antiviral drugs dissolved in the distilled water (0.2 ml) were given p.o. once 1.5 h before infection. Control animals received water. Ribovirin was kindly given by the Lederle American Cyanamid Company, USA. Rimantadin hydrochloride was kindly given by Dr Y. Y. Polis from the Institute of Organic Synthesis, Latvian Academy of Sciences, Riga, USSR.

*Results.* It is seen from figure 1 that both rimantadine and ribovirin effectivity inhibit the reproduction of FPV.

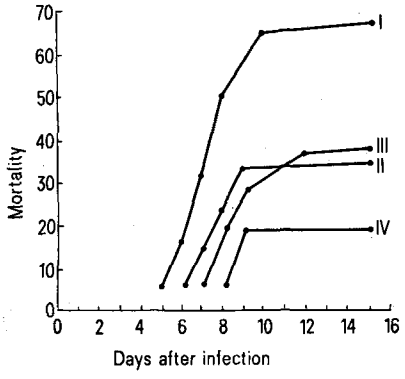


Fig. 4. Protective action of ribovirin and rimantadine on the experimental infection of mice with influenza $A_2$ virus. I, control group; II, rimantadine 10 mg/kg; III, ribovirin 200 mg/kg; IV, both compounds at the same doses. 40 mice per group up.

Decrease to 1.5–2.0 log PFU/ml is achieved with 12.5 μg/ml ($5 \times 10^{-5}$ M) of ribovirin and 0.65 μg/ml ($3.5 \times 10^{-6}$ M) of rimantadine. Ribovirin, in the concentration of 50 and 100 μg/ml, sharply inhibits the synthesis of hemagglutinin and the infectious virus, even if the m.o.i. is 5–10 PFU/cell (not shown in the figure).

The combined use of both compounds gives an additive effect. As is seen from figure 1, the combination of rimantadine (0.65 μg/ml) and ribovirin (12.5 μg/ml) decreases the infectious titre to 4 log PFU/ml, that is 0.7 log PFU/ml more than the sum of the effects of rimantadine and ribovirin at the given doses. Almost complete inhibition of the virus reproduction is achieved by the combined action of the increased doses of rimantadine (1.25–2.5 μg/ml) and ribovirin (12.5–25 μg/ml).

Figure 2 shows how the addition of rimantadine increases the inhibitory action of ribovirin. It is seen from these data that the combination of ribovirin (3–6 μg/ml) and rimantadine (0.65 μg/ml) gives an inhibitory effect which is more than that of ribovirin in the concentration of 25 μg/ml. It is also seen that the combined effect of both compounds is more than the sum of the effects of both compounds at the given doses.

It is also seen from figure 3 that the combination of both compounds is more effective than the use of them alone, though the effect depends on the m.o.i. The protective effect of the compounds in the experimental infection of mice is shown in figure 4. Again it is seen that the protective effect of the combined action of the compounds is considerably higher that the sum of the effects of both compounds given alone.

## Bacterial bioluminescence in chlorophthalmid deep-sea fish: A possible interrelationship between the light organ and the eyes

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*Summary.* A small perianal light organ was found on a ventral part of the body of Chlorophthalmus spp. Simple tests, histological preparations and bacterial culture of the luminous contents clearly indicated that the luminous substance consists of symbiotic bacteria. The unique eyes of Chlorophthalmus spp. suggests a possible interrelationship between the eyes and the newly observed light organ.

Chlorophthalmus spp. (Iniomi), a benthonic genus, occur at moderate depth (150–750 m) throughout tropical and temperate regions of the world. They are normally under 12 inches long[2]. The eyes of C. agassizi has been described by Denton[3] as possessing bright yellow lens. Somiya and Tamura[4] and Somiya[5] have also reported on the optical properties of the yellow lens and several retinal specializations in the eyes of C. albatrossis.

During the R.V. 'Hakuho Maru' Cruise KH72-1, the author had an opportunity to observe various luminous deep-sea fishes and to examine their eyes. This has led the author to speculate on the possible relationship between the specialized eyes and bioluminescence[6]. The experimental fish were freshly caught Chlorophthalmus albatrossis (figure 1A) and C. nigromarginatus from the Kumano sea (off Owase, Mie prefecture, Japan). Macrourid fish (Coelorhynchus hubbsi) were used as controls. The specimens were observed in a dark-room on board ship. A small light organ was found around the anus on the ventral part of the body of the chlorophthalmid. This small spot like bioluminescence was only visible after about 15 min of dark adaptation on the part of the observer. The luminescence was blue-green and continuous as that of macrourid fishes.

Simple tests[7, 8] for differentiating the nature of luminescence (luminous bacteria or luciferin luciferase) were carried out on fish kept on ice. The results from chlorophthalmid and macrourid fish were the same, as follows: a) Luminous emulsions in sea water were obtained by homogenizing the light organ. 1. With the exception of the upper layer exposed to air the luminescence became quiescent gradually when allowed to stand. When the quiescent emulsion was shaken in air, the luminescence was completely restored again. This emulsion retained the ability to luminesce for many hours. 2. On raising the temperature to around 50 °C (5 min), the emulsion ceased to luminesce and the luminescence could not be restored even when cooled again to 20 °C. If the emulsion was cooled below 5 °C, the luminescence was completely extinguished but it was restored when the emulsion was rewarmed to room temperature. 3. When the luminous emulsion was centrifuged at 300 × g for 15 min at room

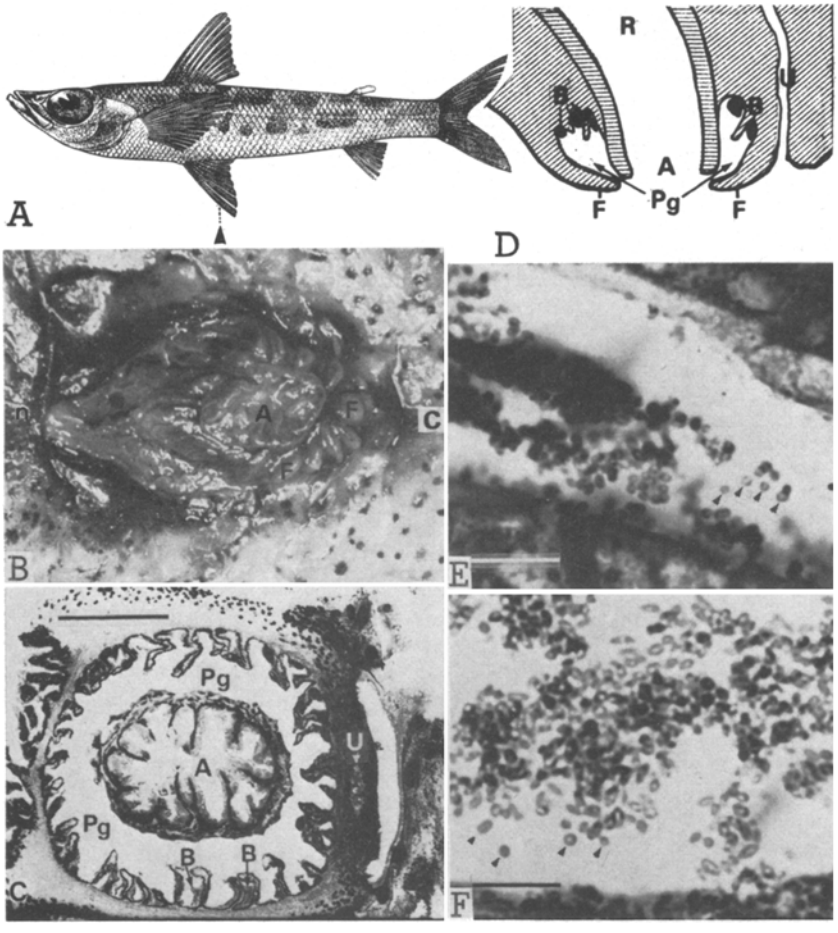


Fig. 1. Location and structure of the perianal light organ. *A* Lateral view of Chlorophthalmus albatrossis (12 cm) showing the position of the anus (after Suzuki[18]). *B* External view of the perianal light organ with many finger-like structures (F) which encircle the terminal end of the rectum (anus) (A). c, caudal; n, nasal. *C* Histological preparation (horizontal section, 7 μm, stained with H. E. method) showing anus (A), perianal circular groove (Pg), and urogenital duct (U). The symbiotic luminous bacteria (B) were found between the base of the finger-like structure (F) in the perianal groove (Pg). (Scale bar = 0.5 mm.) *D* Schematic representation (longitudinal section) of the perianal light organ with colonies of the symbiotic bacteria (B). *E*, *F* Histological comparison of the luminous bacteria of Chlorophthalmus albatrossis (E) and that of macrourid (Coelorhynchus hubbsi) (F). (7 μm, stained with Ziehl-Neelsen method; scale bar = 10 μm.)

temperature, the luminousity was concentrated in the sediment at the bottom of the tube, the supernatant was clear and not luminous. If the precipitate was well mixed with sea water, the whole mixture became uniformly luminous again. 4. When the luminous organ was homogenized in fresh water, the luminescence was immediately extinguished. Also if the luminous emulsion in sea water was diluted with fresh water, the luminescence was quickly extinguished. b) Air-dried light organs did not luminesce when moistened with water.

The luminous contents of the luminous body of a macrourid fish (Coelorhynchus hubbsi) has already been known as the symbiotic luminous bacteria[9]. The results mentioned above strongly suggested that the luminous material of the light organ of the chlorophthalmid also consists of symbiotic luminous bacteria. Actually bacterial culture (in 3% NaCl Nutrient Agar pH = 7.2–7.4) was demonstrated by Dr Yata Haneda of Yokosuka City Museum and the strong blue-green bioluminescence of the cultured luminous bacteria was observed. Furthermore, the emission spectrum of the bioluminescence was measured and revealed the emission max. at 490 nm.

Fish material fixed in 10% formalin or Bouin's fluid was used for macro- or microscopic observation. These observations revealed that the light organ of the chlorophthalmid consists of perianal groove with many finger-like structures which encircle the terminal end of the rectum (anus) (figure 1B, C, D). In the histological preparation, colonies of the luminous bacteria were found in

1 I thank Drs T. Tamura, E. J. Denton, O. Munk, M. Okiyama, K. Suzuki, M. Oguri, T. Tonoue and Mr H. Niwa for their constant guidances and their invaluable helps. This study was started by the stimulative conversation with Dr Yata Haneda. I would like to present the paper to him for the celebration of the perfect
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the perianal groove i.e. between the base of the finger-like structures (figure 1C, D). Precise comparison of the luminous bacteria of chlorophthalmid and of macrourid is shown in figure 1E, F. These indicate that the chlorophthalmids have an open (to the outside) direct emission type light organ with the symbiotic luminous bacteria. Perianal groove structure has already been described as one of main components of the light organ in many Japanese macrourids[10]. Furthermore, the similar anal light organ was reported in argentinoid fish[11,12] and anacanthine fish[13]. These observations indicate that the perianal light organ of the chlorophthalmid would be one of the most primitive type with the symbiotic luminous bacteria.

Chlorophthalmid have one of the most specialized eyes in bony fishes. The retinal specialization is shown in

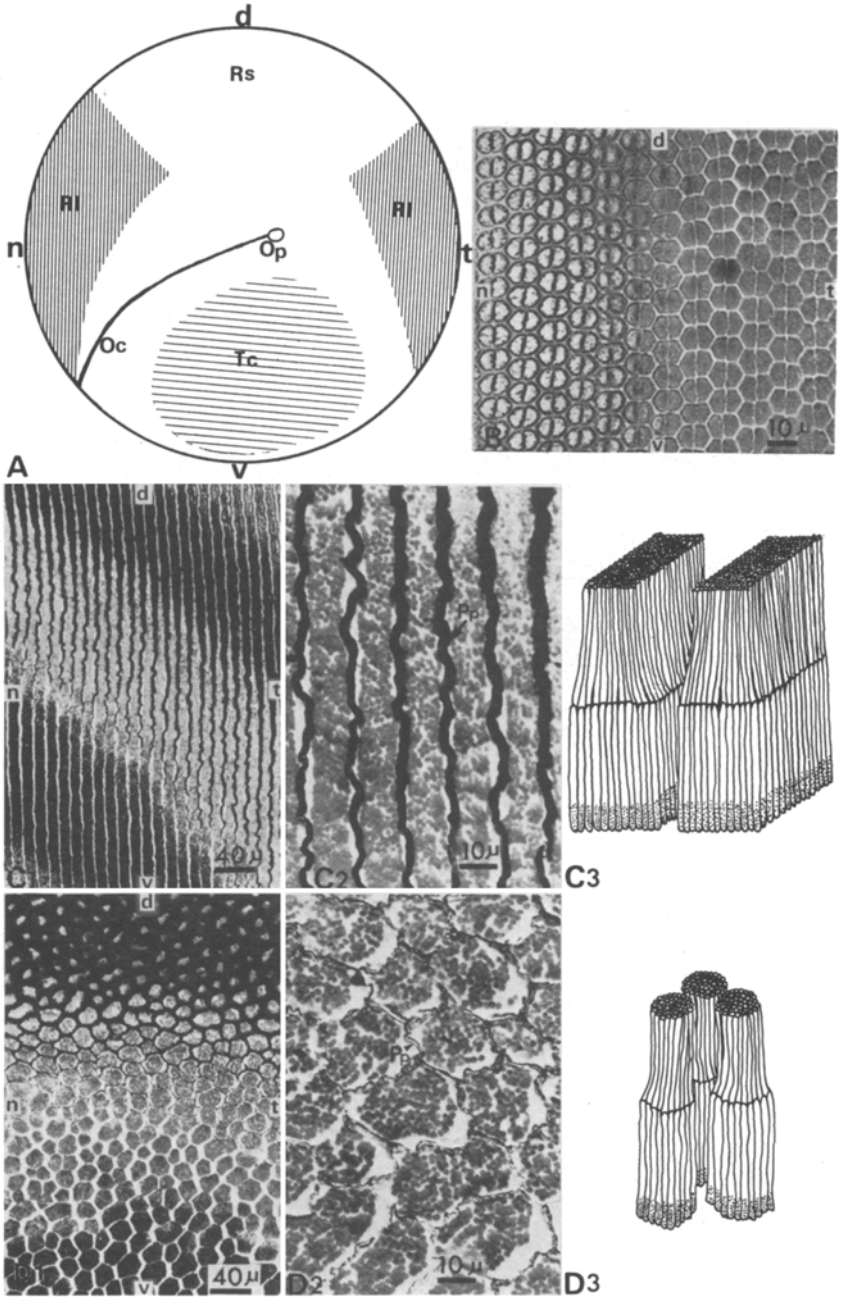


Fig. 2. Schematic representation of retinal eye cup (A) of Chlorophthalmus albatrossis showing the retinal specializations and their histological figures (B, C, D). *A* The retina is divided into 2 areas; one is the twin cone area (Tc) and the other is the rod area. The rod area is also divided into 2 parts i.e. linearod bundles (Rl) and spot-like rod bundles (Rs) according to the form of the tangential sections. The vertical strips correspond to linear rod bundles and the white part to spot-like rod bundles schematically. Oc, Optic cleft; Op, optic papilla. *B* Tangential section of the twin cone area showing many twin cones and no rods. *C* Tangential section of the linear rod bundles (C1, 2) which are arranged vertically in the nasal and temporal direction of the retina. These rod bundles are separated by the processes of pigment epithelial cells (Pp) which contain guanine platelets as reflectors (guanine tapetum). 3-dimensional figure of the rod bundles is shown in C3. *D* Tangential section of the spot-like rod bundles (D1, 2) and the 3-dimensional figure of the rod bundles (D3) are shown. D, dorsal; N, nasal; T, temporal; V, ventral.

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 $3.25 \times 10^{-3}$ 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 $3.25 \times 10^{-3}$ 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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