figure 2. The retina of the fish contains twin cones and rods. Twin cones are observed only in the ventral region (twin cone area) (figure 2A, B). The remaining retina is all rod area which is composed of rod bundles (grouped receptors). Furthermore these rod bundles made a special arrangement shown in figure 2A, C, D. The pigment epithelium contains guanine crystals and makes a guanine type tapetum lucidum[5]. These histological examinations of the retina indicate that chlorophthalmid have the twin cone vision for upper forward and the high sensitive vision depending on guanine tapetum and grouped receptors for other directions.

Additionary chlorophthalmid have the yellow lens in the eyes[3–5]. In the eyes the same discrepancy exists between the light-collecting structure (tapetum and grouped receptors) and the light-absorbing yellow lens, as that of Argyropelecus' eyes[6]. This curious discrepancy also suggests a somewhat importance for the yellow lens. Indeed, the cultured blue-green bioluminescence (emission max. at 510 nm) of C. albatrossis can pass through the yellow lens thoroughly. But light of wavelength shorter than 450 nm barely reaches the retina because of the selective absorption of the yellow lens[3]. Thus, the present finding of the perianal light organ may explain the discrepancy of the eyes. Furthermore, to understand the sensory life of the fish, a possible interrelationship between the light organ and the specialized eyes is discussed.

Function of the small light organ might serve as an intraspecific signal which plays an important role in the school formation as in the case of macrourids[10]. The detection of dim bioluminescence as a signal may drive the evolution of unique eyes. Conversely it may be speculated that the specialized organization of the eyes restrains the primitive light organ from growing up into the well-developed structure, because it is dangerous to possess a large light organ for the intraspecific signal which would easily be intercepted by predators. Indeed the smaller the light organ, the more effective and/or secure the intraspecific signal.

The forgoing considerations (speculations) may be summarized as follows: Chlorophthalmus spp. have the specialized eyes which are necessary to detect the faint signal for the intraspecific communications. In such precise perception, the yellow lens and other retinal specialization (especially twin cone area) may have an active function to find the blue-green signal selectively in such deep-sea environment.

Further studies on such an interrelationship between the vision and the bioluminescence may bring forth useful information on the sensory life in deep-sea fishes. A more detailed report on the perianal light organ of Chlorophthalmus and related genera is under preparation.

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The influence of BrdU on interstitial cell differentiation in hydra[1]

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Summary. Exposure of hydra to 3.25×10^{-3} M BrdU selectively altered differentiation in the animal's pluripotent I-cell population. It is suggested, therefore, that this analog may represent a valid probe to analyze the controls regulating cellular differentiation in in vivo populations of pluripotent cells.

The thymidine analog 5-bromo-2′-deoxyuridine (BrdU) inhibits the expression of cellular differentiation in a wide variety of cells. This inhibition is effected without noticeably modifying either cell division or viability. In most cases suppression of differentiation occurs when BrdU is introduced during a period of cell proliferation and is consistent with an effect mediated through incorporation of the analog into DNA[3].

The freshwater cnidarian hydra possesses a population of presumably pluripotent interstitial cells (I-cells) whose approximate 24 h cell cycle[4] makes them the most rapidly turning-over population of cells in the organism. By virtue of their short cycle time I-cells would presumably exhibit a greater sensitivity than other hydrid cell types to a probe acting on proliferating cells. Therefore, this experimental system would permit an in vivo study of the effects of BrdU on the committment of multipotent cells to specific pathways of differentiation. In this investigation the effect of BrdU on I-cell differentiation into nerve and desmoneme, isorhiza and stenotele nematocytes is examined.

Materials and methods. All animals used for experimentation were non-budding Hydra pseudoligactis. The animals were mass cultured in 20 cm fingerbowls at 20 ± 1 °C according to the method of Loomis and Lenhoff[5] except that distilled water was substituted for tap water. Animals were fed every other day with Artemia salina nauplii and cleaned approximately 4 h after feeding.

Immediately after cleaning, animals were divided into 2 groups and placed in either hydra culture water (HCW) or a solution of 3.25×10^{-3} M BrdU (Sigma Chemical Co., St. Louis, Missouri) in HCW. This concentration was selected as it represented a level of BrdU that had been previously shown not to inhibit regeneration[6]. Furthermore, preliminary experiments in our laboratory revealed it to be the highest concentration at which BrdU-treated animals budded at a normal rate and exhibited no evidence of cell sloughing after a 3-week exposure.

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Quantitative cell data were recorded from macerated hydra and freshly-prepared tentacle whole mounts. At 48 h intervals beginning at the time of initial exposure 10 randomly-selected BrdU-treated animals and 10 HCW controls were cut in half. The distal halves of these animals were macerated according to the method of David[7] and the cells were counted under phase contrast optics. A minimum of 1400 cells was counted for each preparation and classified by cell type as described by Bode et al.[8]. Mature nematocytes were also counted from freshly-prepared tentacle whole mounts. At 48 h intervals beginning at the time of initial BrdU exposure, tentacles were clipped at their bases from both control and BrdU-treated hydra before maceration. These tentacles were mounted immediately and examined with phase contrast optics. To insure uniformity in the counting procedure desmoneme, isorhiza and stenotele nemocytes were counted from the middle third of each tentacle preparation[9]. In all control hydra at least 600 nematocytes were counted per preparation. For BrdU-treated animals, counting ceased when the number of stenoteles reached the control level. Statistical analyses of the differences recorded between the cell types of control and BrdU-treated hydra were performed using a sign test[10].

Results. Prior to beginning experimentation cell counts were recorded on hydra randomly-selected from those to be exposed to BrdU and HCW controls. A comparison of the proportions of each cell type in these hydra revealed no significant differences (designated day 0 in figures 1 and 2). Counts recorded on day 2 showed detectable decreases in the proportions of both large and small I-cells; however, these differences were not statistically significant (figure 1A and B). By day 4, although the proportions of both large and small I-cell remained unchanged in control hydra, BrdU-treated animals exhibited statistically significant ($p < 0.05$) decreases in the proportions of these cell types. Once established these differences were maintained throughout the exposure period. Examination of the proportion of nerve cells present in control and BrdU-exposed hydra revealed that, as found with I-cells, the proportion remained unchanged in control hydra throughout the 16 day study period. BdrU-treated hydra, however, exhibited a pronounced decrease in nerve cell population by day 4 ($p < 0.005$), with no recovery occurring so long as hydra remained in BdrU (figure 1, C). Mature nematocyte counts (from tentacle preparations)

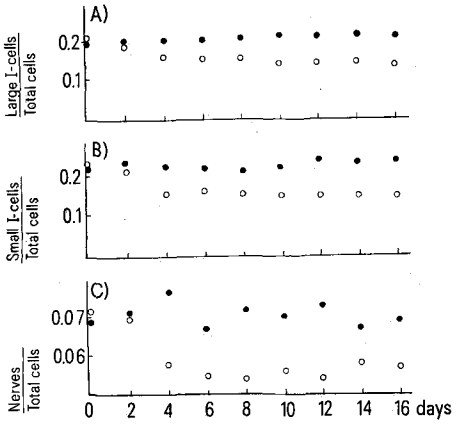

Fig. 1. Comparisons of the relative proportions of 3 cell types in the distal halves of animals exposed to 3.25×10^{-3} M BrdU and HCW controls. In each comparison the open circles represent BrdU-treated hydra, the closed circles represent control animals. A) Large I-cells, B) small I-cells, C) nerve cells.

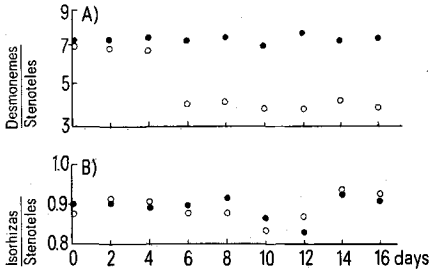

Fig. 2. Comparisons of the desmoneme and isorhiza nematocyte populations, expressed as a proportion of the stenotele population, in the tentacles of animals exposed to 3.25×10^{-3} M BrdU and HCW controls. In each comparison the open circles represent BrdU-treated hydra, the closed circles represent control animals. A) Desmonemes, B) isorhizas.

showed no statistically significant differences in the calculated proportions of either stenotele or isorhiza nematocytes between control and BrdU-treated hydra throughout the study period (figure 2). Such data indicate that the proportions of these 2 nematocyte types changed similarly, if at all, following BrdU exposure. Therefore, because stenoteles represented the larger and more distinctive of the 2 types, they were selected as the standard against which numbers of desmonemes were compared. Counts of BrdU-treated tentacles continued until the number of stenoteles reached that of the control preparation for the specified time interval. As a result the data for mature nematocytes in figure 2 are presented with the nematocyte type (i.e. isorhiza or desmoneme) expressed as a proportion of stenotele nematocytes. It is evident from figure 2 that by day 6 significant differences ($p < 0.05$) existed between the relative proportions of desmonemes in control versus BrdU-treated animals. Once achieved this difference was maintained for the entire study period.

Discussion. Hydrid I-cells represent a pluripotent population of cells from which nerves and nematocytes differentiate. The current model[11] explaining this differentiation envisions a cycling stem cell population from which cells are committed to either a nerve or nematocyte pathway of differentiation. It is evident that 3.25×10^{-3} M BrdU affected hydrid I-cell differentiation, with differentiated derivatives of both pathways being altered by BrdU exposure.

The pattern of response to BrdU revealed that I-cells appeared to be immediately affected by the drug. Once the proportion of I-cells stabilized however, prolonged exposure to the compound did not result in an exaggeration of the response. I-cell derived cell types exhibited a similar pattern of decline and subsequent stabilization, with the decreases in proportions occurring at times predictable from cell cycle analysis.

Therefore, we suggest that the modifications in the cellular composition of hydra observed following BrdU exposure result from BrdU affecting I-cell differentiation. As such, this analog may now be utilized as a probe to further analyze the regulation of cell differentiation in an in vivo pluripotent population of cells.

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