

potent than the non-sulfated form⁹. Therefore, it appears that for the in vitro gall bladder G-17-S is generally more potent than G-17-NS; however, for other target tissues one cannot generalize. Thus, G-17-S and G-17-NS are equipotent in stimulating rat gastric secretion in vivo and in contracting hamster stomach and rat colon in vitro¹⁰. They are also equipotent in stimulating cat gastric acid secretion (Dr. M. GROSSMAN, personal communication). But as a secretagogue of bullfrog gastric mucosa in vitro, G-17-S is 10-times more potent than G-17-NS¹¹.

There are no reports wherein the sulfated form of gastrin, CCK, or their analogues has been found to be less potent than the non-sulfated form.

The molar concentrations of OP-S which were currently

found to be effective in eliciting a response on cat gall bladder, i.e. 2.2×10^{-10} to 5.3×10^{-9} , approximate fasted human plasma levels of CCK^{12, 13}. On the other hand, the concentrations of OP-NS and the 3 gastrin preparations needed to elicit responses probably would not be reached in any physiological situation.

⁹ M. S. AMER, *Endocrinology* 84, 1277 (1968).

¹⁰ E. MIKOS and J. R. VANE, *Nature, Lond.* 214, 105 (1967).

¹¹ L. W. WAY and R. P. DURBIN, *Gastroenterology* 56, 1266 (1969).

¹² R. F. HARVEY, M. HARTOG, L. DOWSETT and A. E. READ, *Lancet* 2, 826 (1973).

¹³ D. A. REEDER, H. D. BECKER, N. J. SMITH, P. L. RAYFORD and J. C. THOMPSON, *Ann. Surg.* 178, 304 (1973).

Loss of Bioluminescence in *Anomalops katoptron* due to Starvation¹

V. B. MEYER-ROCHOW

Department of Biological Sciences, University of Waikato, Hamilton (New Zealand), 1 March 1976.

Summary. After 3 weeks of starvation the bacterial light-organs of the bioluminescent shallow-water fish *Anomalops katoptron* cease to produce light. Because of a reduction of the number of symbionts in the cells of the light organ, it is concluded that the fish supplies its luminescent bacteria with nutrients out of its own metabolism. As a result of starving the fish, the luminescent bacteria decrease in number and finally cease to emit light.

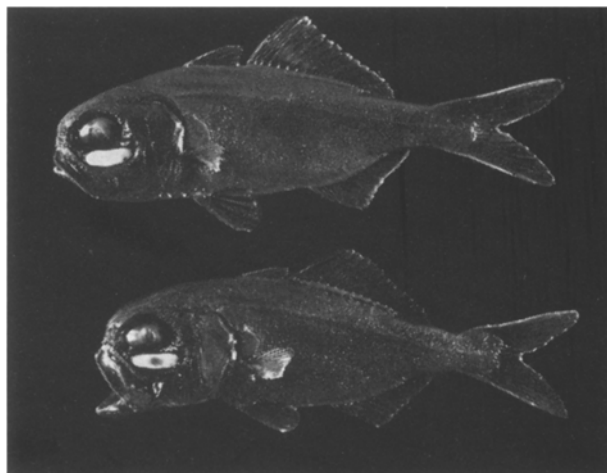
Anomalops katoptron is a bioluminescent shallow-water fish, which is indigenous to the Banda Islands in the South Moluccan Sea. It emits a greenish light from a pair of bean-shaped organs below the eyes. The light-emitting structures contain luminous bacteria². STECHE³ studied the anatomy and histology of the light organ in detail.

The side of the organ which emits light continuously, is creamcoloured, while the opposite face is black owing to a pigmented cell layer. During the day the light is extinguished by rotation of the entire light organ along its long axis, so that the luminous side is turned towards the body, presenting the black pigmented face to the outside. At night the luminous side is turned to the outside, and the light is presumably used to aid in navigation, find food and help keeping the school together.

During the 1975 Alpha Helix South East Asia Bioluminescence Expedition, I kept 7 *Anomalops* in a 73 × 32 × 30 cm glass aquarium, the sides of which were covered with dark-red cellophane. The water was oxygenated and hiding places for the fish, made from dead coral blocks and rocks, existed. The fish were caught during moonless nights by local fishermen. *Anomalops* can be easily herded by the beams of 2 torches into shallow water, where they are caught with hand nets.

Normally the fish were hiding under stones during the day and had their lights extinguished by the method described above. Coinciding with sunset they regularly came out and swam all night, displaying a bright luminescence. At dawn they retreated again to their resting places. Although the light would be displayed when the fish were moved into the dark, even during the day, the activity rhythm appeared to be independent of whether the animals were kept in continuous light or darkness.

After 1 week of starvation the light intensity emitted by the light organs of all *Anomalops* became noticeably weaker. After 2 weeks starvation light production in the central part of the light organ had dropped to a level which made the light organ seem to have a grey spot when studied at night (Figure). After 3 weeks of starvation the light organs, to the human eye, did not appear



Two *Anomalops*, the lower individual starved for 2 weeks. Photograph slightly retouched to demonstrate how the light organ of the starved fish gradually turned non-luminescent, starting in the centre.

to emit any light any longer; they were just of a white creamy colour. Yet, all the fish behaved perfectly normal and continued their precise circadian activity rhythm. After 4 weeks of starvation, (the fish still did not show any signs of malnutrition) some of the non-luminescent light organs were fixed in seawater/collidine-s buffered 2% OsO₄ solution, and later examined in the electron microscope.

¹ Supported by a Queen Elizabeth II Fellowship in Marine Science, and carried out during the 1975 'Alpha Helix' South East Asia Bioluminescence Expedition.

² Y. HANEDA and F. I. TSUJI, *Science* 173, 143 (1971).

³ O. STECHE, *Z. wiss. Zool.* 93, 349 (1909).

Because of fixation problems it is difficult to ascertain whether the fine-structural emptiness of the bacteria represents the true histological state of the symbionts at that time, or whether this was caused artefactually. But it seems important that even though the light organ had not been emitting light for more than 1 week, some bacteria were still present. There is no doubt, however, that their concentration if compared with electron micrographs of fresh *Anomalops* light organ² was considerably reduced.

The relationship between fish and bacteria is commonly described as a symbiosis⁴⁻⁶. While the bacteria can grow in the relative safety of the fish's tissue, the fish benefits from the relationship by being provided with 'torches', useful for finding food during the night. The bacteria are thought to be independent effectors, glowing continuously with no intensity variation⁶. Our observation of a 'dying light organ' shows that over a period of a mere 7 days intensity changes, large enough to be detected by the human eye, occur in the bacterial light organ of *Anomalops*.

According to CORMIER *et al.*⁷, the bacterial bioluminescence system exhibits 4 requirements for light emission: a luciferase, reduced flavin mononucleotide, molecular oxygen and long-chain aldehyde. The quantum yield of the reaction determines the total output of light and in bacteria appears to be dependent upon the availability of aldehyde⁸. Although aldehydes of various chain lengths can be used, the rate of reaction is very dependent upon which aldehyde is used⁷. BERTELSEN⁹ has suggested that bacterial luminescence in the esca of mid-water angler fishes is controlled by the blood flow to this organ.

In the case of *Anomalops* it seems most likely that the symbiotic bacteria receive some vital substances from the fish via the bloodstream. It is known that the light organ in *Anomalops* is richly supplied with blood capillaries running parallel to each other from the pigmented base to the light-emitting organ³. Whether the substances supplied by the fish are directly involved in the process of producing light (like long chain aldehydes or oxygen, for example), or whether they are nutrients enabling the bacteria to grow, is not exactly clear at this stage. The fact that neither HARVEY⁵ nor HANEDA and TSUJI² succeeded in obtaining a culture of luminous bacteria from the light organ (although luminescent bacteria from other fish grew well on their culture media) and the fact that fewer bacteria were present in non-luminescent light organs of starved *Anomalops*, suggests that the fish provides its symbiotic bioluminescent bacteria with vital nutrients. The supply of these nutrients, however, can no longer be maintained if the fish is deprived of food for longer than a week. As a result the luminescent bacteria of *Anomalops* decrease in number and cease to produce light.

⁴ T. D. BROCK, *Biology of Microorganisms* (Prentice Hall Inc., New Jersey 1970).

⁵ E. N. HARVEY, *Bioluminescence* (Academic Press, New York 1952).

⁶ P. J. HERRING, *Proc. R. Soc. Edinb. (B)* 73, 229 (1972).

⁷ M. J. CORMIER, J. E. WAMPLER and K. HORI, *Fortschr. Chem. org. Naturst.* 30, 1 (1973).

⁸ J. W. HASTINGS and Q. H. GIBSON, *J. biol. Chem.* 238, 2537 (1963).

⁹ E. BERTELSEN, *Dana Rep.* 39, 2 (1951).

Electron Microscopy and Electron Probe Analysis of the Ca-Binding Sites in the Cilia of *Paramecium caudatum*

T. TSUCHIYA¹

Department of Physiology, School of Medicine, Teikyo University, Itabashi-ku, Tokyo 173 (Japan), 16 March 1976.

Summary. Electron-dense deposits were observed at the base of cilia of *Paramecium* fixed in glutaraldehyde solution containing 5 mM CaCl₂. The deposits were probed by X-ray microanalyzer and it was clearly demonstrated that the deposits consisted of calcium and phosphorus.

Evidence has been accumulating that, in both protozoan and metazoan animals, the modification of ciliary movement is achieved by the change in concentration of intracellular free Ca ions²⁻⁴. Experiments with Triton-extracted models of a ciliate *Paramecium* indicate that the change in direction of the effective stroke of cilia (ciliary reversal) is brought about by an increase in Ca ion concentration^{2,5}. It still remains, however, to be investigated whether in the inward movement of extracellular Ca or the release of intracellularly stored Ca is the main immediate source for the cause of the ciliary reversal in living *Paramecium*⁶⁻⁸. Recent electron microscopic studies on *Paramecium* fixed in a glutaraldehyde solution containing CaCl₂ have shown the localization of electron-dense deposits at the ciliary base, suggesting the presence of intracellular Ca-binding sites^{9,10}. As the first step to explore the possibility that the intracellular Ca-binding sites are involved in the control of ciliary movement, the present experiments were undertaken to determine the inorganic constituents of the electron-dense deposits found in the cilia of *Paramecium* by utilizing techniques of electron probe X-ray microanalysis.

Specimens of *Paramecium caudatum* reared in a hay infusion were kept in a solution containing 2 mM CaCl₂ and 1 mM Tris-HCl buffer (pH 7.2) and prefixed in a 2.5% glutaraldehyde solution containing 5 mM CaCl₂ and 80 mM s-collidine (pH 7.5). Then, the specimens were washed with a solution containing 5 mM CaCl₂, postfixed in a 1% OsO₄ solution containing 5 mM CaCl₂ and 80 mM s-collidine, dehydrated in ethanol, and embedded in Epon 812. Both the thick sections (150-200 nm thick) for X-ray microanalysis and the thin sections for conventional electron microscopic observation were cut on a Porter-Blum MT-2 microtome. The thin sections were weakly stained with uranyl acetate and lead citrate, and examined with a Hitachi HS-9 electron microscope, while a layer of carbon was evaporated on the thick sections without staining for X-ray microanalysis. The X-ray analytical system consisted of a JEM 100C transmission microscope fitted with a JEM ASD scanning attachment and an EDAX 707A energy dispersive spectrometer. The diameter of X-ray beam focused on the section was about 200 nm with an acceleration voltage of 40 kV. Analyses were performed with an integrated detecting time of 200 sec.