Because of fixation problems it is difficult to ascertain whether the fine-structural emptyness 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 organ[2] was considerably reduced.

The relationship between fish and bacteria is commonly described as a symbiosis[4–6]. 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 continously with no intensity variation[6]. 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 aldehyde[8]. Although aldehydes of various chain lengths can be used, the rate of reaction is very dependent upon which aldehyde is used[7]. BERTELSEN[9] 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 organ[3]. 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 wether they are nutrients enabling the bacteria to grow, is not exactly clear at this stage. The fact that neither HARVEY[5] nor HANEDA and TSUJI[2] succeeded in obtaining a culture of luminous bacteria from the light organ (although luminesent bacteria from other fisch 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 numer and cease to produce light.

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

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Summary. Electron-dense deposits were observed at the base of cilia of *Paramecium* fixed in glutaraldehyde solution containing 5 m*M* $CaCl_2$. 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 ions[2–4]. 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 $CaCl_2$ 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 m*M* $CaCl_2$ and 1 m*M* *Tris*-HCl buffer (pH 7.2) and prefixed in a 2.5% glutaraldehyde solution containing 5 m*M* $CaCl_2$ and 80 m*M* s-collidine (pH 7.5). Then, the specimens were washed with a solution containing 5 m*M* $CaCl_2$, postfixed in a 1% OsO_4 solution containing 5 m*M* $CaCl_2$ and 80 m*M* 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 ASID 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.

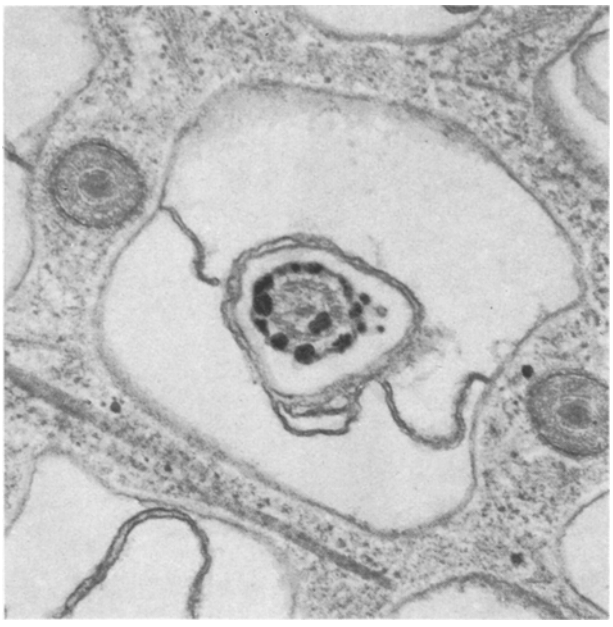

Fig. 1. Tangential section through the cell surface of *Paramecium caudatum*. Many electron-dense deposits are seen at the inner side of the ciliary membrane and a few deposits between outer doublet microtubules and the central pairs. Weakly stained with uranyl acetate and lead citrate. ×50,000.

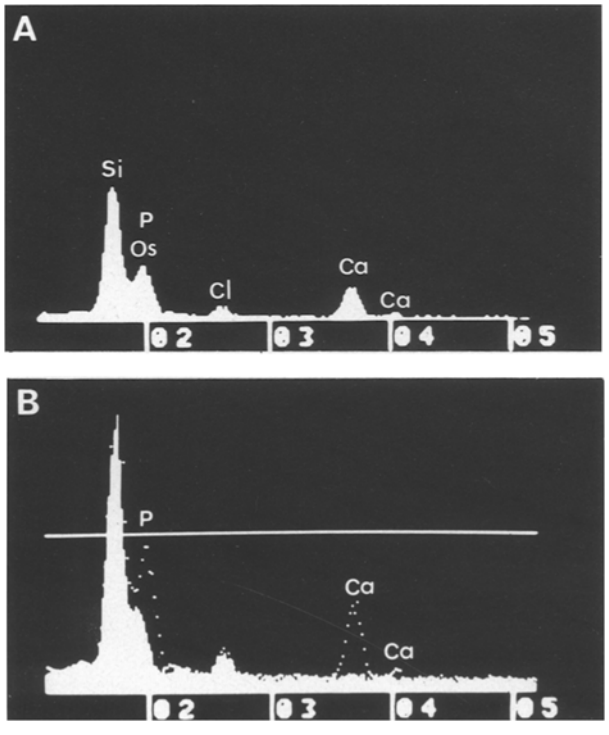

Fig. 2. A) X-ray energy spectrum obtained in the cilium containing the electron-dense deposits. Ca Kα (3.69 keV), Ca Kβ (4.01 keV) lines are seen. The peak at about 2.0 keV is the summation of P Kα1 (2.01 keV) and Os Mα (1.91 keV) lines. Cl Kα1 (2.62 keV) and Si Kα (1.74 keV) lines are attributed to the resin. B) X-ray energy spectrum in the cilium without the deposit. The spectrum in the cilium with the deposits are shown by dots for comparison. The absence of Ca and P lines is clearly seen in the cilium without deposit.

In almost all the cilia examined, electron-dense deposits were observed at the basal portion, though the amount of the deposit varied from cilium to cilium (Figure 1). The deposits were most frequently seen at the inner side of ciliary membrane just above the 'ciliary necklace', and also found between the outer and central microtubules. No deposits were present in the cilia of the specimens fixed in a glutaraldehyde solution without $CaCl_2$. The deposits at the basal part of the cilia almost completely disappeared, if the sections were treated with 5 m*M* EGTA. Detailed description of the deposits will be given elsewhere[9].

Electron probe microanalysis of the electron-dense deposite clearly demonstrated the presence of Ca and P as shown in Figure 2. When the probe was placed on the basal region of the cilium with the deposits, the energy spectrum showed distinct peaks at energy levels corresponding to Ca Kα (3.69 keV), Ca Kβ (4.01 keV) Cl $K\alpha_1$ (2.62 keV), Si Kα (1.74 keV) lines; the peak near (2.0 keV appeared to result from summation of P $K\alpha_1$ 2.01 keV) and Os Mα (1.91 keV) lines (Figure 2A). If, on the other hand, the distal region of the cilium without the deposits was probed, the energy spectrum showed Cl $K\alpha_1$, Os Mα and Si Kα lines, but not Ca Kα, Ca Kβ, and P $K\alpha_1$ lines (Figure 2B). In Figure 2B, the energy spectrum of the cilium with the deposits is also shown by dots for comparison, clearly showing that Ca and P are present only in the deposits. The Si Kα and Cl $K\alpha_1$ lines observed in all the sections may be ascribed to Epon embedding the specimen.

The presence of Ca and P in the electron-dense deposits suggests that the deposits contain calcium phosphate. In this connection, it is of interest that the deposits in the trichocysts and the ciliary granular plaques of *Paramecium* also contain Ca, P and S[11], though the presence of S was not detected in the present study. The electron-opaque deposits along the plasma membrane of squid giant axon are also shown to contain Ca and P[12]. Although the present results have clearly shown the existence of Ca-binding sites in the cilia of *Paramecium*, their role in the control of ciliary motion is not clear. The localization of the deposits just above the 'ciliary necklace' linking the ciliary membrane and the outer doublet microtubules[13] suggests the possibility that Ca ions may be released from the intracellular binding sites during excitation of the ciliary membrane to influence the ciliary movement.

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