Biphasic Feeding Response in a Sea Anemone: Control by Asparagine and Glutathione

Abstract. *Two phases of the feeding response in the sea anemone* Anthopleura elegantissima *are controlled by different chemical activators. Asparagine controls the contraction and bending of tentacles which bring food to the mouth; reduced glutathione controls ingestion of food once it has contacted the mouth. A complete feeding response occurs only when both chemical activators are present.*

Feeding behavior in many coelenterates is under chemical control (*1–6*). Most respond to one compound (*1, 3, 4*), such as reduced glutathione (GSH) or proline. Some respond to two different compounds (*6*) although there seem to be no differences in behavioral responses to the two compounds. I describe two phases of the feeding response in a sea anemone *Anthopleura elegantissima*; each phase is controlled by a different chemical feeding activator. Asparagine induces bending of tentacles, which brings food into contact with the mouth; reduced glutathione induces swallowing after this contact has been made.

Specimens of *Anthopleura*, collected from docks and pilings at Holiday Harbor, San Pedro, California, were kept in open jars placed on racks in 20-gallon aquariums. A constant temperature of 17° ± 1°C was maintained by using the method of Bakus (*7*). The medium, Instant Ocean (Aquarium Systems, Inc., Wycliffe, Ohio), was prepared without addition of the liquid trace elements provided by the manufacturer. Animals were not fed during the course of the experiments. Suspect activators of feeding were isolated by chromatographic techniques (*5*).

Table 1. Chemical activation of the ingestion response in *Anthopleura*.

Compound on paper	Response to paper (No.)		Positive responses (%)
	Ingestion	Rejection	
None	3	16	18
Artemia homogenate	20	3	87
Reduced glutathione	66	15	82
S-Methyl glutathione	1	15	6
Glutamate	1	9	10
Glycine	0	10	0
Cysteine	0	10	0
Mixture of glutamate, glycine, and cysteine	1	10	9
Valine	0	10	0
Proline	0	10	0
Asparagine	9	47	19

Anthopleura gives identical feeding responses to live *Artemia salina* nauplii and to tissue fluids (*8*) of these animals spotted and dried on filter paper squares (1 mm²). This response consists of the following sequence: On contact with treated paper (or nauplius) the tentacle contracts and bends over the central axis of the body until it contacts the mouth. At the same time the mouth begins to swell, exposing the actinopharynx. The mouth elongates in the direction of the approaching tentacle until contact is made. The treated paper (or nauplius) is transferred from tentacle to mouth (actinopharyngeal portion) and is ingested.

The ingestion phase of the feeding response is induced by GSH. Filter papers treated with suspect chemical activators were placed on the mouths of anemones that had already rejected a similar piece of untreated filter paper. *Anthopleura* ingested papers spotted with GSH in 82 percent of the trials (Table 1). None of the other compounds tested elicited positive responses significantly above background (*9*). No positive responses were observed to any of the three amino acid components of GSH—glutamic acid, cysteine, and glycine—or to a mixture of the three. Likewise, other known coelenterate feeding activators, valine (*5*), proline (*3*), and leucine (*10*), were ineffective. *S*-Methyl glutathione, which can substitute for GSH in the feeding response of *Hydra*, did not induce a response in *Anthopleura*.

Anthopleura placed in solutions of GSH (in concentrations as low as 10^{-5} mole/liter) ingested pieces of clean filter paper placed on their mouths; maximum sensitivity was to $10^{-3}M$ GSH. The glutathione receptors of *Anthopleura* appear to be localized, because ingestion occurred only when paper treated with GSH was placed on the mouth. Treated paper placed on tentacles or even on the outer portion of the oral disk did not induce swallowing.

Because anemones carry out a complete feeding response in the presence of *Artemia* extract and only the ingestion phase of the response in the presence of GSH, I assumed that another factor might control the carrying of food to the mouth by the tentacles. An assay for this phase of feeding was devised.

Pieces of untreated filter paper were placed on tentacles of anemones. If these induced no response, paper spotted with a suspect feeding activator was presented in the same way. A positive response consisted of tentacle bending and transfer of paper from tentacle to mouth. Of the suspect compounds tested, asparagine induced the largest percentage of positive responses (Table 2). (Anemones respond to asparagine in solution in concentrations as low as 10^{-5} mole/liter, with maximum sensitivity at 10^{-3} mole/liter.) Aspartic acid, an analog of asparagine, also induced a significant number of positive responses. On the other hand, glutamine (next larger homolog of asparagine) induced no positive responses.

A complete feeding response can be induced in *Anthopleura* by manually placing filter paper treated with asparagine on tentacles of anemones bathed in GSH solutions (Table 3). The asparagine on the paper causes it to be brought to the mouth. It is then ingested in response to the varying concentrations of glutathione, with maximum responses at 10^{-3} and $10^{-4}M$ GSH.

Like those of other coelenterates, the

Table 2. Chemical control of tentacle bending and transfer of filter paper from tentacle to mouth. (Glutathione and 18 amino acids were inactive.)

Compound on paper	Tentacle bending (No.)		Positive responses (%)
	Bending	No bending	
None	2	15	11
Artemia extract	24	3	89
Asparagine	45	7	87
Aspartate	18	12	60
Glutamine	0	16	0

Table 3. Response to GSH in solution and asparagine-treated filter paper placed on tentacles.

GSH (mole/liter)	Tentacle bending (No.)		Response to paper (No.)	
	Bending	No bending	Ingestion	Rejection
10^{-3}	13	4	11	2
10^{-4}	10	0	7	3
10^{-5}	16	0	6	10

receptors of *Anthopleura* show specificity for the size and shape of activator molecules. Glutamate, although it induces no positive feeding responses in *Anthopleura* (Table 1), is an effective competitive inhibitor of the ingestion response of anemones both to reduced glutathione and to *Artemia* homogenate (*10*). This points to GSH as the specific component of *Artemia* tissue fluids that induces ingestion. The same type of inhibition of the response to GSH by glutamate is seen in *Hydra* (*11*). Therefore, like *Hydra*, the GSH receptors of *Anthopleura* must be most sensitive to the γ-glutamyl moiety of the molecule. Unlike the case in *Hydra* (*12*), in *Anthopleura* the thiol group of GSH cannot be methylated. The molecule's primary chain length also appears to be a very important feature. Loomis (*1*) found that *Hydra* gives no response to asparthione (next smaller homolog of GSH). Likewise, the sea anemone *Boloceroides* gives no response to leucine, the next larger homolog of its feeding activator, valine (*5*). *Anthopleura* is sensitive to asparagine, but glutamine (next larger homolog of asparagine) does not elicit positive feeding responses (Table 2).

Preliminary results (*10*) indicate that reduced glutathione may affect the cilia of the mouth and actinopharynx. Experiments with colloisol dyes (Farbwerke Howchst, Frankfurt) have shown that nonfeeding anemones in clean seawater have ciliary currents flowing out of the mouth and across the oral disk toward the tentacles. Dye squirted above the oral disk does not enter the coelenteron but is eventually rolled up in mucus and moved off the oral disk. This current is reversed in the area of the mouth when animals are placed in solutions of glutathione. Dye enters the coelenteron and is retained there for over an hour (*10*). Reversal of ciliary currents in coelenterate feeding behavior was proposed in 1896 by Parker (*13*).

The effects of asparagine and reduced glutathione on the behavior of *Anthopleura* are very different. Asparagine is a feeding incitant, that is, a substance that induces the initial contact between mouth and food, or tasting (*14*). Reduced glutathione is a feeding stimulant, that is, a substance that induces ingestion or continued feeding (*14*). Asparagine induces tentacle bending and transfer of treated paper from tentacle to mouth. Ingestion of this paper does not occur, however, unless glutathione is present. Glutathione induces swallowing but only when present directly on the mouth.

The experiments with *Anthopleura* illustrate an extension of the role played by chemical activators in the feeding behavior of a coelenterate, the division of the feeding response into two phases, each phase controlled independently by a different chemical activator. It is probable that other phases of coelenterate feeding behavior, for example, the "preparatory feeding" phase described by McFarland (*15*), will be found to be controlled by specific chemical activators as well.

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8. Approximately 50 to 100 nauplii were washed several times in distilled water and homogenized in 1 ml of water. This homogenate was spotted and dried on Whatman No. 1 filter paper.
9. A small percentage of anemones (less than 20 percent) ingested untreated filter paper. An even smaller percentage (5 to 15 percent) rejected paper treated with *Artemia*.
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16. This work was partially supported by PHS biomedical sciences support grant RR-07012-04. I thank Drs. Gerald Bakus and Howard Lenhoff for critically reading this manuscript and for helpful suggestions, and Dr. John Mohr for giving me the colloisol dyes.

22 March 1971; revised 1 June 1971

Genetic Aspects of Increase in Rat Liver Aldehyde Dehydrogenase Induced by Phenobarbital

Abstract. *In the supernatant fraction of homogenized rat liver, the activity of aldehyde dehydrogenase that is dependent on nicotinamide adenine dinucleotide (E.C. 1.2.1.3) is increased up to tenfold after administration of phenobarbital for 3 days. The effect is genetically controlled and is inherited as an autosomal dominant characteristic. The mechanism is apparently unrelated to other drug-induced increases in enzyme activity such as that which occurs in the hepatic microsomal systems for drug metabolism.*

Aldehyde dehydrogenases that are dependent on nicotinamide adenine dinucleotide (NAD) are enzymes of broad substrate specificity located in highest concentrations in the liver although they exist in other tissues in smaller amounts (*1*). The enzyme activity is present in both the supernatant and mitochondrial fractions of liver homogenates.

There is evidence that the enzymes recovered from these two subcellular fractions differ in their substrate specificities (*2*) and in their physical properties (*1*). However, neither has been completely purified. The enzymes are capable of the oxidation of a large number of aldehydes to the corresponding acids. Among these aldehydes are glyceraldehyde, an intermediate in fructose metabolism (*3*); acetaldehyde, a product of ethanol metabolism (*4*); the aldehydes generated from biogenic amines as a result of monoamine oxidase activity (*5*); and many other aromatic and aliphatic aldehydes (*6*).

Recently Redmond and Cohen (*7*) reported that repeated injections of phenobarbital into mice resulted in a twofold increase in aldehyde dehydrogenase activity in whole liver homogenates. This report describes a similar but much greater effect in rat liver and demonstrates that it is confined to the soluble enzyme present in the supernatant and is inherited as a dominant characteristic in this species.

Rats of the Fischer, Long-Evans, and Sprague-Dawley strains were obtained from Simonsen Laboratories. The optimum weight of the rats used in these studies was about 200 g. Rats were injected intraperitoneally with phenobarbital (20 mg/ml) in a dose of 100 mg per kilogram of body weight, once daily. Three doses were required for a consistent response, and even a dose of 100 mg/kg was not sufficient to elicit the maximum response. Controls