrastructural study of the surfaces of gastrodermal and epidermal cells. We have also attempted to relate these observations to the pattern of incorporation.

Materials and methods

Autoradiography

Hydra. Hydra were either soaked in a medium containing the label at a concentration of 100 \( \mu \text{Ci/ml} \) or injected with 1.0 \( \mu \text{l} \) of culture water containing 0.2 \( \mu \text{Ci} \) of tritium-labeled nucleotides or 14C labeled amino acids. The animals were sacrificed at 30 min after injection, since preliminary observations showed that longer periods of incubation, up to 12 hr, did not enhance specific labeling, or at 30 min, 3 hr, 6 hr, 12 hr, and 24 hr after soaking was initiated.

Marine hydroids. The marine hydroids Clava, Tubularia, Pennaria and Sertularia were soaked in a medium containing 10 \( \mu \text{Ci/ml} \) of label/ml or were injected with 1.0 \( \mu \text{l} \) of sea water containing 0.2 \( \mu \text{Ci} \) of label. They were sacrificed at 30 min after the beginning of the experiment. Specimens of Pennaria were also soaked for 2 hr in the label-containing medium.

Planula larvae. Larvae of Pennaria were soaked for 30 min in a medium containing 10 \( \mu \text{Ci/ml} \) tritiated thymidine. (Longer incubation periods did not enhance specific labeling.)

Autoradiographic technique. All animals were fixed in Bouin's fluid and were embedded in paraffin using standard techniques. Sections of 5 \( \mu \text{m} \) were prepared, deparaffinized, and coated with Kodak NTB-2 Nuclear Track Emulsion. Coated slides were air-dried and exposed for 2 weeks at 4 \( \text{C} \). Following exposure, slides were developed for 1 min in undiluted Dektol at 70 \( \text{C} \).

Electron microscopy

Hydra were fixed and embedded as described in Haynes and Davis (1969). Marine species and planula larvae were fixed as described by Summers (1970). Sections were stained with either lead citrate or lead citrate and uranyl acetate. Specimens were observed with an RCA EMU 3G electron microscope.

Autoradiographic observations

The two methods of administration produced distinctly different results in Hydra. When samples of tritiated thymidine, tritiated uridine or tritiated amino acids,

![Graph](https://example.com/graph1.png)

**FIG. 1.** Relative amounts of \(^3\text{H}\) uridine and \(^{14}\text{C}\) algal hydrolysate incorporation by the cells of *Hydra*. In each case the label was introduced into the gastrovascular cavity and the animal was sacrificed 30 min later. The intensity of label (based on grain counts) was scored for seven body regions and the intensity of each region was divided by the maximum observed in the animal. Thus, the maximum region in each animal has a label intensity of 1 and less heavily labeled regions have lower intensities.
and 14C algal hydrolysate are injected into the gastrovascular cavity, maximum labeling occurred in both the epidermis and the gastrodermis within 30 min following the introduction of label into the animal. Both the uridine and the thymidine were clearly localized over the nuclei of all cell types. The distribution of the labeled cells following the injection of uridine is shown in the graph (Fig. 1). The distribution of labeled cells following the injection of thymidine is essentially the same as that described by Campbell (1965). The labeled amino acids were localized within the cytoplasm and their distribution along the body column is shown in Figure 1. Examples of labeling with tritiated thymidine and uridine are presented in Figure 2A, B. While all cell types are labeled, the heaviest label occurs in the gastrodermal secretory cells and the interstitial cells and young cnidoblasts of the epidermis.

When animals were soaked in the label for a period of 30 min, no label was detected in the epidermis or the gastrodermis. After prolonged incubation (6 to 24 hr), label began to appear in both the epidermis and the gastrodermis. The distributional pattern of labeling of the amino acids along the body column is similar to that found with injection, but the label is much lighter and all cells appear uniformly labeled. With uridine, the incorporation is diffuse and label is associated with both the nuclei and the cytoplasm. Incorporation of thymidine was not detectable until incubation had proceeded for 24 hr. Occasional nuclei showed label above background, but the major portion of the label was cytoplasmic.

In Clava, Tubularia, and Pennaria, soaking for 30 min in a medium which contained tritiated thymidine produced strongly labeled nuclei in epidermal (Fig. 2C, D, E) and moderate label in gastrodermal cells. In Tubularia and Pennaria, incorporation occurred only in hydranths; it did not occur in regions surrounded by the perisarc. In Pennaria, when the exposure to the label was extended to 2 hr, we still failed to label cells in the coenosarc proximal to the perisarc secreting zone. In Sertularia, the only thecate hydrozoan we have thus far investigated, soaking produced a slight, but still detectable, label in the gastrodermal and epidermal cells of the hydranth. No label was detected in the coenosarc of Sertularia.

Injection studies were carried out with thymidine on both Clava and Tubularia. In both species the cells of the epidermis and gastrodermis were labeled within 30 min after the label was introduced into the gastrovascular cavity. In Clava particularly, the intensity of the gastrodermal label was greater in the injected animals than in the soaked animals (compare Fig. 2E, F). The most significant and consistent difference between the soaked and injected animals was found in the gastrodermis of the hypostomal region. In both Clava and Tubularia the gastrodermal cells of the hypostome were prominently labeled following injection of tritiated thymidine. Labeling in this area was less intense and was occasionally absent in animals soaked in the label for a period of 30 min.

Ultrastructural observations

When the epidermal and gastrodermal surfaces of the species under study were observed with the electron microscope, we identified three different morphological types of cellular surface. The first type occurs on the apical surface of the gastrodermis of all five species. The second is confined to the epidermal surface of Hydra. A third type is present on the epidermis of the marine species we have observed.

Gastrodermis. We will describe the apical surface of a gastrodermal cell of Tubularia as an example of this type (Fig. 3). The gastrodermal digestive cells are tall columnar cells containing a large vacuole in the basal and central regions. The free or apical surface which faces the gastrovascular cavity has a net-like appearance when viewed with the light microscope. With the electron microscope the surface of the cell is seen to be highly irregular, possessing a large number of cytoplasmic projections.
Immediately beneath the apical surface are many large tubular and vesicular profiles. These appear to be interconnected, some of them communicating directly with the gastrovascular cavity. As a result of this complex tubular infolding of the apical surface.
FIG. 3. Electron micrograph of the apical surface of a gastrodermal digestive cell of *Tubularia*. Note numerous microvilli (MV) and that the apical plasmalemma is invaginated as a series of tubules. Subapical cytoplasm also contains numerous discoidal coated vesicles (DCV). In one of these the pegs on the inner surface of the membrane can be seen. (27,000x)

Cal cellular surface there is an amplification of cell surface exposed to the gastrovascular cavity. Numerous large, discoidal coated vesicles occur in the apical cytoplasm. In sections these appear as dumbbells. On the inner surfaces of the bounding membranes of the vesicles are regularly spaced pegs. These pegs can be seen most clearly where fixation has caused swelling of the vesicle and the separation of the membranous walls. These structures have been described in *Hydra* by Slautterback (1967) and they are found in all species studied.

Epidermis. The apical surfaces of the

FIG. 2. *A*, The incorporation of tritiated thymidine by *Hydra* at about 15 hr after feeding. The label was injected into the gastrovascular cavity 30 min prior to sacrifice. Label is found in the nuclei of both gastrodermal (left) and epidermal (right) cells. *B*, Incorporation of tritiated uridine into the cells of *Hydra*. Uridine was injected into the gastrovascular cavity 12 hr after feeding and the animal was sacrificed 30 min later. Label is localized primarily in nuclei and can be seen in both apical (left) and basal (right) gastrodermal cells. *C*, Incorporation of tritiated thymidine in *Pen-naria* hydranth soaked for 30 min. Epidermal nuclei are clearly labeled (arrow). *D*, Incorporation of tritiated thymidine in *Tubularia* hydranth soaked for 30 min. Nuclei of the epidermis are labeled (arrow). *E*, Incorporation of tritiated thymidine (arrow) into *Clava* hydranth following soaking for 30 min. *F*, Incorporation in *Clava* hydranth following injection of label into the gastrovascular cavity. Labeled cells are seen in both the epidermis and the gastrodermis (arrows). (*A*, 260x; *B*, 580x; *C–F*, 200x)
epithelio-muscular cells of the *Hydra* epidermis are either smooth or show some very gentle folds (Fig. 4A). There is no indication of cytoplasmic processes or microvilli arising from the epidermal surface. Directly beneath the apical plasmalemma of the epithelial muscle cells are large aggregates of secretory vesicles. Our observations indicate that the release of vesicular contents contributes to the formation and maintenance of a thick layer of extracellular material completely surrounding the exterior surface of the animal. This coat is often resolved into two distinct zones: a dense zone immediately adjacent to the apical surface of the cell, and a diffuse zone forming the outermost portion of the cell coat. Histochemical evidence (Burnett, 1959) indicates that this material is a mucopolysaccharide or mucoprotein.

The type of apical surface found in the epidermis of the marine species that we observed is significantly different from that
in *Hydra*. While there are considerable species differences in the exact form of the extracellular coats, they all conform to a general pattern. The apical surface of the epidermal cells is highly folded and numerous microvillous projections ramify within the extracellular coat. Although the extracellular coat is variable in thickness, it is always penetrated by these processes which extend almost through the coat but never project beyond the coat. More than one layer is always demonstrable within the coat. This layering is seen in *Tubularia* (Fig. 4B) where the coat is made up of two layers resembling those found in *Hydra* but in reversed position. *Pennaria* possesses a very thick coat which is cuticular in nature (Fig. 5). The thick inner portion is homogeneous and apparently unstructured, while the thin outermost layer has a definite striated appearance.

**Discussion**

The method by which labeled compounds are administered clearly influences the pattern of incorporation and the amount of total label found in an autoradiograph. With *Hydra* the only method of successfully administering nucleotide and amino acid precursors to non-regenerating animals is by injection into the gastrovascular cavity. Marine species can be labeled by soaking, but there is indication that better results, particularly in terms of gastrodermal labeling, can be ob-

![Image](https://academic.oup.com/icb/article-abstract/14/2/783/2014155/16)
tained if the label is injected into the gastrovascular cavity. The absence of any label in *Pennaria* proximal to the perisarc secreting zone and the lack of success in labeling the stalk of *Tubularia* by soaking (Tweedell, 1970) indicates that the marine hydranths do not absorb the compounds we have utilized across the perisarc. The failure of label to be transported by the gastrovascular cavity to the stalk after the label has entered the gastrodermis from the epidermis indicates that the label is not released from the gastrovascular cavity during a 2-hr period of soaking.

The differences in label incorporation seem to be correlated with the different types of cellular surfaces observed with the electron microscope. The apical surface of the *Hydra* epidermis, which lacks any microvilli or projections, is not penetrated by the labeled compounds. Apparently the extracellular coat secreted by these cells retards the absorption of amino acids and nucleotides by the *Hydra* epidermis. They may penetrate slowly, as some labeling is observed after the animals are soaked for six or more hours. In marine forms, some of which possess a very thick extracellular coat, the uptake of materials is extensive when the hydranths are soaked in a medium containing label. This may be the result of the fact that their epithelial cells all possess processes that penetrate most of the way through the extracellular coat.

In all species studied it is the gastrodermal surface which takes up the labeled compounds most freely. This surface lacks any sort of extracellular coat and it is extensively folded and invaginated. It presents the greatest surface area for absorption. In addition the apical cytoplasm of all these species possesses discoidal coated vesicles which, as Slatterback (1967, 1969) has demonstrated in *Hydra*, can add to the surface membrane of the cell and participate in the active uptake of material in bulk from the gastrovascular cavity.

We are presently conducting electron microscopic studies on the incorporation of labeled compounds to determine the extent to which the coated vesicles participate in the uptake of materials and to determine if the pathway of entrance of material into the epidermis of marine species is through the processes of the epidermal surface.

One final conclusion concerning the administration of labeled compounds is that the epidermis and the gastrodermis both reach their maximum labeling within 30 min after the label is introduced into the gastrovascular cavity. This indicates that small molecules can be transported from the gastrovascular cavity to the epidermis in relatively short periods of time. It is interesting to compare the uptake of labeled small molecules with the uptake of label introduced in the form of macromolecules. In the latter case, prolonged periods are required for the transport of material from the gastrovascular cavity to the epidermis (Murdock and Lenhoff, 1968).

**AUTORADIOGRAPHIC ANALYSIS OF THE SECRETORY CYCLE OF ZYMGEN CELLS**

**Introduction**

The zymogen cells are secretory cells scattered between the digestive muscle cells of the gastrodermis of all hydrozoans. On the bases of their morphology and their response to feeding, they are believed to be responsible for the production and liberation of the enzymes that carry out the extracellular phases of digestion (Semal-Van Gansen, 1954; Haynes and Davis, 1969). Three different stages in the differentiation of the zymogen cell have been recognized: a basal reserve cell which is a small basophilic cell situated close to the mesoglea and lacking any secretory product; immature zymogen cells located between the mesogleal and apical surfaces and containing variable amounts of secretory materials; and finally, mature cells, filled with secretory material, and found as part of the lining of the gastrovascular cavity. These mature cells secrete their product after feeding. The gastrodermis then reestablishes a population of mature
secretory cells prior to the next feeding. We have conducted an autoradiographic study of the reproduction and differentiation of the secretory cells as part of an analysis of the dynamics of the zymogen cell’s maintenance-replacement cycle. In particular we are concerned with evaluating the relative parts played by the resynthesis of secretory products in depleted cells, the differentiation of immature cells, and the division of mature cells in the reestablishment of the mature zymogen cell population in *Hydra*.

Labeled materials for these experiments were administered by injection since previous experiments (above) indicated that this was the most reliable method for the introduction of nucleotides.

**Results**

By counting the numbers of mature secretory cells, the numbers of immature cells, and the numbers of discharged cells, we were able to establish that the replacement cycle for the secretory cells requires about 16 hr and that a normal population could be reestablished in this amount of time following a single heavy feeding (Fig. 6). During this time the total number of secretory cells remains constant.

To assess the role that mitosis might play

![Graph of relative numbers of mature secretory cells and discharged secretory cells](https://academic.oup.com/icb/article-abstract/14/2/783/2014155)

**FIG. 6.** Graph of the relative numbers of mature secretory cells and discharged secretory cells in *Hydra* plotted as a function of time after feeding. In each case the number of cells is presented as a percentage of the total secretory cells. At feeding the mature secretory cells represent 90% of the secretory cells and discharged cells are not present. Following feeding the discharged secretory cells increase in number and the mature secretory cells decrease in a parallel manner. After about 8 hr they reverse and the mature cells increase as the discharged cells decrease. In 16 hr the population of secretory cells has returned to its original composition. Immature cells make up a steady 10% of the total throughout.
in the reestablishment of the population of mature zymogen cells, we counted mitotic figures in zymogen cells to determine a mitotic index for this cell type and administered tritiated thymidine to determine the time at which DNA is synthesized in them following feeding. We found a sharp increase in the mitotic index between 16 and 19 hr after feeding. This followed a period of thymidine incorporation that peaked between 12 and 15 hr after feeding. These results are illustrated in Figure 7.

The time at which the synthesis of proteins was initiated and the specific cells in which proteins were being synthesized were determined by following the synthesis of RNA. Tritiated uridine was administered at hourly intervals following feeding. These results are shown in Figure 8. The mature secretory cells show two peaks of uridine incorporation. The first begins at 4 hr following feeding. The second begins at about 17 hr after feeding. Depleted secretory cells show a peak incorporation of uridine at about 4 to 6 hr following feeding. Immature secretory cells show a period of uridine incorporation beginning at 7 to 8 hr after feeding and continuing until about 15 hr after feeding.

Discussion

The data obtained through use of autoradiography to measure the times of DNA and RNA synthesis in the secretory cells in relation to the time of feeding enable the establishment of the basic cycles of synthesis and replacement of these cells. It is clear that the secretory cell population is reestablished completely in the 16 hr following feeding. Since the synthesis of DNA does not peak until 14 to 15 hr after feeding, mitosis cannot play a major role in the replacement of discharged secretory cells. The replacement must be accomplished through the differentiation of immature cells and/or the resynthesis of secretory products by the discharged cells.
The timing of RNA synthesis (presumably messenger RNA) in the different populations of cells indicates that both of these routes play a role. Depleted secretory cells reach their maximum numbers at about 6 to 8 hr after feeding. In this same period these cells show a peak in uridine incorporation. In fact it appears that the depleted cells begin to synthesize RNA almost as soon as they have discharged their secretory products. The peak synthesis of RNA is followed by a disappearance of these cells and the reappearance of mature secretory cells. We interpret these results as an indication that many of the discharged cells are capable of synthesizing messenger RNA and of resynthesizing their zymogenic secretory products.

The incorporation of uridine into the immature cells must represent the initiation of protein synthesis (i.e., mRNA synthesis) in relatively undifferentiated cells which will complete their maturation and contribute to the new population of mature cells. They undoubtedly will replace the zymogen cells which have died or have been discharged and will contribute to the growth of the Hydra. At the present we do not have enough data concerning absolute numbers of cells to estimate the number of new cells which must differentiate following each feeding.
fertilization. At this stage the young planula has a pyriform shape. The broad end of the larva will develop into the base of the polyp and the narrow end will form the hydranth. We will refer to the future basal end as the posterior end of the planula and to the future hypostomal end as the anterior end of the planula. Early embryonic stages and planulae of Pennaria are solid masses of cells and it is therefore impossible to administer isotopes by injection.

Results

When the planulae are allowed to remain in sea water containing tritiated thymidine, a maximum label is achieved after only 30 min. In younger larvae (immediately following the completion of gastrulation), exposure to tritiated thymidine shows that DNA synthesis is occurring almost exclusively in the epidermis (Fig. 9A). This epidermal incorporation indicates a gradient of DNA synthesis with the greatest incorporation being in the anterior end of the embryo. This proliferation is concomitant with rapid elongation of the planula. As the planula elongates, the epidermal gradient of thymidine incorporation is preserved but the absolute number of labeled nuclei slowly decreases (Fig. 9B, C).

Thymidine incorporation is first detected in the cells of the gastrodermis as the planula begins to elongate (Fig. 9B). The distribution of label in the gastrodermis follows a gradient but the direction of the gradient is opposite to that found in the epidermis, i.e., the highest region of thymidine incorporation is found in the posterior portion of the planula gastrodermis (Fig. 9C).

When the planula reaches a total length of about 1.0 mm the incorporation of thymidine into both the epidermis and gastrodermis becomes quite low and only occasional scattered labeled cells can be identified (Fig. 9D). At this stage the
planula attaches by its basal end and undergoes metamorphosis into a hydranth.

**Discussion**

The incorporation of thymidine by the planula larva clearly indicates a double gradient in the synthesis of DNA. The gastroidermal cells synthesize DNA in a gradient running from posterior to anterior while the epidermal cells synthesize DNA in a gradient running from anterior to posterior. Since the division of cells in the epidermis of the planula is initiated before the division of cells in the gastroidermis and since the basic morphogenetic changes which convert the stubby pyriform young planula into the slender mature planula are well underway before the initiation of gastroidermal cell divisions we conclude that the gradient of cellular division in the epidermis is primarily responsible for the morphogenetic changes that occur during the maturation of the planula larva.

Preliminary observations with the electron microscope indicate that the extracellular coat of the planula larva is poorly organized and that the cellular surface possesses numerous microvillous projections. The successful introduction of label into both epidermis and gastroidermis may be due to the presence of these cytoplasmic projections.

**References**


