Tyrosine Enteroreceptor of Hydra: Its Function in Eliciting a Behavior Modification

Abstract. *Hydra that still contain partially digested food in their gastrovascular cavities, undergo a modified feeding response when they ingest additional prey. A localized constriction is formed just below the sites of tentacle attachment, preventing the loss of gastrovascular contents. This response involves the interaction of an ectodermal receptor system for glutathione and an endodermal receptor system for tyrosine.*

The reduced form of the tripeptide glutathione is the specific activator of the coordinated feeding response in some species of *Hydra* (*1, 2*). Most experiments demonstrating this function of glutathione have been performed on animals fasted from 1 to 2 days (*3*). Because hydra are relatively sessile, feeding in nature is undoubtedly a fortuitous and discontinuous process, with the capture and ingestion of food occurring whenever swimming prey happen to strike the hydra's outstretched tentacles. Thus, it seems probable that a hydra, while still retaining in its tubular gastrovascular cavity partially degraded food from recently ingested prey, may capture more live prey. We now report a chemically controlled modification of the feeding behavior of such hydra. This modification, which we call "neck formation," involves the interaction of an external receptor-effector system for glutathione and an internal receptor-effector system for tyrosine.

Three species of hydra were used: *H. pirardi* (Brien) and *Chlorohydra viridissima* (Schulze), both grown in "M" solution (*4*), and *H. littoralis* (Hyman), grown in a solution containing $10^{-3}M$ $CaCl_2$ and $10^{-4}M$ $NaHCO_3$, at a *p*H of 7.4 (*3, 5*). Hydra at approximately the same stage of development were taken from cultures increasing exponentially with time and were fasted 1 to 2 days before experimentation. We prepared shrimp extract by homogenizing 1.0 ml of chilled, packed *Artemia salina* nauplii in a glass tissue-grinder containing 2.0 ml of "M" solution and centrifuging the homogenate at 9000*g* for 60 minutes. The clear, middle layer was removed and used in experiments calling for shrimp extract.

At the beginning of each experiment, a fasted hydra was given five *Artemia* nauplii. Within 2 hours after ingestion of the nauplii, the hydra's gut became enlarged to about two or three times its normal volume, and the body wall of the animal appeared distended laterally (*6*).

If during the 3rd and 4th hours after ingestion a single nauplius is captured by such a hydra, there results a localized constriction ranging over an area from just below the sites of tentacle attachment throughout the upper one-third of the body tube. The extent of the constriction varied among each of the three species tested. The most pronounced response was shown by *C. viridissima*, the weakest in *H. littoralis*, whereas *H. pirardi* gave an intermediate response.

In *C. viridissima* the constriction is such that it essentially seals off the gut and its contents from the outside medium during mouth opening. In neck formation, the sequence of events is: (i) a nauplius is captured on the tentacles of the distended hydra; (ii) as the nauplius is brought to the mouth, the neck forms; (iii) next the mouth opens; (iv) the shrimp is ingested, the mouth closes, and the shrimp is transported down into the large lumen at the lower part of the gut; and finally (v) the neck constriction disappears (Fig. 1). The entire process usually takes place within a few minutes.

The agents causing neck formation were revealed through the following experiments. Specimens of all three species of hydra, distended with fluids and food from a previous meal, formed a neck constriction after capturing a single *Artemia* nauplius. Specimens of each species also formed neck constrictions if, instead of being given an *Artemia* nauplius, they were flooded with diluted (1 : 5) shrimp extract. Both distended *H. pirardi* and *H. littoralis* also formed necks when placed in a solution of $10^{-5}M$ reduced glutathione (GSH).

In all of the subsequent experiments we used *H. pirardi* because: (i) their relatively large size facilitates the injection or removal of substances from the hydra's gut; (ii) they form unmistakable constrictions; and (iii) they give a good feeding response to GSH.

We tested each substance for activity in inducing neck formation by injecting it through the mouth opening into the hydra's gut by means of a fine pipette attached to a microliter syringe. The animals were immediately placed in fresh "M" solution containing $10^{-5}M$ GSH.

To determine whether the internal stimulus to neck formation was merely the physical distension of the hydra's gut, animals were distended with the "M" culture solution, air, or mineral oil. In no case did the addition of GSH externally lead to neck formation. If, on the other hand, fasted animals were distended either with the fluid gut contents removed from the coelenteron of a hydra fed 2 hours previously, or a shrimp extract (1 : 5), neck constriction followed the addition of GSH. Hence, perhaps some substance released in the hydra's gut from the ingested prey was one of the causative factors in the neck formation.

To determine the nature of the sub-

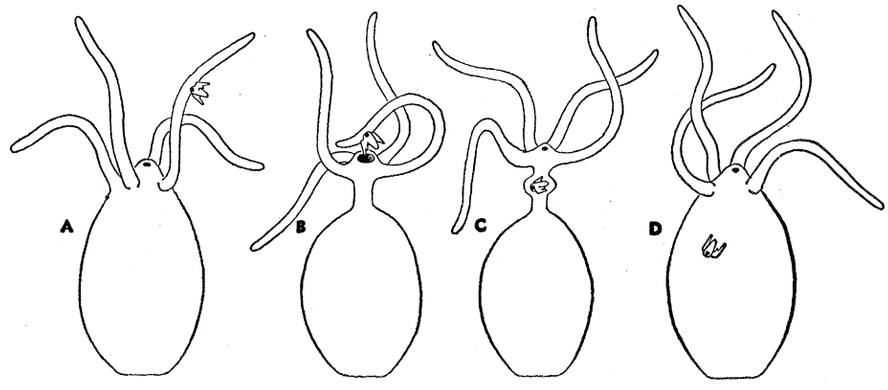

Fig. 1. Diagrammatic representation of "neck formation" in hydra, which occurs during the capture and ingestion of prey approximately 3 hours after the ingestion of a previous meal. (A) Capture of nauplius, (B) formation of neck, (C) ingestion of shrimp, and (D) disappearance of neck.

stance in the shrimp extract, we followed the basic procedures used by Loomis (*1*) and Fulton (*7*) when they determined the compounds that induce feeding in *Hydra littoralis* (*1*) and *Cordylophora lacustris* (*7*). We found that the substance is stable to boiling, is dialyzable, and, when chromatographed on Whatman No. 1 paper in a single dimension in a mixture of butanol, acetic acid, and water (120 : 40 : 50 by volume), has an R_F of about 0.56. Testing for amino acids that might have similar R_F's, we found that neither histidine nor valine were active, nor was GSH ($10^{-4}M$) placed within the gut. The only compound present in shrimp extract that had an R_F near 0.56 and also promoted neck constrictions was the aromatic amino acid tyrosine (*p*-hydroxyphenylalanine), which was active at *p*H 6.4, 7.8, and 10.4.

To determine the degree of specificity of the endodermal receptor for tyrosine, we tested solutions ($10^{-4}M$) of various tyrosine analogs. Good neck formation was elicited by *o*-tyrosine. A solution of *m*-tyrosine, however, seemed to give the most pronounced neck constriction. Also active were dinitrotyrosine, diiodotyrosine, and 3-aminotyrosine. Weak and transient activity was elicited by monoiodotyrosine. These experiments indicate that the electrophilic group on the aromatic ring can be somewhat diverse and randomly distributed. No constriction was induced by phenylalanine, leucyl tyrosine, or tyramine. Tyrosine hydroxamide induced an occasional transient response in a few animals. Epinephrin also produced weak and transient responses.

We conclude that along the hydra's gut there are enteroreceptors specific for tyrosine. The hydroxyl, the α-amino, and the α-carboxyl groups are all necessary for the amino acid to be active. Hence, since at least three groups are required for activity, the tyrosine molecule has the three points for attachment, often associated with a specific binding of a small molecule to an active site.

The adaptive value of neck formation to hydra seems obvious. Without the neck constriction, were the distended hydra to open its mouth to receive new prey, it would lose some of the food particles being circulated in the gut. On the other hand, if more prey were captured and not swallowed, there would be an inefficient expenditure of vital nematocysts, as well as a loss of more food. By forming the neck constriction, the hydra forfeits neither part of its previous meal nor nematocysts, but is able to swallow new food as it is captured.

The initiation of neck constriction by reduced glutathione is but another of the growing list of responses in hydra controlled in part by this tripeptide. It is the specific activator of the sequence of tentacle-bending and mouth-opening in hydra's feeding behavior (*1*). This tripeptide also inhibits the animal's contraction in response to light and mechanical agitation (*8*). The so-called sweeping movements (*3*) of the "tentacle concerts" are initiated by extremely low concentrations ($10^{-9}M$) of glutathione (*9*). Electrophysiological correlates to the additions of glutathione to hydra have been shown (*10*); and now, we show glutathione has an essential function in neck formation.

The occurrence in hydra of two chemoreceptor systems that must act in concert represents, to our knowledge, the first such instance reported for the lower invertebrates. The existence of this system gives insight into how a complex chemical coordinating system might have developed, that is, by evolving receptor sites to ubiquitous small molecules emitted from the capture prey. Whether or not the glutathione-tyrosine system represents an early prototype of the more complex interacting hormonal systems of the higher invertebrates remains a question.

RICHARD S. BLANQUET*
HOWARD M. LENHOFF
Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida

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6. This inflation of the gut with fluids seems to provide hydra a means for circulating food particles in the gut, and for regurgitating of indigestible matter, such as nauplii exoskeletons. Within 5 to 6 hours after ingestion, hydra are fully distended and take on a bloated appearance. It is at about this time that regurgitation occurs.
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* Present address: Department of Biology, Georgetown University, Washington, D.C.

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Cyclic Changes in Enzyme Activity in Synchronized Mammalian Cell Cultures

Abstract. *Activity of the enzymes glucose-6-phosphate dehydrogenase and lactate dehydrogenase increases intermittently in synchronized cultures of Chinese hamster cells. During G_1 phase (3 hours after mitosis), midway through S phase (7 hours after mitosis) and again in late S phase (10 hours after mitosis), rapid increases in activity were observed and correlated with a net increase in cell size and total protein. The evidence suggests that this is a general phenomenon which affects a whole population of proteins and may be the expression of an endogenous cellular rhythm.*

Using synchronized mammalian cells, we previously studied the relationship between DNA replication and the capacity of the cell to synthesize RNA (*1*). These studies led us to question whether enzyme levels also reflect the increased gene dosage resulting from replication of DNA and, in general, how enzyme activity changes as a function of the cell cycle. The enzymes lactate dehydrogenase (LDH) (EC 1.1.1.27) and glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) were measured during one cycle of synchronous growth and division, and the changes in activity were compared with several other parameters of cell growth, such as mean cell volume and total protein.

Synchronized cultures were obtained by treatment of an exponential population of Don C Chinese hamster cells with Colcemid for 2½ hours. Cells blocked in mitosis were selectively removed by a brief trypsinization. The collected metaphase cells were then suspended in medium at 37°C at a concentration of 1.25×10^5 cell/ml and inoculated into culture vessels at 5×10^4 cells per square centimeter of culture surface. Division of the attached cells resulted in a surface density of 1×10^5 cell/cm^2 for most of the experiment. Evidence indicates that the cells suffer no lasting effects from brief arrest with Colcemid (*2*).

Enzyme activity (Fig. 1) does not increase in a simple and regular fashion through the cycle but rather undergoes sharp periodic oscillations. Peaks occur repeatably at the end of G_1 (3 to 4 hours after mitosis), midway through S (7 hours after mitosis), and again in late S phase (10 hours after mitosis).