

# THE CHEMICAL CONTROL OF FEEDING IN THE PORTUGUESE MAN-OF-WAR, *PHYSALIA PHYSALIS* L. AND ITS BEAR- ING ON THE EVOLUTION OF THE CNIDARIA<sup>1</sup>

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The remarkable demonstration by Loomis (1955) that reduced glutathione (GSH) induces a feeding response in *Hydra* led us to examine the feeding responses of other cnidarians. Our object was to see whether the chemical control of feeding occurred in other hydrozoans and to identify the chemicals involved. The first organism selected was the Portuguese man-of-war, *Physalia physalis* L. Many different types of zooids suspend from the crested pneumatophore, or float, of this colonial hydrozoan (Figs. 1-4). Among the most numerous are the gastrozooids (Fig. 5), which are the only members of the colony capable of ingesting food. These gastrozooids have the usual polyp form, but lack tentacles. In the feeding process, the prey is drawn up to the gastrozooids which apply their mouths to the surface of the prey; the lips of the gastrozooids then spread out until they envelop the prey (Fig. 4) and digestion proceeds. This process has been described briefly by Wilson (1947). The present report describes the chemical control by GSH of the behavior of both isolated and attached gastrozooids, and discusses the possible evolutionary significance of these findings.

## MATERIALS AND METHODS

*Physalia* were captured off the coast of Woods Hole, Massachusetts, in the summers of 1957 and 1958 and were maintained in aquaria supplied with fresh running sea water. A total of fifteen colonies were studied. *Hydra littoralis* were grown by the methods of Loomis and Lenhoff (1956).

Experiments testing the effects of GSH and cysteine were performed in the following manner:

- 1) Groups of gastrozooids were removed from the float with forceps and scissors. Single polyps were separated, collected in a finger bowl, and rinsed several times to remove any fluids that had oozed from the cut surface. This washing prevented most of the spontaneous opening of the gastrozooid mouth which occasionally occurred after the gastrozooids were isolated. Apparently this spontaneous mouth opening is a response to some substance released from either the cut surface of the gastrozooid itself, or from recently ingested food in the cavity of the gastrozooid.

- 2) The rinsed gastrozooids were distributed randomly in a series of finger bowls, each containing 90 cc. of fresh sea water (non-aerated). Only newly isolated gastrozooids with closed mouths were used.

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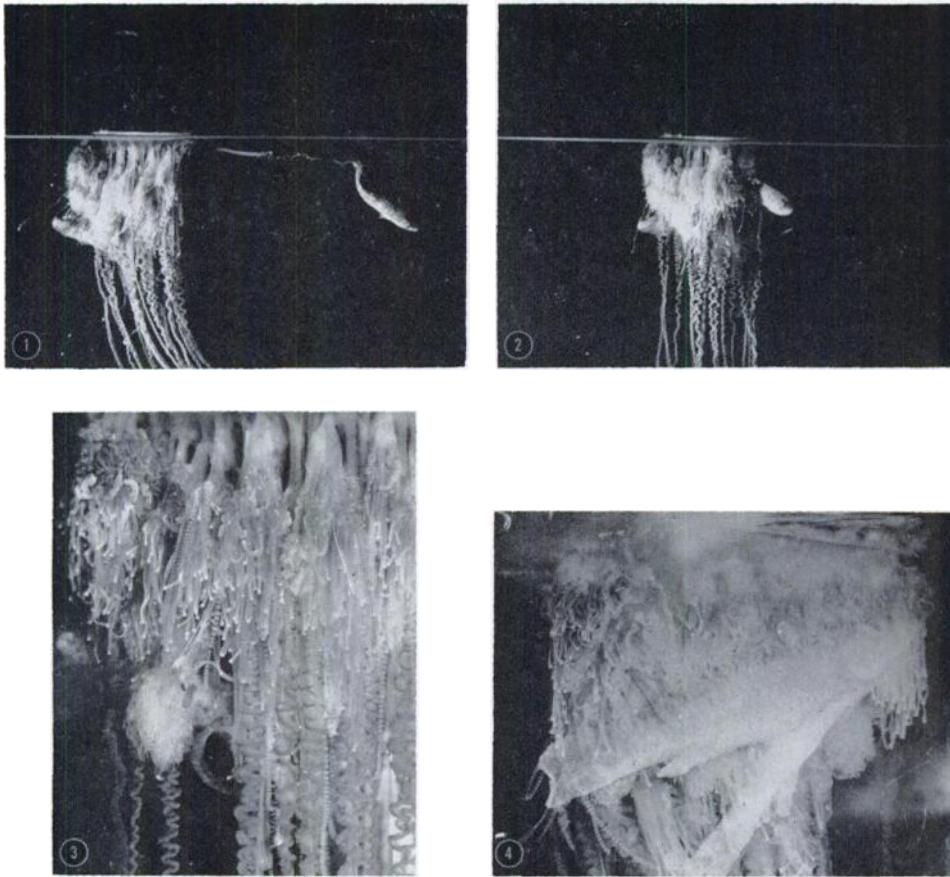


FIGURE 1. *Physalia* fishing tentacle captures small fish.

FIGURE 2. Fishing tentacle draws fish into colony of zooids.

FIGURE 3. Enlargement of *Physalia* zooids. Note the long coiled fishing tentacle, and the many small finger-like, white tipped gastrozooids.

FIGURE 4. Gastrozooids envelop captured fish. Note, in the area of the caudal fin, the mosaic pattern formed by the rims of the spreading gastrozooids in contact with each other. Compare this arrangement with that of the isolated spreading gastrozooids shown in Figure 7.

3) After the gastrozooids were added to the finger bowls, 10 ml. of a freshly-prepared, neutralized solution of GSH or cysteine were added to give final concentrations ranging from  $10^{-3}$  to  $10^{-8}$  *M*.

#### EXPERIMENTAL RESULTS

##### 1. Observations of the "feeding response" of *Physalia* gastrozooids

In the experiments to be described, the gastrozooids exhibited a specific "feeding response," which is described here in detail. Several of the stages are illustrated in Figures 5-8.



FIGURE 5. Isolated gastrozoid. The mouth is at the uppermost part at the end of the narrow cylindrical neck.

FIGURE 6. Gastrozoid induced to spread by GSH. Note the many small batteries of nematocysts along the rim of the gastrozoid lip.

FIGURE 7. Several GSH-induced spreading gastrozooids cover a large surface area.

FIGURE 8. Gastrozooids turned partially inside out by GSH.

Upon addition of GSH or fresh fish blood, the isolated gastrozooids immediately began to writhe. Within five minutes their mouths opened. Whenever a mouth contacted a solid object, such as a fish or a glass surface, it attached itself and began spreading (Fig. 6) as if to enclose the object in conjunction with the other spreading gastrozooids (Fig. 7). The gastrozooids, normally about 1 to 2 mm. in diameter, frequently spread to a diameter of 20–25 mm. By this process many small gastrozooids could surround and digest a large fish (Fig. 4). The spreading phenomenon usually took about one-half hour from initial contact with the solid object until the maximum diameter was reached. This spreading of the gastrozooids may persist for only a few minutes or last for two or more hours. The duration probably depends in part upon the nature of the surface to which the gastrozooid is attached (*i.e.*, smoothness, etc.) and upon their nutritional state. Occasionally spreading gastrozooids detached from the solid object and folded back over themselves, sometimes completely inverting so that endoderm was on the outside and ectoderm on the inside (Fig. 8). A similar phenomenon was described by Loomis (1955) in *Hydra*.

Some outspread gastrozooids migrated slowly over the surface of the solid object leaving a "mucous" trail. The polyp probably secreted extracellular proteolytic enzyme in this mucus to partially break down its prey.

## 2. Feeding response of the intact animal

A small piece of filter paper, soaked in a  $10^{-8}$  M solution of GSH, was placed several centimeters from the gastrozooids of an intact *Physalia*. A typical feeding response occurred, with active squirming of the gastrozooids and then a spreading of their mouths on the paper and on the wall of the aquarium.

## 3. Demonstration of a chemical feeding mechanism using live prey

Ten gastrozooids and a small killifish (*Fundulus* sp.) (8 cm.) were placed in a finger bowl. After a half hour no feeding response of the gastrozooids was observed. At this time a fragment of a fishing tentacle armed with a large number of nematocysts (Fig. 3) was dropped onto the fish. The fish thrashed about for a moment and became immobilized. Within 10 to 15 minutes most of the gastrozooids began spreading on the surface of the finger bowl, apparently in response to some substance released from the pierced fish. Had the gastrozooids been close to the fish (as occurs when the intact colony draws the prey up to the float) then doubtless their mouths would have enveloped the fish (Fig. 4).

## 4. The effect of different concentrations of reduced glutathione

Ten gastrozooids were placed in several concentrations of GSH. The number of gastrozooids spreading was counted at intervals. As shown in Table I, after approximately two and one-half hours, 80–90% of the gastrozooids in  $10^{-5}$ – $10^{-6}$  M GSH had spread their mouths over the surface of the glass bowl. The fact that higher concentrations failed to elicit this response is not unusual, and finds a parallel in *Hydra* where concentrations of GSH above optimum caused a tight closing of the animal's mouth. Also, as in *Hydra*, concentrations of GSH less than  $10^{-7}$  M were ineffective.

TABLE I  
*Number of gastrozooids spreading in different concentrations of reduced glutathione after various time intervals\**

Time (min.) . . . . .	10	35	70	105	155
Reduced glutathione concentration	Number spreading				
$10^{-3} M$	0	0	0	0	0
$10^{-4} M$	0	0	0	0	0
$10^{-5} M$	0	1	5	6	9
$10^{-6} M$	1	2	2	6	8
$10^{-7} M$	0	0	0	0	0
$10^{-8} M$	0	0	1**	0	0
0 <i>M</i>	0	0	0	0	0

\* Ten isolated gastrozooids were exposed to each concentration.

\*\* Spread only slightly.

A delayed spreading phenomenon was often observed at high concentrations of GSH. For example, 9 of 10 gastrozooids, which initially failed to respond to  $10^{-3} M$  GSH, responded 7 hours after the start of the experiment. Occasionally a few gastrozooids took as long as 24 hours to respond. Perhaps these delayed responses occurred when the "excess" GSH had oxidized, giving the optimum concentration.

##### 5. *A comparison of the effects of cysteine and of reduced glutathione*

The effects of another biological reducing agent, cysteine, were tested. The results shown in Table II clearly demonstrate that cysteine failed to induce a feeding response at concentrations at which GSH was active.

##### 6. *Differences in the feeding response of animals in different physiological states*

In one series of experiments the effects of GSH were studied on gastrozooids from two *Physalia* in different physiological states. The first (ca. 16-cm. float) was maintained without food in the laboratory for one week, while the second (ca. 24-cm. float) was kept under the same conditions for one day. The results in Table III indicate that the one-day captive responded rapidly to  $10^{-4} M$  GSH, while the

TABLE II  
*A comparison of the effects of cysteine and of reduced glutathione on the spreading of gastrozooids\**

Concentration	Number spreading at 90 minutes	
	Cysteine	Reduced glutathione
$10^{-4} M$	1**	0
$10^{-5} M$	0	8
$10^{-6} M$	0	4
$10^{-7} M$	0	2

\* Ten isolated gastrozooids were exposed to each concentration.

\*\* Spread only slightly.

7-day captive did not. Also, the response was quicker in the one-day captive animal at all concentrations of GSH. These differences may have been due to the nutritional state or over-all well-being of the one-day captive animal as compared to the 7-day captive animal.

Further studies of more than a dozen *Physalia* revealed striking differences in the responsiveness of the gastrozooids to GSH. Sometimes newly-captured *Physalia* exuded a copious mucus secretion for several days. Gastrozooids from these mucus-covered animals were usually unresponsive to GSH. By contrast, in captives which produced little mucus the gastrozooids always responded. In one

TABLE III

*A comparison of the effect of different concentrations of reduced glutathione after various time intervals on the gastrozooids of a 7-day captive and 1-day captive Physalia\**

Time (min.) . . . . .	7-day (16-cm. float)		one-day (24-cm. float)	
	10	35	10	35
Concentration	Per cent spreading			
$10^{-4}$ M	0	0	66	83
$10^{-5}$ M	10	50	33	83
$10^{-6}$ M	20	50	50	50-66

\* Ten isolated gastrozooids of the 7-day captive and 6 of the one-day captive were used at each concentration.

case an animal was kept in the laboratory for two weeks and its gastrozooids responded throughout this period.

## DISCUSSION

### 1. Feeding response

In *Physalia*, the feeding response of the gastrozooid involves mouth-opening, spreading, and food ingestion. The food is drawn to the gastrozooids by the dactylozooids. Each gastrozooid then writhes and stretches until its mouth comes in contact with a solid object, whether it be the fish that the colony has captured, or the wall of the container in which the gastrozooids were placed. In *Hydra* the tentacles writhe and sweep inward toward the mouth. The mouth then opens and ingests the prey *whenever the prey is brought in direct contact with it* (Ewer, 1947). Thus in contrast to *Physalia* where the whole gastrozooid writhes, in *Hydra* only the tentacles writhe. Moreover, in *Hydra*, the body tube and mouth do not spread when exposed to GSH, but do so only when the food, or some other solid object, is brought to the mouth by the writhing and contracting tentacles. For example, when 10 *Hydra* were placed in each of a series of Petri dishes having 25 ml. of GSH of  $10^{-8}$  to  $10^{-7}$  molarity, none of the *Hydra* exhibited the spreading phenomenon, although their mouths opened. This failure to spread was not due to the tentacles blocking the mouth, because removal of the tentacles did not increase spreading. However, when 10 *Hydra* were crowded in a 0.5-ml. volume of  $10^{-4}$

M GSH so that their mouths were forced to contact the wall of the container or the surface of the water, 6 animals spread within 3 minutes.

It must be stressed that mere mouth opening or writhing does not indicate a feeding response in *Physalia* or in *Hydra*. The only true index of a feeding response is an actual attempt to feed—that is, to spread the mouth over or around the prey. Many deleterious chemicals cause writhing or mouth opening. For example, in *Physalia*, high concentrations of alloxan (which, besides inhibiting the true feeding response, subsequently killed the gastrozooids) caused occasional “mouth opening” but no characteristic feeding response. This “gaping” has also been observed in *Hydra* by using Tween, and other harmful compounds (Lenhoff, unpublished observations).

## 2. The action of reduced glutathione

The data leave little doubt that GSH induces a feeding response in *Physalia* just as it does in *Hydra*. We cannot be certain that GSH is the only biological compound that will induce the response in *Physalia* but the clear response of the gastrozooids to low concentrations of GSH, coupled with Loomis' finding (1955) that no other commonly occurring compound of many tested worked on *Hydra*, make this likely. Since a gastrozoid of *Physalia* is four to six times as long as a *Hydra*, and thicker, it should be practical to explore the detailed mechanism of the feeding response with greater ease than in the case of *Hydra*. For example, it should be possible to locate the receptor cells sensitive to GSH and to explain the mechanics of the transformation of the cylindrical gastrozoid into a disc.

## 3. Phylogenetic considerations

The Siphonophora are commonly regarded as the most specialized order of the Hydrozoa in that they attain the highest degree of polymorphism and present the greatest number of medusoid and polypoid types. While there is some disagreement regarding the phylogenetic relations within the group, there appears to be no question but that they have clear hydrozoan characters (Hyman, 1940; Totten, 1954). Furthermore it is generally accepted that save for the Chondrophora (*e.g.*, *Velella*) which are now thought to have close affinities with tubularian hydroids (Totten, 1954; Rees, 1957), the Siphonophora proper, including *Physalia*, early diverged from the cnidarian stem and evolved in directions quite different from other hydrozoans (Hyman, 1940). On the other hand, *Hydra* itself is commonly considered a highly specialized gymnoblastic hydroid, in all likelihood a fresh-water-adapted tubularian (Hyman, 1940). Thus among the Hydrozoa it would be hard to find two forms which diverged earlier from one another during evolution—two forms which are very specialized and not generalized members of their class. Recognizing this, the demonstration of a GSH-induced feeding response in both forms assumes special interest. It suggests that either (1) this GSH-induced response is primitive (Loomis, 1955) and has persisted through the course of geological time since these animals diverged because they both retain a primitive carnivorous feeding habit, or (2) that this is simply convergence. The former suggestion is not only more attractive but is more likely, and implies that GSH-stimulation of feeding is a very ancient coordinating system and will be found among many of the



Hydrozoa and possibly among Scyphomedusae and Anthozoa as well. This conclusion finds support in recent observations that *Campanularia flexuosa*, a calyptoblastic hydroid unrelated to either *Hydra* or *Physalia*, gives a feeding response to GSH. It must be emphasized, however, that the presence of a GSH response in these three hydrozoans does not rule out the possibility that other small molecules in the fluids released from captured prey may function in the feeding response of other cnidarians. The essential feature of the primitive feeding mechanism is that the prey must release fluid when pierced (see below).

These experiments support the opinion offered earlier (Schneiderman and Gilbert, 1958) that the evolution of chemical control mechanisms has proceeded by particular groups of animals adapting available and often ubiquitous molecules to special tasks. Under this view the evolution of hormonal coordination involves primarily the evolution of receptor systems sensitive to specific molecules rather than any evolution of hormones as such.

The chemical similarities between the nematocyst-GSH feeding mechanisms of *Physalia* and *Hydra* invite inquiry into the nature of their possible common ancestor. Both of these organisms sting and capture their prey by means of nematocysts. It is of interest in this connection that the nematocyst capsule of *Hydra* (Lenhoff *et al.*, 1957) and *Physalia* (Lane and Dodge, 1958; Lenhoff and Kline, 1958) are composed of similar kinds of unusual hydroxyproline-rich, collagen-like proteins. After the prey is penetrated, the GSH in the fluids flowing from the wound stimulates the feeding response.

Since *Hydra* and *Physalia* both have a nearly identical chemical control of their feeding behavior, and since their nematocysts are of a unique chemical composition, it seems probable that both animals evolved from a common hydrozoan stem-form which also possessed these chemical characteristics. It is noteworthy that the nematocyst-GSH mechanism can only be used to capture prey which has sufficient body fluids to release enough GSH on being stung to elicit the feeding response (*i.e.*, organisms which have either a pseudocoelom, or vascular system) (Loomis, 1955). Therefore, either (1) the primitive nematocyst-bearing hydrozoan stem-form ate prey which was more highly evolved than itself, or alternatively, (2) the primitive nematocyst-bearing hydrozoan stem-form fed on some presently unknown lower form, perhaps a large protozoan, with a great deal of body fluid. We favor the first alternative and prefer to believe that the hydrozoan stem-form fed on animals with "vascular" fluids (*i.e.*, nematodes, and members of higher phyla).

These facts permit us to speculate about the feeding habits of the ancestral cnidarians, *i.e.*, the forms from which the Hydrozoa and the other classes of Cnidaria evolved. Ordinarily one hesitates to base phylogenetic schemes on feeding habits which in most phyla are notoriously labile. However, the universal use of nematocysts to capture prey by all contemporary cnidarians supports the view that the feeding habits of members of this phylum are far more stable than those of other animals. The ancestral cnidarians most probably fed on animals which were of a lower grade of organization than themselves, and hence could not have employed the nematocyst-GSH mechanism. Most likely they were filter feeders like many present-day Anthozoa. Under this view, nematocyst-bearing cnidarians with a GSH-mechanism evolved from filter-feeding ancestors at the same time as did higher forms. Thus cnidarians with nematocysts (a diagnostic feature of the



phylum) may be removed from their position as the ancestors of higher metazoans. Whether they are members of a regressive line of evolution which have degenerated because of a sessile habit (Hadzi, 1953), or are an offshoot of a progressive line of evolution, remains to be proven. Since all these events took place in the Pre-Cambrian Era, none of these suggestions can be ruled out at present and doubtless others will arise.

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#### SUMMARY

1. *Physalia* gastrozooids, both isolated and *in situ*, exhibit a feeding response when exposed to low concentrations of reduced glutathione ( $10^{-5}$  –  $10^{-6}$  M).
2. Cysteine did not elicit the response.
3. The feeding response of the gastrozooid consisted of opening of its mouth and the subsequent spreading of its lips over a large area in an attempt to envelop the prey. This response resembles a similar GSH-induced feeding response found in *Hydra*.
4. The sensitivity to GSH depended upon the physiological state of the *Physalia*.
5. The significance of this primitive chemical coordinating mechanism in relation to the evolution of the Cnidaria and of the Metazoa as a whole is considered.

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