should require opsin incorporation into the rhabdomere. Secondly, the labeling density on the plasma membrane is significantly higher in the newly emerged than in 10 d old flies (Table 1). Thirdly, the plasma membrane is lined with the uniformly oriented actin filaments with the plus ends towards the rhabdomere (Fig. 6c, d) and anti-NINAC labeling [13]. As suggested by Adams and Pollard [1] in Acanthamoeba myosin I, the myosin-like NINAC could move the membrane-embedded opsin towards the plus end of the actin filaments, i.e., towards the rhabdomere.

In fact, actin is probably involved in the rhabdomere morphogenesis. In a crab, Hemigrapsus sanguineus, the rhabdom volume increases at dusk and decreases around dawn [3]. The volume increase was inhibited by treating the isolated eye at dusk with cytochalasin D, which disrupts actin filaments. As the cytochalasin D treatment had no effect at night on the enlarged rhabdom, the inhibition of the volume increase should be attributed not to the disintegration of once established rhabdom but to the disruption of some process(es) in the rhabdom morphogenesis [16]. The most plausible process mediated by the presumptive actin-myosin interaction is the transport of opsin. When applied also at dusk, colchicine, a microtubule inhibitor, failed to stop the volume increase, suggesting that the actin was more directly involved in the rhabdom morphogenesis than the microtubules [16]. Further analyses using other inhibitory drugs are in progress.

The actin-NINAC interaction also explains another route of opsin transport through the SRC, which is an opsin-containing network that wraps the entire base of the rhabdomere [14, 24]. The SRC is connected to the rER with membrane tubules forming an extensive endomembrane system. The opsins in the SRC are probably originated from the rER, the site of opsin synthesis, through the membrane tubules, although no opsin had been so far localized in the tubules. Here we detected antiopsin labeling in the region between the rhabdomere and the cell body. The labeling appeared to be associated with the vesicles or pieces of membranes (Fig. f, g). These endomembranes are most likely parts of the tubules, for the tubules appear in transverse sections as elongated or swollen vesicles [14], or even as fragmented membranes if one side of the tubule was tangentially sectioned. The antiopsin labeling on the rER probably represents new opsins that will be transferred into the tubules (Fig. 6b, f).

The region between the rhabdomere and the cell body is furnished also with the NINAC [13]. By the presumptive interaction between actin and NINAC, the opsins embedded in the endomembranes can be transported towards the rhabdomere.

ACKNOWLEDGMENTS

We thank Dr. T. Tanimura for providing the monoclonal anti-Drosophila Rhl opsin antibody. Drs. T. Tanimura, S. Stowe, and E. Eguchi provided helpful comments in the initial stages of the work. We also thank two anonymous referees for many valuable suggestions on the manuscript. The work was supported by the Grants to K. Arikawa from Whitewall Foundation (Florida, USA), Kihara Foundation for Life Sciences (Yokohama), and the Ministry of Education, Science, and Culture of Japan.

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Experimental Perturbations of the *Litonotus-Euplotes* Predator-Prey System

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ABSTRACT—A model previously proposed to demonstrate the interactions between *Litonotus* (predator) and *Euplotes* (prey), led to a new round of experiments. The different experimental approaches used to solve these questions (starved cells; killed cells; enzymes; lectins; ions; inhibitors) resulted in quite a new model of the cell interactions which accounts for the different steps of the phenomenon: the main point demonstrated by these experiments is that the cellular cortex of both predator and prey is involved in many of the successive steps of the cascade reactions enabling *Litonotus* to prey upon *Euplotes*

INTRODUCTION

Efforts spent in attempting to deepen our understanding of predation among protozoa are completely justified by the basic importance of the process. Protozoa, indeed, were not only the first primary consumers in the primeval Oceans, but the first predators as well [12]. Such a new trophic niche is quite an important one, due to the two consequences it leads to: (a) it creates new empty spaces for new organisms to settle in, (b) it triggers a sort of evolutionary competition between preys and predators (to escape and to strike each other, respectively) as to their morpho-functional acquisitions. Many examples have been already studied and the knowledge of the *Didinium-Paramecium* [1], *Dileptus-Colpidium* [34], *Enchelys-Tetrahymena* [5], *Chaenea-Uronema* [5]; *Homoalo-zoon-Paramecium* [4] predator-prey systems, cannot but help us to complete our overall picture of this phenomenon considered from a more specific siceological point of view: in this perspective, indeed, the study of predator-prey interactions also lends itself to be used in an attempt to penetrate the adaptive strategies, conditioning the reciprocal (co)evolution of predators and preys. Let us recall the example of the bat-moth relationships, as a truly paradigmatic one, to clarify our idea [28].

In this context we studied another predatory model, namely that of *Litonotus lamella-Euplotes crassus*, focusing our attention successively on (a) the ultrastructure of the toxicysts of the predators [11], (b) the ultrastructural analysis of the consequences to *Euplotes* of toxicyst discharge by *Litonotus* [32]; (c) the peculiar digestion process [33] and, finally, (d) the behavioural patterns following each other along a path characterised by the succession of several basic steps, namely casual encounter (CE); toxicyst discharge (TD); research (R); engulfment of the prey (PE)[8]. The predator-prey interaction model proposed in the previous paper focused our attention on several closely related problems, which represented the targets of the next round of experiments: (a) how is the toxicyst-discharging system triggered and controlled? (b) which are the spatio-temporal sequences of a toxicyst-discharge phenomenon?

The unique nature of our pet-organism protozoa (i.e. indeed perfect eukaryotic cells and complete organisms, at the same time) offers a double advantage: (a) it enables us to use all those techniques typical of experimental studies on cell interactions to investigate also the relationships between entire organisms and (b) it allows us to transfer any results obtained for these truly sophisticate organisms to the general field of cell biology.

MATERIALS AND METHODS

Both *L. lamella* and *E. crassus* were grown, collected and used as already described by Ricci and Verni [24]. The observations were made with a Wild M5 (20–60X) stereomicroscope, and a Leitz Orthoplan (400X) microscope (together with its Nomarsky interferential contrast), coupled to a Panasonic TVC camera and a VHS videorecorder. Unless otherwise indicated, the prey organism was *E. crassus*. The following specific procedures were followed for the different kinds of experiments:

*Exp. 1* The effects of starvation on preys and on predators were studied using normal and starved *Litonotus*, exposed to both normal and starved *Euplotes*. Normal populations of predators were used 4 days after the last feeding, while the starved ones were tested after 11 days. Normal *Euplotes* were not fed for 24 hr, while the starved ones were used 7 days after the last feeding.

*Exp. 2* To study the role played by the body itself, of both the predator and the prey, in the specific toxicyst discharge (TD) processes at the very moment when the two organisms come into direct contact with each other and the TD itself is triggered and actually occurs, both *Litonotus* and *E. crassus* were frozen, and then thawed at room temperature (the experimental populations were immersed in liquid nitrogen for 2 min): in this way the structurally and chemically preserved, but physically inert bodies of both *Litonotus* and *Euplotes* were tested with living prey or predator respectively. In some of these experiments homogenized *Euplotes* were also used.

*Exp. 3* Whenever a predator contacts a prey TD occurs: does TD affect the microenvironment where it occurred? How far for TD area is such an effect perceived? How long does it persist? How is the behaviour of *Euplotes* affected? To solve these problems, many TD
events were videorecorded and the videotapes scored frame by frame according to the standard technique for behavioural studies reported elsewhere by Ricci [20]. In this way we quantified: a) the subcircular area where the TD effects are perceived by Euplotes; b) their duration; c) the changes in the behaviour of the prey.

Expt. 4 To assess the role possibly played by calcium concentration in the sea water, 3 different standard set ups (cf. Expt. 5) were prepared: the first contained standard marine water (control), the second 15 mM calcium chloride, the third the same concentration of CaCl₂ plus 0.1 mM EDTA, to inhibit the effects of the calcium. Previous experiments, carried out with 5, 10, 15, 20, and 25 mM Ca²⁺ and with 0.01, 0.1 and 10 mM EDTA had shown that the best results were obtained with 15 mM Ca²⁺ and with 0.1 mM EDTA. In other words these two concentrations were the lowest capable of inducing clearcut results. Five experiments were then carried out and videorecorded, to measure: (a) the time lag between the introduction of Litonotus and the first instance of TD (this period of time will be referred to as TDₜ from this paper, it somehow measures the efficiency of Litonotus in intercepting the prey); (b) the time lag between the introduction of Litonotus and the actual engulfment of the killed prey (this period of time will be referred to as Iₜ in this paper, it somehow measures the efficiency of Litonotus feeding on the prey); (c) the length of the backward motion of Litonotus following TD; (d) the number of TD per Litonotus.

Expt. 5 The effects of trypsin (Sigma, TR253; concentration 2.5, 2, 1.5, 1 and 0.5%) were also studied. About 50 Litonotus were incubated in the different concentration for various time periods (15, 30, 60, 90, 120, 180, 210 and 240 min); they were washed 3 times and then used in a 50 µl droplet with concentrated Euplotes, to study the TD, Iₜ and the percentage of inhibited (namely not-toxicist discharging) Litonotus. When 100% TD inhibition was induced, the Litonotus still incubated by trypsin were washed free of the enzyme fresh water and then used in Euplotes populations to monitor their recovery period.

Expt. 6A The effects of concanavalin-A (Con-A, Sigma C2010; concentration 2, 1.5, 1, 0.5 and 0.25%; treatment time 15, 30, 60, 90, 120, 150, 180 and 210 min) on Litonotus were studied by washing the treated cells after different periods of time and measuring TD, Iₜ and the percentage of inhibited cells, when these experimental Litonotus were transferred into a 50 µl droplet of concentrated preys. The same treatments were also carried out on incubating Litonotus in the same dosages of Con-A and for the same times as before, in the presence of 40 mM of α-methyl-D-mannoside, well-known as a specific competitor of Con-A. The same parameters were measured.

Expt. 6B Concentrations of 2 and 1% of Con-A were also used to incubate Euplotes for 15, 30, 60, 90, 120, 150, 180, 210, 270, 330, and 390 min; these populations were washed three times and then exposed to Litonotus, to measure the TD, Iₜ, the percentage of non-discharging Litonotus.

Expt. 7 In a final experimental approach, Litonotus was treated with different concentrations (0.5, 0.25, 0.125, 0.06, 0.03 and 0.015%) of cycloheximide (Chx)(Sigma C-6255) for different times (1 to 9 hr); they were used singly with populations of Euplotes to measure TD, Iₜ and the percentage of TD inhibition. When 100% TD inhibition was obtained the still incubated cells were washed free of Chx and then used with Euplotes to study the kinetics of their recovery in terms of the percentage of TD inhibited cells.

RESULTS

Expt. 1 The effects of different starvation of Litonotus and Euplotes.

Previous microscope observations (Verni, unpublished results) had shown that the longer the starvation, the more caudal the distribution of toxicysts: the effects were statistically significant. On the basis of these findings, the consequences of starvation were studied more specifically.

The results obtained in this round of experiments demonstrated that (a) severe starvation affects the efficiency of predator's TD: Table 1, the III and the IV columns vs the I and the II; (b) the starvation of the prey affects, to a limited extent, the ingestion capability of Litonotus (Table 1, Iₜ, the II vs the I column and the IV column vs the III), while it does not affect the corresponding TD, Iₜ.

Expt. 2 The predatory interactions between Litonotus and frozen-thawed Euplotes.

Litonotus cannot be frozen and then thawed, without being disrupted: no result could be obtained, except that the area where a disrupted Litonotus lies is avoided by the preys. Normal Litonotus exposed to a population of frozen-thawed Euplotes demonstrated that: (a) Litonotus can ingest them without discharging any toxicysts (Fig. 1B); (b) Iₜ is longer than twice as much as that of the control (Fig. 1: B vs A); (c) when freshly prepared homogenate of Euplotes is added to the system, Iₜ is strikingly reduced (Fig. 1C).

Expt. 3 The TD-affected area.

![Fig. 1. The TDₜ (white bars) and the Iₜ (shadowed bars) of Litonotus (L) in presence of [A] normal Euplotes (E), [B] frozen-thawed Euplotes (fE) and [C] fE and homogenated Euplotes (hE).](image-url)
The results we obtained are the following: (a) the effects of a TD event extend around the TD point over a sub-circular area of about 300μm in diameter, (b) the same effects increase up to their maximum for 90 sec after the TD event and last for about 3 min; (c) the behaviour of the prey is clearly affected by the TD event as demonstrated by the high frequency of avoidances induced in Euplotes by TD immediately afterwards (Fig. 2, shaded areas); the avoidances correspond to the behavioural pattern called Side Stepping Reaction [19] and indicated as SSR; (d) these SSR occur according to a temporal pattern parallel to that of Euplotes avoiding the area where a frozen-thawed disrupted Litonotus is placed (Fig. 2, white areas).

The experiments reported here led to a widening and deepening of the general knowledge of the predator-prey relationships between Litonotus and Euplotes [24]. First of all, it was found that starvation acts on both elements of the system, although differentially: (a) in Litonotus it affects both the cortical areas where TD occurs (the more severe starvation, the more caudal the TD) and TD At, a parameter somehow measuring the “hunting efficiency” of Litonotus, (the longer the starvation, the more inefficient it becomes); (b) starved Euplotes are ingested in longer times.

**Table 1.** The effects of starvation, on the predation of Litonotus lamella on Euplotes crassus, measured by the durations of the TD At and of the Ld (described in Materials and Methods section).

<table>
<thead>
<tr>
<th></th>
<th>+ normal Euplotes</th>
<th>+ starved Euplotes</th>
<th>+ normal Euplotes</th>
<th>+ starved Euplotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD At</td>
<td>x 1'25&quot;</td>
<td>1'20&quot;</td>
<td>2'21&quot;</td>
<td>2'15&quot;</td>
</tr>
<tr>
<td></td>
<td>s 55&quot;</td>
<td>50&quot;</td>
<td>1'12</td>
<td>1'18&quot;</td>
</tr>
<tr>
<td></td>
<td>n 35</td>
<td>26</td>
<td>29</td>
<td>32</td>
</tr>
<tr>
<td>Ld</td>
<td>x 2'30&quot;</td>
<td>3'</td>
<td>3'42&quot;</td>
<td>4'9&quot;</td>
</tr>
<tr>
<td></td>
<td>s 1'</td>
<td>1'5&quot;</td>
<td>1'15&quot;</td>
<td>1'18&quot;</td>
</tr>
<tr>
<td></td>
<td>n 35</td>
<td>26</td>
<td>29</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table 2.** The effects of Ca\(^++\) (15 mM) on the 4 parameters indicated on the left; BM = the length of the backward motion of a Litonotus lamella after discharging its toxicysts: it is measured in Relative Units (RU): 1 RU = 1 species-specific length of Litonotus lamella ≈ 250 μm. The number of observations for each parameter was of 50.

<table>
<thead>
<tr>
<th>parameters measured</th>
<th>Control</th>
<th>15 mM Ca(^++)</th>
<th>15 mM Ca(^++) &amp; 0.1 mM EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD At (sec)</td>
<td>43 ± 17</td>
<td>41.5 ± 18</td>
<td>42 ± 26</td>
</tr>
<tr>
<td>BM (RU)</td>
<td>1.1 ± 0.7</td>
<td>2.5 ± 0.7</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>Ld (sec)</td>
<td>132 ± 30</td>
<td>186 ± 80</td>
<td>139 ± 33</td>
</tr>
<tr>
<td>(n^2) of TD per Litonotus</td>
<td>1.2 ± 0.4</td>
<td>2.4 ± 0.5</td>
<td>1.2 ± 0.5</td>
</tr>
</tbody>
</table>

**Expt. 4** The role of Ca\(^++\) in the environment.

The results given in Table 2 clearly show that calcium ions affect the general physiology of the Litonotus-Euplotes system at least at three different levels: (a) TD At is not affected; (b) the length of the backward motion is doubled; (c) Ld increases by about 50%; (d) the number of TD per Litonotus is doubled. EDTA inhibits calcium effects, as expected. As considered in the Materials and Methods section, the differential effects of calcium ions on TD At (no effect) and on Ld (strong inhibition), actually demonstrate the importance of both parameters in interpreting correctly the effects of calcium ions on the Litonotus-Euplotes system.

**Expt. 5** The effect of trypsin on Litonotus.

The enzyme was already known to affect the physiology of Litonotus; progressively higher concentrations and longer treatments, indeed, induce (a) a progressive darkening of the cytoplasm, (b) a rounding of the body shape, (c) reduced locomotion, (d) immobilization and (e) lysis of the cell (Ricci and Verni, unpublished results). It was found (Fig. 3) that the different concentrations used for the different times indicated in Material and Methods, clearly affect TD At (the higher the concentration, the longer TD At) but not Ld (the difference between TD At and Ld is indeed rather constant). For a certain concentration a longer treatment affects the percentage of cells not discharging their toxicysts (Fig. 4), while TD At tends to be more or less constant, the different values depending solely upon trypsin concentration. Once
100% inhibition is induced (namely, when no Litonotus can discharge its toxicysts) they still behave quite normally and their physiology seems to be unaffected: after washing, these completely inhibited populations recover 80% of their TD activity within 30–60 min, and 100% of their potentialities within 60–120 min.

Similar treatments with trypsin were also conducted on Euplotes; the enzyme either has no effect at all on both TD Δt or Idt, and it kills the preys.

Expt. 6A The effects of Con-A on Litonotus.

The results of the experiments are the following: (a) the higher the Con-A concentration, the stronger the effect, on TD Δt, but not on Idt (Table 3) (b) for the same concentration, the longer the treatment, the stronger the effect on TD Δt but not on Idt (Table 3) (c) as the Con-A concentration
when Litonotus treated with Con-A are washed free from the lectin, the inhibitory effects are removed after a period whose length is roughly proportional to the Con-A concentration: while, for instance, 1% Con-A inhibits completely the TD after 90 min and the recovery period, after washing, is about 4 hr, 0.5% Con-A inhibits TD completely after 3.5 hr, but the populations start discharging their toxicysts again only 40 min after washing; (f) the effects of Con-A are specific, being completely inhibited by α-methyl-D-mannoside, the specific competitor of Con-A.

**Expt. 6B** The effects of Con-A on Euplotes.

The results showed that this lectin affects the prey in the same way as it proved to do with the predator: (a) Con-A treated Euplotes induce a reduction of TD Δt and the percentage of TD predators, according to a clearcut dose-dependence; (b) the longer the treatment period of Euplotes with a certain concentration of Con-A, the longer the TD Δt of Litonotus and the higher the percentage of non TD predators; (c) when Con-A treated Euplotes do not induce any TD at all, they are washed: the recovery periods required for Litonotus to discharge its toxicysts again are proportional to the Con-A concentration used on Euplotes; (d) the effects of Con-A on Euplotes are specific, being completely inhibited by α-methyl-D-mannoside.

**Expt. 7** The effects of cycloheximide on Litonotus.

When Litonotus is treated with cycloheximide TD is severely affected: (a) the higher the Chx concentrations, the longer TD Δt (Table 4) and the larger the amount of non TD Litonotus (Fig. 6). Also the interference effects of Chx are easily reversed upon washing and the recovery times are proportional to the concentrations used for a certain treatment.

**DISCUSSION**

The experiments reported here led to a widening and deepening of the general knowledge of the predator-prey relationships between Litonotus and Euplotes [24].

First of all, it was found that starvation acts on both elements of the system, although differentially: (a) in Litonotus it affects both the cortical areas where TD occurs (the more severe starvation, the more caudal the TD) and TD Δt, a parameter somehow measuring the "hunting efficiency" of Litonotus, (the longer the starvation, the more inefficient it becomes); (b) starved Euplotes are ingested in longer times.

---

**Table 4.** The inhibitory effects of Chx on TD of Litonotus: the TD Δt (min) on organisms treated by different concentrations (% shown in the left column) for different hours (indicated in the first line)

<table>
<thead>
<tr>
<th>%</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>1'</td>
<td>1''</td>
<td>3'</td>
<td>3''</td>
<td>5'</td>
<td>5''</td>
<td>6'</td>
<td>8''</td>
<td>&gt;30'</td>
</tr>
<tr>
<td>0.03</td>
<td>1'36''</td>
<td>1'54''</td>
<td>4'00''</td>
<td>6'00''</td>
<td>6'00''</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
</tr>
<tr>
<td>0.06</td>
<td>2'</td>
<td>1'54''</td>
<td>5'00''</td>
<td>6'00''</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
</tr>
<tr>
<td>0.125</td>
<td>3'50''</td>
<td>3'24''</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
</tr>
<tr>
<td>0.25</td>
<td>4'24''</td>
<td>3'48''</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
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<tr>
<td>0.5</td>
<td>&gt;30'</td>
<td>&gt;30'</td>
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<td>&gt;30'</td>
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In general it seems possible to conclude that a basic physiological "health" of both predator and prey represents a sort of prerequisite for predation to occur efficiently.

Freezing-thawing experiments provided several clues to a deeper understanding of our predation model. Frozen-thawed *Euplotes* (a) maintain their shape, (b) cannot induce TD in *Litonotus*, and (c) still suitable prey for *Litonotus* to eat. These results seem to suggest that some "activation energy" (related to the kinetic energy of a normally swimming *Euplotes*) might be required for TD. Similar results on the other hand, have also been found by Ricci et al. [26] regarding the mechanisms which trigger specific cell differentiation (giant cell) in *Oxytricha bifaria*. Experiments carried out with paralyzed mutants of *Euplotes* [23] are expected to give more precise answers to the question: the fairly small amount of energy involved in this process, however, should not to be considered exceptional since the world of ciliates has already proved to be ruled by unexpectedly small forces, as demonstrated by Machemer [16] and by Ricci et al. [27]. According to these results, moreover, TD does not represent a step necessarily occurring before ingestion, although TD itself seems to facilitate somehow the actual ingestion, as demonstrated by *ld* of *Litonotus* engulfling frozen-thawed *Euplotes*: the values of these *ld* are, on average, more than twice those of the controls. A further clue to the comprehension of the factors involved in the *Litonotus-Euplotes* interactions is given by the use of the homogenate of *Euplotes*, which strikingly reduces *ld* of *Litonotus* engulfling frozen-thawed *Euplotes*. These evidences seem to suggest that a cytoplasmatic factor (or complex of factors) of *Euplotes* (called e) is capable of "activating" *Litonotus*. The nature of this "activation" is at present being studied through a behavioural approach, namely investigation of the specific locomotory pattern of *Litonotus* exposed to the homogenate of *Euplotes*, and related to the normal searching activity of the predator already reported by Ricci and Verni [24].

The findings of experiment 3 show that a "toxic" (in its broadest sense) area persists, where a *Euplotes* has been killed by a *Litonotus*: its spatial extension and temporal duration recalls quite closely that of the area where a frozen-thawed *Litonotus* is placed. This finding strongly suggests that *Litonotus* releases a factor (or complex of factors), whenever it "breaks down" or discharges its toxicysts. According to our present understanding, a *Litonotus* must depolarize its membrane to discharge its toxicysts and this, in turn, cannot but lead it to creep backwards for a while. To "buffer", somehow, this unavoidable physiological handicap, *Litonotus* may be supposed to release a substance (called λ) at the TD point, capable of guiding it towards the prey: on the other hand the same λ might represent the "stay away" signal for *Euplotes*, thus producing what we called the "toxic area". Similar "repulsive" effects have been described also for *Colpidium* avoiding killed *Dileptus* [6]. The use of 15 mM Ca^{2+} produced clearcut results, in terms of a larger number of TD per *Litonotus* and longer backward locomotion following the TD: these observations strengthened our hypothesis of electrically controlled TD for *Litonotus*, according to both what is already known for *Dileptus* [6] and to the more specific report of Hara et al. [13] for *Didinium*.

The dramatic effects of proteolytic enzymes on a wide range of biological phenomena of ciliates are already known: binary fission [36], conjugation [15, 22, 35] and feeding processes [6, 7, 30, 37]. The data reported here show that while no effect can be detected when trypsin acts on *Euplotes*, (Expt.5) the same enzyme affects (a) the physiology of *Litonotus* (cytoplasm, body shape, and locomotion) in much the same way as in *Dileptus* [6], (b) the average TD dt and (c) the recovery periods of washed populations, with a generally clearcut dose dependence. An exception is represented by the difference between *ldt* and TD dt, which is fairly constant at the different trypsin concentrations: this observation indicates that TD and ingestion differ from each other from a physiological point of view, as also suggested by the results of the experiments carried out with frozen-thawed *Euplotes*. The most surprising effect of trypsin, however, is the finding that induced TD dt depends only on the concentration itself and not on the length of the treatment, which is on the contrary capable of inhibiting (completely and reversibly) the TD of the *Litonotus* population. Such a puzzling result (strong numerical inhibition of *Litonotus* vs constant TD dt at a certain concentration of trypsin, upon longer treatment times) might be accounted for by a working hypothesis, based on some kind of accumulation of proteins, possibly involved in the TD processes (called TDP). Similar accumulations have already been hypothesized by Beisson and Rossignol [2] for the trichocyst discharging of *Paramecium*, by Heckmann and Siegel [14] for the preconjugant cell interactions of *Euplotes crassus*, and by Ricci et al. [25] for the differentiation of carnivorous giants in *Oxytricha bifaria*. According to our hypothesis, the higher the trypsin concentration (i.e. the larger the number of TDP molecules) the larger also the number of digested cortical TDP. If *Litonotus* can substitute them at a certain velocity, recruiting them from a cytoplasmic pool, the effect of trypsin can somehow be counterbalanced, at least for a certain time: the average number of newly-exposed and ready-to-work TDP can be expected to be inversely proportional to the number of trypsin molecules in the environment (and not related to the length of the treatment!). According to this way of thinking the reason why the trypsin concentration is directly proportional to the time required to reach the physiological threshold of TDP per surface unit requested for the TD itself can be easily explained. Only when the entire cytoplasmic pool of presynthesized TDP is exhausted is a *Litonotus* inhibited from discharging its toxicysts. The temporal trend of the progressive inhibition of the entire population, on the contrary, would depend upon the relative differences occurring among different *Litonotus* with regard to the pool of presynthesized TDP and to the TD threshold. This general working hypothesis seems to be supported and strengthened

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