

reported elsewhere<sup>3</sup>. The presence of the 12 *C. papposus* among the 36 *A. rubens* and food dramatically reduced the feeding activity in *A. rubens*. Individuals of *A. rubens* were quicker to begin feeding but they were soon displaced by contact with *C. papposus* when it began to feed on the *C. fornicata*. Once the sunstars became established on the food, all *A. rubens* attempting to feed were prevented from doing so by contact with the *C. papposus*. *C. papposus* effectively exploited the avoidance behaviour of *A. rubens* to gain access and reserve the same food resource for itself. Actual attacks on *A. rubens* by *C. papposus* were very rare during the experiments.

The extensive starfish dredging surveys of the Crouch have revealed that the 2 species have a discrete distribution<sup>6,21,22</sup>. This has been related to different recruitment strategies of the species and heavy predation by *C. papposus* on newly settled *A. rubens*<sup>6,22</sup>. Interspecific avoidance among adults should now be considered an important factor also. The gut contents of adult *C. papposus* in the Crouch overlapped considerably with that of *A. rubens*, especially on molluscan prey, and *C. papposus* showed little evidence of starfish predation<sup>20,21</sup>. Indeed, recent work by Hancock<sup>5</sup> has suggested that *C. papposus* will very readily take molluscs and that it is not such a specialized predator on *A. rubens* as previously thought<sup>6,22</sup>.

From the experiments reported here and accumulated field evidence, it is suggested that *C. papposus* in the Crouch is not so much an active predator of *A. rubens* but could be an active competitor with *A. rubens*. *C. papposus* exploits its fright substance in excluding *A. rubens* from a common food source. Also, *C. papposus* disperses its own population to perhaps decrease intraspecific competition for food and exclude *A. rubens* from the immediate area. In a detailed field study<sup>23</sup> the importance of aggression in the competitive co-existence of 2 starfish species has been clearly shown. The dominant competitor decreased the feeding

rate and affected the distribution of the other species after aggressive encounters. Non-predatory encounters between starfish<sup>11,23</sup> require further study as their effects may alter starfish predatory activities and thus may be important to the ecology of some benthic communities. These encounters reveal an interesting behavioural complexity of these animals.

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#### Starfish contact durations

Contact type	n	Mean (sec)	Range (sec)
<i>Asterias-Asterias</i>	100	39.8	3-291
<i>Crossaster-Crossaster</i>	100	9.0	1-27
<i>Crossaster-Asterias</i>	100	6.1	1-22

The ranges do not include the single longest contact in all cases (n=99). The temperature during tests varied between 11 and 13 °C.

## Studies on the cytochemical localization of adenylate-cyclase activity in *Dugesia lugubris* s.l.<sup>1</sup>

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**Summary.** The localization of adenylate-cyclase activity in *Dugesia lugubris* s.l. has been investigated cytochemically using 5'-adenylyl-imidodiphosphate as substrate. The enzyme was localized in mucous gland cells, in rhabdite cells, in intercellular spaces and also in nerve endings of this planarian. The presence of adenylate-cyclase on the membrane suggests that it might mediate different stimulus-secretion coupling by increasing cyclic AMP synthesis in specialized areas of the planarian.

Adenylate-cyclase has been found in a very wide variety of organisms, ranging from bacteria to mammals. It appears to occur throughout the animal kingdom and in most cases a major regulatory role has been established for the enzyme<sup>4</sup>. A number of systems have been studied in higher animals, in which the enzyme mediates the action of many hormone messengers<sup>5</sup>. The adenylate-cyclase of neuronal tissue has

been shown to be sensitive to a number of putative neurotransmitters, including norepinephrine, dopamine, serotonin, histamine and octopamine<sup>6</sup>. A number of similarities have been found between the physiological receptor for a particular neurotransmitter and the regulatory subunit of the adenylate-cyclase.

Such similarities constitute important evidence that a par-

ticular neurotransmitter-sensitive adenylate-cyclase may mediate the physiological action of the neurotransmitter<sup>7,8</sup>. The presence of neurotransmitters such as dopamine, nor-epinephrine and serotonin has been demonstrated histochemically in planarians<sup>9</sup>. The presence of cyclic AMP and an adenylate-cyclase system has been demonstrated for the first time in *Polycelis tenuis* by Franquinet et al.<sup>10</sup>. Franquinet et al.<sup>11</sup> have also shown that adenylate-cyclase of *P. tenuis* is activated by serotonin and guanine nucleotides. Recent studies have shown that adenylate-cyclase activity can be demonstrated cytochemically at the electron microscope level in the liver<sup>12</sup>, and in the islets of Langerhans of the pancreas<sup>13</sup>. Furthermore, it has been shown that the specificity of the cytochemical method can be increased by using an artificial substrate, adenylyl-imidodiphosphate (AMP-PNP), instead of ATP<sup>13</sup>. AMP-PNP, an analogue of ATP, in which nitrogen is substituted for oxygen between the terminal phosphates, serves effectively as a substrate for adenylate-cyclase, but not for ATPase<sup>14</sup>. The aim of this investigation is to localize the adenylate-cyclase in a fresh water planarian, *Dugesia lugubris*, because it could be useful for further work to determine its precise regulatory role in the physiology of the organism.

**Materials and methods.** Chemicals: 5'-adenylyl-imidodiphosphate has been purchased from ICN Ltd, Irvine, California. All other chemicals were of analytical grade.

The animals were starved for 7 days and then sacrificed, and strips of 1 mm thickness were taken off at level of the auricles. These strips were cut in smaller pieces which were pre-fixed for 45 min at room temperature in 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, and washed in cacodylate buffer, 0.05 M with 8% glucose, then left 12 h at 4°C. These pieces were then incubated basically according to Howell and Whitfield<sup>13</sup> in 80 mM tris-maleate, pH 7.4 containing 8% glucose, 2 mM theophylline, 2 mM magnesium sulfate, 0.5 mM 5'-adenylyl-imidodiphosphate and 4 mM lead nitrate. To this basic medium was added 10 mM sodium fluoride. Incubations were performed at 30°C for 45 min, after which time the pieces were washed briefly with a similar tris-maleate-glucose buffer before postfixation in 1% osmium tetroxide in cacodylate-nitrate-glucose buffer, pH 7.4. The pieces were then dehydrated in ethanol and embedded in epon-araldit. Thin sections, after a brief staining in a saturated solution of uranyl-acetate in 50% ethanol, were examined with a Philips EM 300 electron microscope at 60 kV.

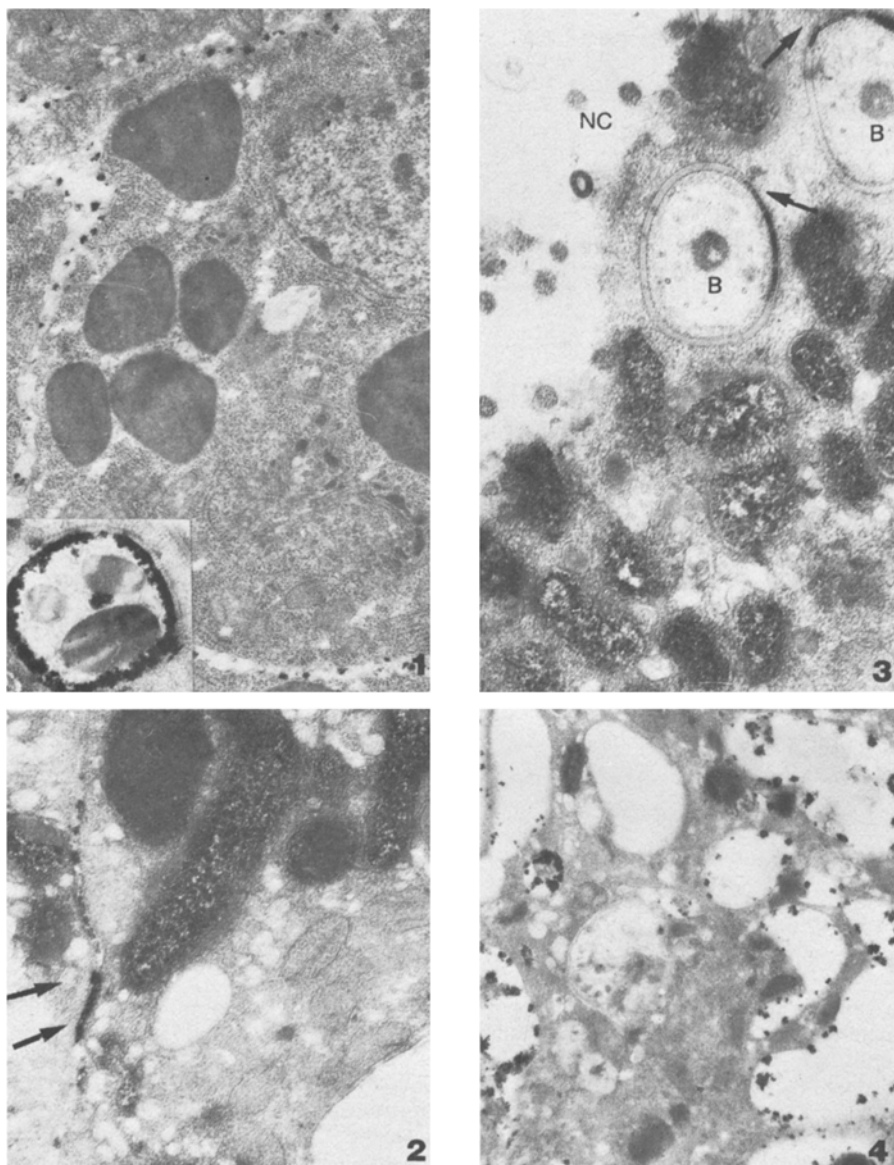


Fig. 1. *Dugesia lugubris*. Mucous gland cell body with secretory granules. Adenylate-cyclase activity on the cell membrane, stained with uranyl-acetate  $\times 21,000$ . Inset is a mucous cell process. High adenylate-cyclase activity along the membrane. Stained with uranyl-acetate.  $\times 39,000$ .

Fig. 2. *D. lugubris*. Presence of adenylate-cyclase activity on the lateral membrane of 2 contiguous rhabdite cells. The highest activity is present along the septate junctions (arrows). Stained with uranyl-acetate.  $\times 39,000$ .

Fig. 3. *D. lugubris*. Adenylate-cyclase activity (arrows) along a side of the membrane of nerve endings. B, basal body; NC, neural cilia. Stained with uranyl-acetate.  $\times 43,000$ .

Fig. 4. *D. lugubris*. Presence of adenylate-cyclase activity around the intercellular spaces. Stained with uranyl-acetate.  $\times 20,000$ .

**Results and discussion.** The adenylate-cyclase has been localized in mucous gland cells (figure 1), in rhabdite cells (figure 2), in nerve endings (figure 3) and in intercellular spaces (figure 4) of the planarian *Dugesia lugubris*. Figure 1 shows that the membranes of mucous gland cell bodies and of the terminal tract of their glandular processes are positive to the enzymatic reaction. It is possible to assume that the adenylate-cyclase controls the early and final stages of the mucous secretion phenomenon.

The enzymic activity is also present on the lateral membranes of contiguous rhabdite cells. Its highest activity has been found along the septate junctions, suggesting that the adenylate-cyclase is important for the ionic exchange between the rhabdite cells (figure 2).

Adenylate-cyclase activity is present on the membranes of nerve endings, connected to the brain by nerve fibres, which give rise to neural cilia (figure 3). This could be interesting, since Pigon et al.<sup>15</sup> have localized in these clumps of neural cilia, which are concentrated in the

cephalic margin, the control of the fissioning process in planarians. This enzyme activity is also present around the intercellular spaces (figure 4). Since the large intercellular clefts in planarians, in which a vascular system is absent, have been suggested to have a function in ion and water transport<sup>16</sup>, the adenylate-cyclase could mediate the absorption of these compounds from the cells.

Therefore, considering the presence of neurotransmitters in planarians, and that Franquinet et al.<sup>11</sup> have recently shown that adenylate-cyclase in *Polycelis tenuis* is activated in vitro by serotonin, such enzymic cytochemical localizations give a further proof for the presence of a particular neurotransmitter-sensitive adenylate-cyclase and might provide a basis for further work to determine particular physiological roles of adenylate-cyclase in this organism.

Moreover, further work on adenylate-cyclase from planarians might be useful for understanding the central mechanism of action of this enzyme.

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## Social behaviour versus temperature in the ciliate *Colpidium campylum*

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**Summary.** When *Colpidium campylum* are put in an observation chamber, they form groups of hundreds or thousands of individuals. In a temperature gradient, these groups find their preferred temperature range within a few hours, whereas isolated individuals can achieve this in min. This difference is caused by the social tendency of these ciliates.

When subjected to a temperature gradient, ciliates of the species *Colpidium campylum* Bresslau 1922 search for a range of preferred temperature as other free living animals do. As these ciliates normally live in groups, we investigated the locomotion of groups in an observation vessel (50 × 8 × 0.3 cm) as well as that of isolated individuals in a glass capillary (l = 50 cm, Ø = 1 mm). The ciliates observed in the capillary were exposed to a linear temperature gradient from 0 to 45 °C, whereas the group-behaviour was observed in 2 temperature gradients (6–26 °C or 23–40 °C). Before setting the temperature gradient, the pattern of aggregation was determined at a homogeneous temperature of 24 °C.

When single individuals of *C. campylum* were placed in the capillary with the temperature gradient, they swam in a helical course from one end of the capillary to the other and back. At the warm end of the gradient, the turning point could be determined exactly at 42 °C, because of the phobic reaction. At the cold end, however, the speed of the

ciliates decreased until they reached the ice and turned back. After they had been placed in the capillary, they swam from warm to cold through the preferred temperature range (24–28 °C) only a few times. Soon the turning points approached the 2 limits of the thermopreferendum, and after 3–5 min the individuals remained within the preferred temperature range.

The behaviour of *C. campylum* in a temperature gradient is strongly influenced by its social tendency<sup>2,3</sup>. This fact could be demonstrated by the following experiment: A large number of individuals (10<sup>4</sup>–10<sup>5</sup>) was dispersed equally in the observation vessel at a constant temperature of 24 °C. Within 2 h the ciliates formed groups with a macroscopic diameter of 1–6 mm at the bottom of the vessel. Contrary to the related ciliate, *Colpidium colpoda*, *C. campylum* aggregated in several layers, one upon the other<sup>4</sup>.

At the initial homogeneous temperature, the distribution of the groups on the observation plane was equal (dotted columns of the diagram). When a linear temperature