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BEHAVIORAL AND ELECTROPHYSIOLOGICAL STUDIES OF HYDRA. III. COMPONENTS OF FEEDING BEHAVIOR[1]

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The mechanisms controlling feeding responses in coelenterates were the subject of much research at the turn of the century (Nagel, 1892; Loeb, 1895; Jennings, 1905; Parker, 1896, 1917). Recently interest in coelenterate feeding behavior was rekindled by a report that in the freshwater *Hydra,* a feeding response is specifically activated by reduced glutathione (GSH) (Loomis, 1955). This finding led to extensive study of the effects of this tripeptide as a feeding stimulator in *Hydra* (Lenhoff, 1961a, 1961b, 1965, 1967, 1968a, 1968b), and a search for other simple molecules activating feeding in other coelenterates (Fulton, 1963; Mariscal and Lenhoff, 1968; Linstedt, Muscatine and Lenhoff, 1968; Pardy and Lenhoff, 1968). The uniqueness of GSH as an initiator of feeding in *Hydra* was questioned both by Forrest (1962) and Burnett, Davidson and Wirnick (1963). However, many of their questions have been answered in Lenhoff's later experiments (Lenhoff, 1965, 1968a).

In the past decade, there has been a mounting interest in electrophysiological studies of hydroids (Josephson, 1961, 1965a, 1965b, 1967, Josephson and Mackie, 1965; Passano and McCullough, 1962, 1963, 1964, 1965; Rushforth, 1967, 1971; Mackie, 1968). These investigations have characterized, in considerable detail, the properties of pacemaker activities underlying stereotyped, rhythmically recurring behavioral events. Electrical recording techniques have given us considerable insight into the types of potentials associated with spontaneous behavior in several hydroids. There is little information, however, concerning the electrical correlates of feeding responses in these animals. The present research was undertaken to study sequences in the feeding behavior in *Hydra* and to determine if changes in electrical activity accompanied such sequences. Similar studies were performed on the marine hydroid *Tubularia* for comparative purposes (Rushforth, in preparation).

Materials and Methods

Most of the experiments were performed using *H. littoralis,* the animal most extensively used by Loomis and Lenhoff in their investigations of feeding behavior. However, since previous papers in the present series have investigated the electrical activities of *H. pirardi* and *H. pseudoligactis,* these two species were also

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used, particularly in the electrophysiological studies of feeding responses. All animals were cultured using methods previously described (Rushforth, 1971).

The animals used had been fed 24-hours previously with *Artemia salina* nauplii. Since Lenhoff (1965) showed that the feeding response in *Hydra* was influenced by the microenvironment surrounding the animal, particularly in crowded cultures, an effort was made to thoroughly wash the hydra before making observations. Individual hydra were rinsed by pipetting successively in three finger bowls each containing 200 ml of buffered culture solution. The animals were then placed in a fourth finger bowl, also containing 200 ml of the same solution (1.5×10^{-3} M $CaCl_2$, 1.2×10^{-3} M $NaHCO_3$ and 1.2×10^{-4} M Na_4 EDTA and 10^{-3} M histidine buffer, pH 6.2), and were left undisturbed for an hour before the start of an experiment.

Single nauplii were sucked into a fine glass holder made from a pasteur pipette. One end of the pipette was drawn out into a fine bore, the other end being attached to flexible plastic tubing. By releasing the suction gradually, the nauplius was gently directed onto a selected region of a given tentacle. By means of stop-watches the times for various resulting behavioral sequences were recorded. All observations were made using a dissecting microscope. An extract of *Artemia salina* was prepared from a dense 1-ml suspension of nauplii in 5 ml of distilled water. The preparation was homogenized in a small tube using a ground glass plunger, and the resulting suspension was centrifuged at 3000 rpm for 15 minutes. Aliquots of 1 ml of the supernatant, approximate total protein content of 6 mg/ml, were diluted ten fold with culture solution and a sample of 1 ml of this solution was administered to the hydra.

Electrical recordings were made from individual hydra using methods previously described (Rushforth, 1971). The animal's movement was only restricted by its base being attached to the recording electrode. Single *Artemia,* homogenates of *Artemia,* or GSH were pipetted into the environment of the animal while its behavior and electrical activity were recorded. Minor modifications of these experimental procedures will be described in the appropriate section in the presentation of the results.

Results

The most concise description of the feeding response of *Hydra* was given by Josephson: "First the prey strikes the polyp, usually on one of the outstretched tentacles, and becomes there attached by nematocyst discharge. The portion of the tentacle proximal to the prey then contracts, often spiralling inward, which brings the prey near the mouth. As the prey nears the mouth, the surrounding tentacles concertedly flex in the oral direction. This sometimes results in adjacent tentacles contacting the prey and pushing it towards the mouth. Concerted tentacle flexion may be repeated several times during and after ingestion of the prey. Finally the mouth opens, creeps around the prey, and closes about it" (Josephson, 1965c, page 34).

In the present study we have investigated: (1) tentacular movements following nematocyst discharge; and (2) inhibition of the normal endogenous contraction activities of the tentacles and body column, which takes place concurrently with the reflexly-linked, local responses of feeding.

1. Tentacular movements

On attachment of the *Artemia* to the tentacle by nematocyst discharge, there is a latent period before the first observable movement of the tentacle. This time period, measured in seconds, we define as the tentacle reaction time. The tentacle movement usually consists of contraction proximal to the point of contact of the *Artemia,* but there may be bending or spiralling movements associated with

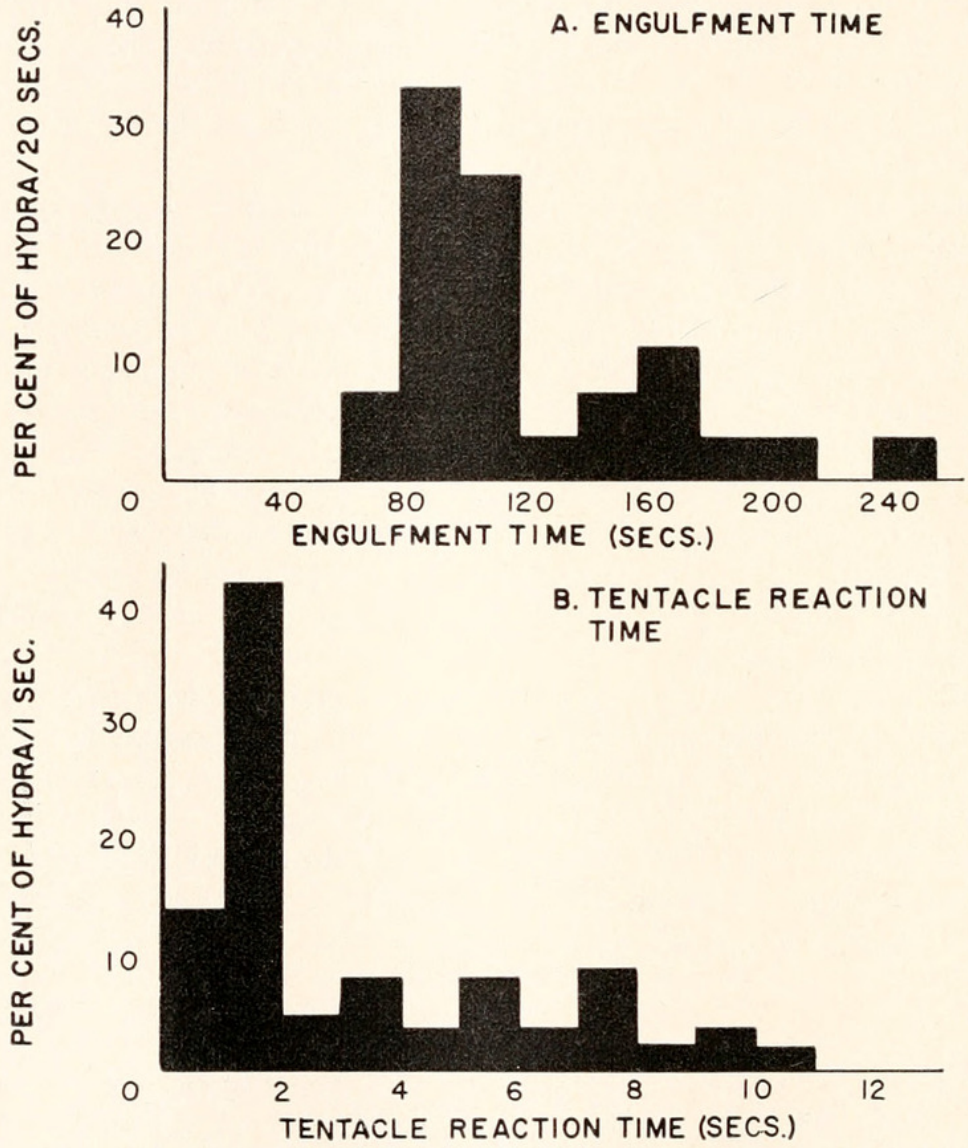

FIGURE 1. Distributions of engulfment and tentacle reaction times for *H. littoralis;* A: histogram of the per cent distribution of engulfment times for a group of 27 hydra; B: histogram of the per cent distribution for tentacle reaction times for a group of 102 hydra.

the shortening, depending on the position of the attachment. Such activities of the stimulated tentacle bring the prey near to the mouth and precede a concerted flexion of the group of tentacles (termed a concert). As such concrts repeatedly occur, the mouth opens and begins to creep around the prey. At this stage the concerted tentacular movements often are replaced by uncoordinated writhing activities. Such writhing movements of the tentacles have been reportd by several other workers (Ewer, 1947, Loomis, 1955, Lenhoff, 1961b). The mouth continues to creep around the *Artemia,* finally closing about it. The time in

seconds from prey attachment to mouth closing we have termed the engulfment time. The distributions of engulfment times (A) and the reaction times (B) for *H. littoralis* are given in Figure 1. Both distributions are skewed to the right with modal class intervals of 80–100 seconds or 1–2 seconds, respectively.

The tentacle reaction initiated by *Artemia* capture depends on the part of the tentacle involved in the capture. This was systematically examined by arbitrarily subdividing the tentacle into three portions: the basal; middle and distal regions. Each of the three regions was tested with each of 30 hydra, the order of observing the regions was selected at random. The results are shown in Table I. They indicate the wide range of tentacle movement used to bring the prey organism to the mouth of the hydra. The fact that contraction of the tentacle is limited to the portion below the point of prey attachment, strongly suggests polarized conduc-

TABLE I

Tentacle movement and position of Artemia *attachment in* H. littoralis

Position of attachment	Number of observations			
	Tentacle movement			Total
	Bending or bending and contracting	Contracting	Spiralling and contracting	
Basal	10	18	0	28
Mid	3	22	3	28
Distal	2	13	15	30
Total	15	53	18	86

(In two *Hydra* the *Artemia* detached and were not engulfed.)

tion in the oral direction. The three forms of tentacle movement may result primarily from mechanical stimulation by the prey, but they do not occur without nematocyst discharge. If a clean glass rod is lightly touched to the tentacle, there is only slight localized contraction at the point of stimulation. However, if the stimulation is greater, causing nematocyst discharge and tentacle adhesion to the rod, then contraction below the point of attachment occurs, similar to that in the capture of an *Artemia*.

Although the type of tentacle movement depends on the site of prey attachment, the latent period before tentacle movement is independent of the type of tentacle movement. The mean reaction times are not significantly different ($P > 0.10$) among hydra exhibiting the following tentacle movements: (1) bending and contracting (3.2 sec, *Artemia* on basal region); (2) contracting (2.5 sec, *Artemia* on middle tentacle region); and (3) spiralling and contracting (3.0 sec, *Artemia* on distal portion). It was also found that neither the reaction time nor the engulfment time changed significantly ($P > 0.10$) with successive capture for a group of five *Artemia*. Thus, there is no evidence of fatigue or facilitation of movements in the feeding response, at least for the first few prey captured.

2. Concerted tentacle flexions

In the majority of hydra investigated, an *Artemia* placed on a single tentacle was engulfed within 80–100 seconds. However, concert activity initiated with the first tentacle movements, continues long after the prey has been swallowed (Fig. 2, upper graph). Only 15–20 minutes after the prey has been engulfed does the concert frequency decrease to the pre-feeding level.

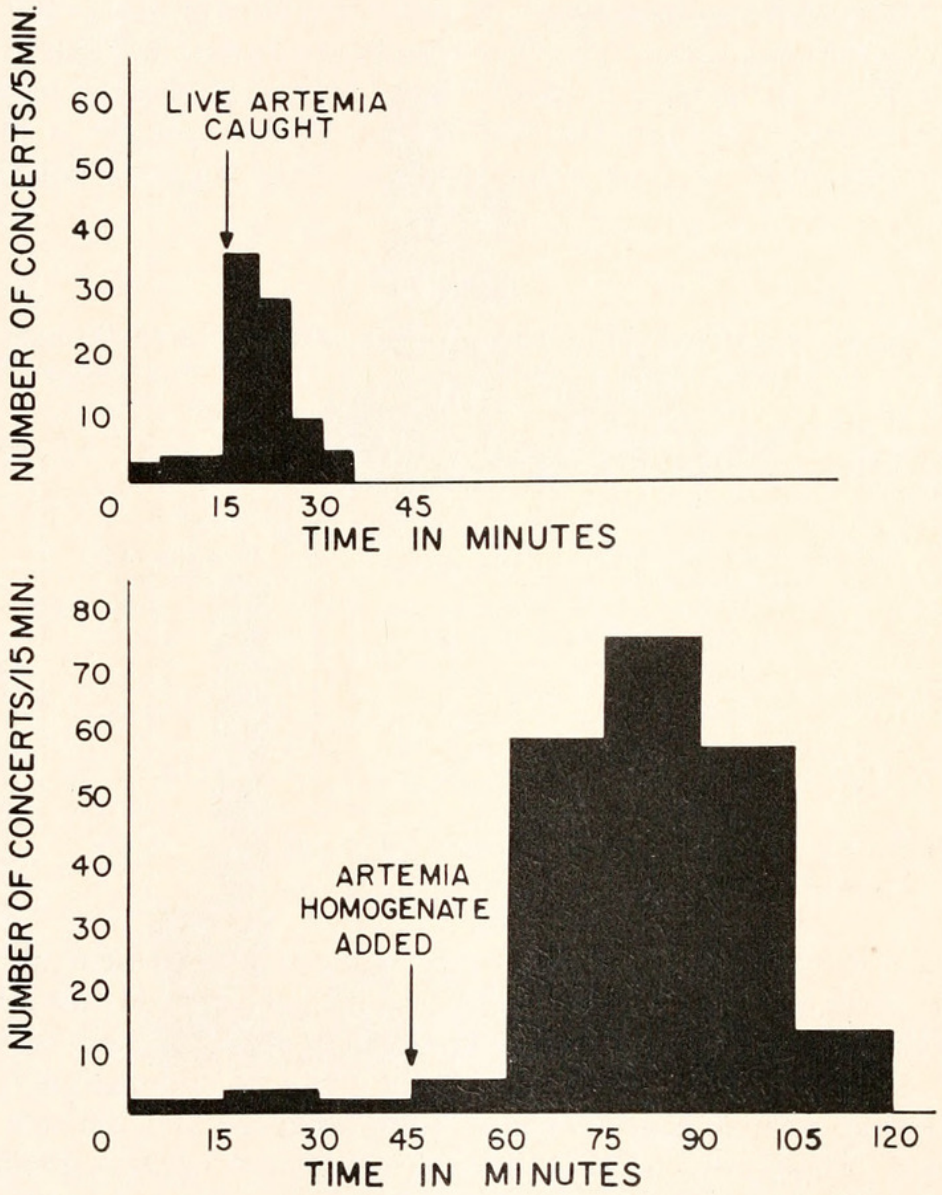

FIGURE 2. Effect of feeding stimuli on concert frequency in *H. littoralis;* upper graph: number of concerts/animal/5 min before and after a single *Artemia* is captured, based on a group of 10 hydra; lower graph: number of concerts/animal/15 min before and after exposure to homogenate of *Artemia,* based on a group of 5 hydra.

Concerted tentacle flexions consist of a highly coordinated oral sweeping movement by all of the tentacles, or all tentacles except that involved in prey capture. Such concerts are quite dissimilar from uncoordinated writhing activities, occasionally seen when the hydra feeds on a single *Artemia.* If several specimens of *Artemia* are simultaneously captured by the polyp, initial concert activity soon gives rise to writhing movements, which continue after all the prey are engulfed.

In turn, the writhing activity becomes less vigorous, and concerts return at enhanced frequencies. Concert frequencies are gradually restored to base line levels, 30–60 minutes later.

Both concerted oral flexion and tentacle writhing can be chemically induced, by either *Artemia* extract or GSH (Fig. 2 and 4). On exposure to *Artemia* extract

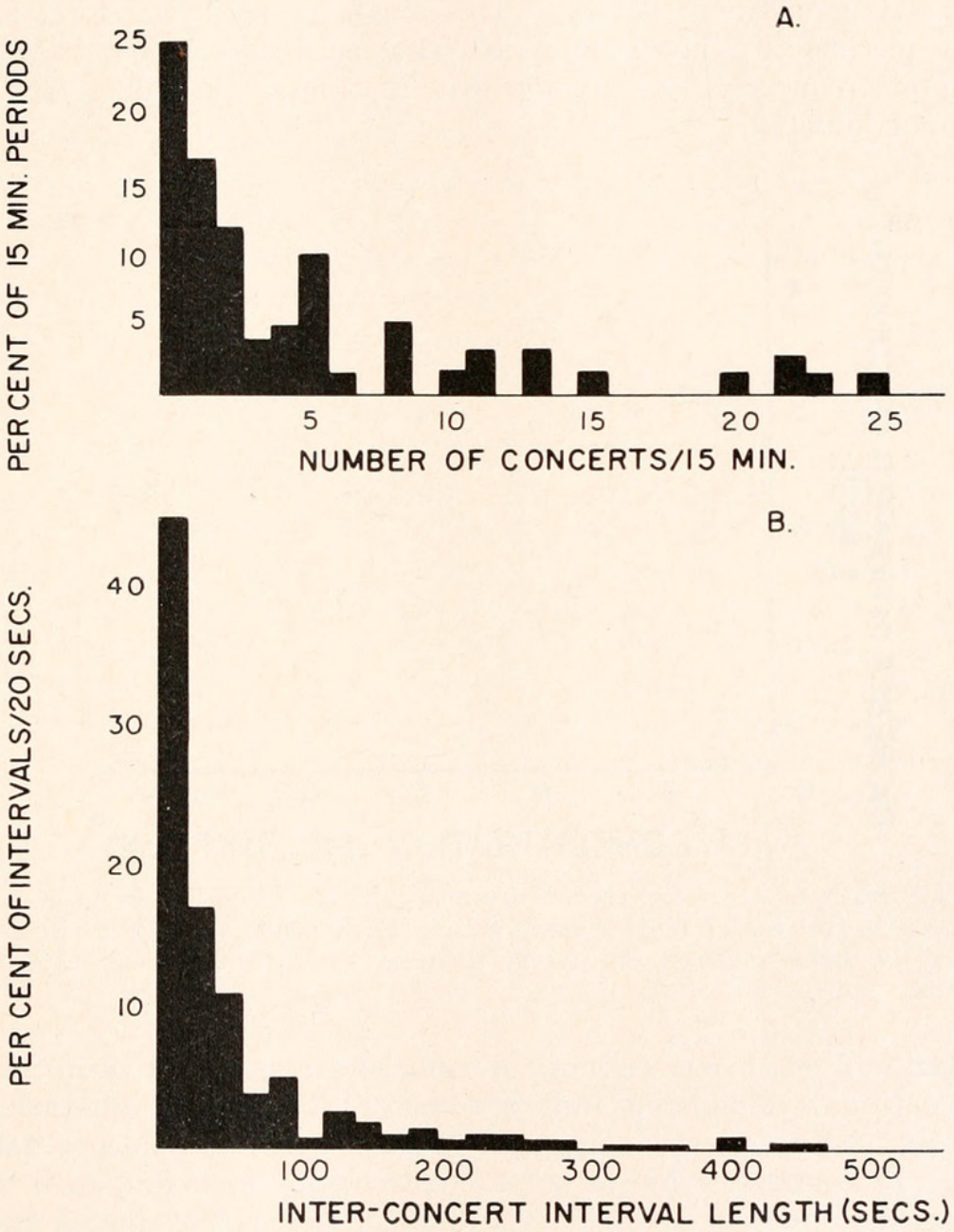

FIGURE 3. Concert frequency in *H. littoralis;* A: per cent distribution of concerts/15 min periods for 60 hydra; B: inter-concert interval distribution for 60 hydra.

there are a few initial concerted tentacle flexions, followed by considerable tentacle writhing. Such writhing is the predominant tentacle activity in the first 15 minute period following exposure to the homogenate. Thus, the concert frequency is only slightly above the pre-stimulation level during this period. However, for the subsequent 45 minutes, concert frequency is greatly enhanced, and is still above control values an hour after administration of the extract. This behavioral se-

quence is also observed when the hydra is exposed to GSH in concentrations greater than 10^{-8} M, or when several live *Artemia* are fed simultaneously to the animal.

A single concert may be induced by prodding a tentacle with a clean glass rod so that nematocyst discharge occurs. The stimulated tentacle adheres to the rod and contracts below the point of attachment, and a concerted flexion of the other tentacles immediately follows. This single concert, therefore, appears to result from mechanical stimulation. Following such stimulation, additional concerts occur at frequencies not greater than prestimulation values, and writhing activity is not initiated.

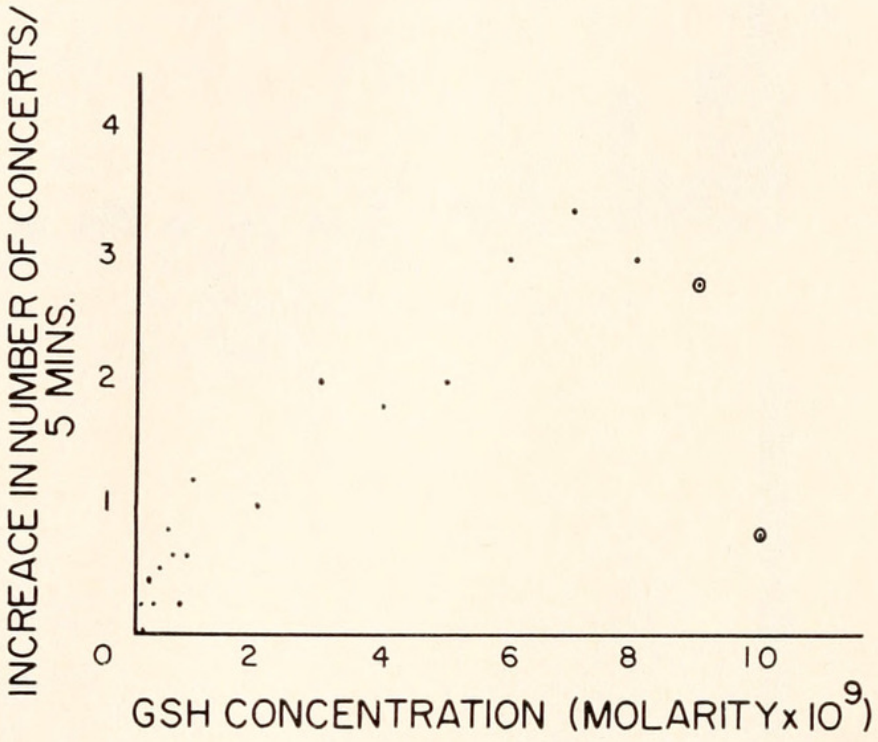

FIGURE 4. Effect of GSH on concert frequency in *H. littoralis;* increase in concerts/animal/5 min as a function of GSH concentration. Each point is based on 50 hydra. The circled values are those readings of concert frequency, in which tentacle writhing accompanied concerts.

Prolonged writhing movements of the tentacles are only seen when the hydra is exposed to chemicals stimulating feeding responses, or noxious substances in high concentrations. This activity is not part of the normal spontaneous behavior of the animal. In contrast, concerts occur endogenously in hydra, their frequency being increased by feeding stimuli. The distribution of spontaneous concerts in unfed *H. littoralis* is shown in Figure 3A. This histogram is highly skewed to the right, a result of the concerts tending to come in bursts. This is clearly seen from the inter-concert interval histogram plotted for these animals in Figure 3B. This distribution is also skewed to the right, with a modal class of intervals having lengths shorter than 20 seconds. These intervals, comprising about 50 per cent of all the inter-concert intervals, represent intervals between concerts in a burst. The longer intervals constitute inter-concert-burst intervals.

In studying the mouth opening response in *H. littoralis* elicited by GSH, Lenhoff postulated a limited number of receptor-effector systems in the animal,

localized in the area immediately around the mouth and tentacles (Lenhoff, 1961b). He observed that concentrations of GSH greater than 5×10^{-6} M activate all these systems, 10^{-6} M GSH eliciting a half-maximum response. Experiments with glutathione analogs and related amino acids were used by several workers to document the specificity of glutathione in initiating the feeding response in *Hydra* (Loomis, 1955; Cliffe and Waley, 1958; Lenhoff and Bovaird, 1961). However, both Forrest (1962) and Burnett *et al.* (1963), questioned such specificity, finding that a wide range of substances elicited feeding reactions in several species of *Hydra.* On the other hand, spontaneous column contractions, and those induced by light and mechanical stimulation, were found to be specifically inhibited by GSH and its S-methyl analog (Rushforth, Krohn and Brown, 1964; Rushforth, 1965). It was, therefore, of interest to determine: (1) the degree of specificity of GSH for inducing concerts, and (2) the range of concentrations of GSH over which concert activity was significantly increased.

The following regime was used to assay for GSH and other substances as possible initiators of concerts. The number of concerts were determined for a group of 10 hydra during a five minute period. Then the test substance was pipetted into the dish to give a specified concentration, and the number of concerts/animal was recorded for the subsequent five minute period. The change in the number of concerts between the two time periods was used as a measure of induced concert activity by the substance at the specific concentration. The effect of GSH on concert frequency is shown in Figure 4. There is an increasing rise in concert frequency with higher concentrations of GSH, starting at 1×10^{-10} M GSH levelling off to a plateau value at about 8×10^{-9} M GSH. Although there is considerable variability in concert frequencies, as shown in the wide scatter of points, both the half-maximal and the saturation values are much lower than those for mouth opening or inhibition of contractions, which are in the range of 10^{-6} M GSH (Lenhoff, 1961b; Rushforth, 1965). Thus, this assay shows that extremely small concentrations of GSH increase the frequency of spontaneous concerts. With GSH concentrations above 8×10^{-9} M, concert frequencies decrease as the coordinated sweeping movements of the tentacles change into vigorous writhing activity. Analogs of GSH also increase concert frequency, maximal response levels for S-methyl GSH, S-acetyl GSH and oxidized GSH being attained at concentrations of approximately 8×10^{-8} M, 1×10^{-7} M and 7×10^{-9} M respectively. However, a number of other substances tested at concentrations up to 10^{-6} M had no effect on concert frequencies. These were: proline, glycine, NaCl, NaOH, urea, nicotinic acid, glutamic acid, sodium citrate, and acetic acid, many of which were found by Forrest (1962) and Burnett *et al.* (1963), to elicit feeding activity in *Hydra.*

3. Inhibition of contractions during feeding

(a). *Tentacle contractions.* In unstimulated hydra, spontaneous activity of the tentacles consists primarily of single contractions of individual tentacles, or bursts of contractions of one or more tentacles. There is an increased frequency of such tentacle contractions before the contraction of the body column of the hydra (Rushforth, in preparation). During the capture and engulfment of a single *Artemia* endogenous tentacle contractions are supplanted by localized activities

of the stimulated tentacle and concerted oral flexions of the rest of the tentacles. With greater stimulation, by multiple *Artemia* or GSH, the tentacle activities consist of uncoordinated writhing movements for extended time periods, gradually giving rise to concerts. During this behavioral sequence, the normal spontaneous contraction activities of the tentacles are suppressed. Those contractions are gradually restored to prestimulation levels, approximately 15 minutes after *Artemia* engulfment (Fig. 5).

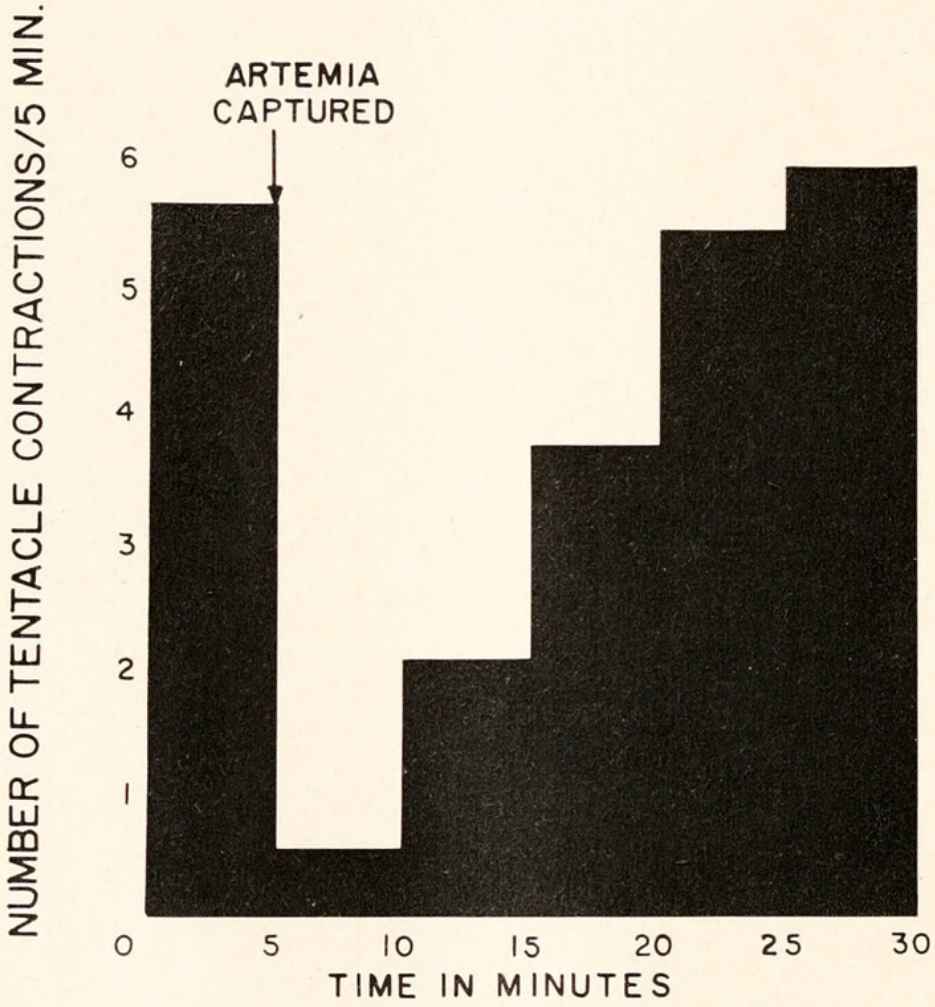

FIGURE 5. Effects of exposure to live *Artemia* on the frequency of tentacle contraction in *H. littoralis;* number of tentacle contractions/animal/5 min, 15 min before and after capture of a single *Artemia,* for a group of 10 hydra.

(b). *Column contractions.* Column contraction is perhaps the most striking spontaneous activity of hydra. In *H. pirardi* most contractions occur in bursts, while in *H. pseudoligactis* contractions tend to occur as single widely-spaced events (Rushforth, 1971). In *H. littoralis* the temporal pattern of contraction activity appears intermediate between that of these two species (Rushforth, 1966). With all species, contractions of the body column are inhibited during feeding (Rushforth *et al.,* 1964). The results of the following experiments clearly show that such inhibition is chemically mediated. One group of 10 hydra was flooded with a large number of *Artemia salina* nauplii and the hydra were allowed to feed for a 15 minute period. If the culture water was not changed at this point, the column contraction frequencies were significantly lower ($P < 0.01$) than those

of unfed hydra, over a subsequent 90 minute period (Fig. 6B). If, in contrast, the culture water in which the hydra had been feeding for a 15 minute period was changed, the contraction frequencies were significantly higher ($P < 0.01$) than unfed controls (Fig. 6A). This indicates that the ingestion of *Artemia* itself enhances column contractions while substances liberated to the medium during feeding inhibit them. Inhibition of column contractions occurs when hydra are exposed to: (1) extracts of *Artemia;* or (2) 10^{-5} M GSH (Fig. 6C). Further experiments showed that suppression of column contractions with feeding stimuli

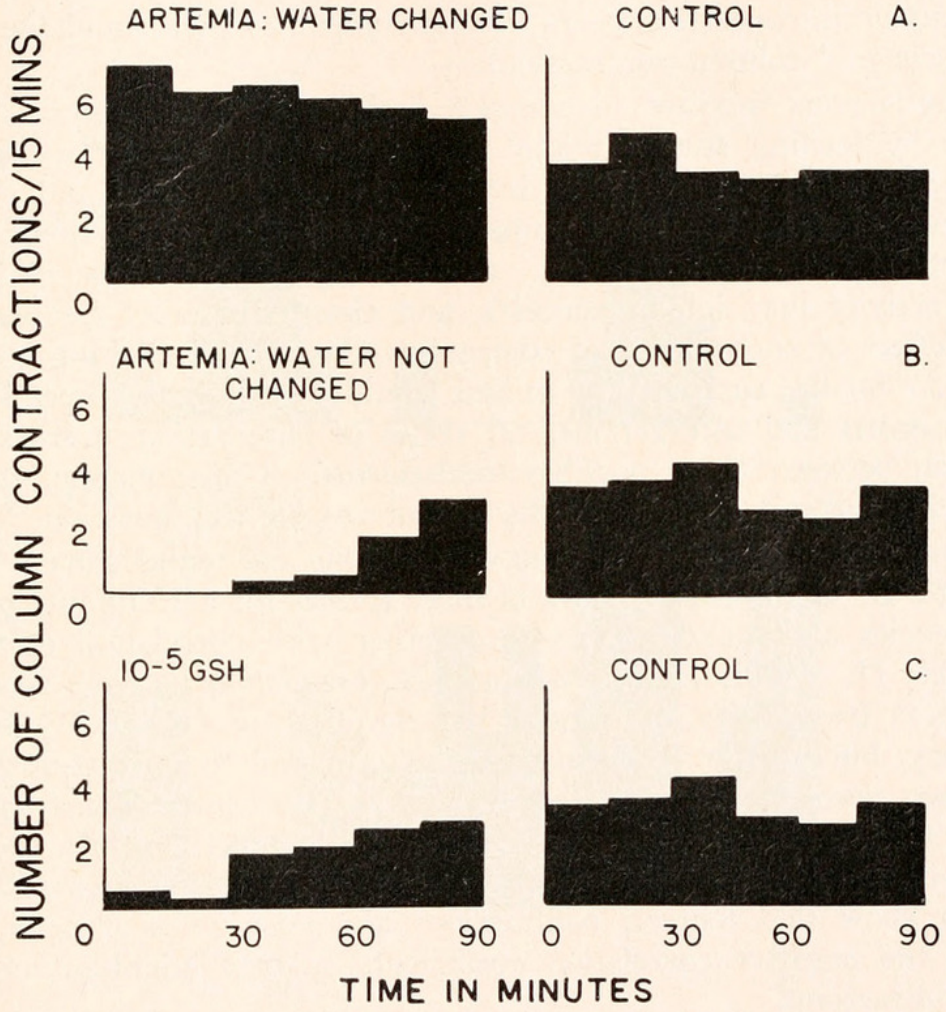

FIGURE 6. Effect of feeding stimuli on column contraction frequency in *H. littoralis;* A: numbers of column contractions/animal/15 min for 10 hydra fed excess *Artemia,* after the culture water was charged (time 0) compared with those for 10 control hydra; B: same as A above when the culture water was not changed; C: column contraction frequency after exposure to 10^{-5} M GSH.

occurred with species other than *H. littoralis.* The culture water in which *H. pirardi, H. pseudoligactis* or *H. fusca* had been feeding on excess *Artemia* when *filtered,* significantly reduced column contraction frequencies of *H. pirardi.* In addition, 10^{-5} M GSH inhibited column contractions in all three species.

4. Relationship between concerts and tentacle and column contractions

The experiments whose results were described above indicate that feeding stimuli enhance the frequency of concerts while inhibiting tentacle and column

contractions. A further experiment was performed to determine the temporal sequence giving rise to increased concert frequency and suppression of tentacle and column contractions when *H. littoralis* was fed a single nauplius. The frequencies of concerts (A), tentacle contractions (B), and column contractions (C), were observed for two control periods of 100 seconds, for each of five hydra. Each animal was given a single *Artemia* and the frequencies of the behavioral events were recorded for the next 30 minutes. The averaged frequencies are plotted in Figure 7. Over the first half of this time period both the frequency of column contractions and tentacle contractions are reduced (Fig. 7B and 7C). A subsequent reduction in concerts appears to be accompanied by a simultaneous restoration of tentacle and column contractions.

The simultaneous increase in concerts and decrease in column and tentacle contractions by feeding stimuli might be explained by one or more of several mechanisms: (1) concerts might be inhibitory to both column and tentacle contractions, such contractions being suppressed as a secondary effect of induced concerts with feeding stimuli; (2) alternatively, mechanisms giving rise to contraction activity may inhibit concerts, and elevated concert frequency may be an indirect effect of suppression of contractions by stimuli eliciting feeding activities; and (3) finally, such feeding stimuli could jointly have direct but opposite effects on concerts and contractions. A series of observations were made to try to distinguish between these possible mechanisms. Unfortunately, these studies were somewhat indirect and the results do not resolve this question.

The frequencies of concerts, column contractions and tentacle contractions were determined for 60 unstimulated hydra of three species for a 15 minute period. The mean frequencies and standard errors, together with correlation coefficients are given in Table II. While *H. littoralis* and *H. pseudoligactis* have similar concert and contraction frequencies, *H. pirardi* has significantly ($P < 0.01$) smaller concert frequency, but greater frequencies of column and tentacle contractions than the other two species. For all three animals, the contraction frequencies for column and tentacles are positively correlated while concert values are negatively correlated with the two types of contractions. Since other studies (Rushforth, in preparation) show that tentacle contractions are most frequent prior to column contractions the negative correlation coefficients suggest inhibition between concerts and contractions.

Passano and McCullough (1964) previously showed that the frequency of contraction bursts of *H. pirardi* decreased with starvation. It was therefore, of interest to determine the relationship between concert activity and the nutritional state of the animal. If concerts were suppressed by tentacle and column contraction, or they were mutually inhibitory, an increase in concerts with starvation, might result from decreased contraction activity. It seemed quite plausible that concert frequency might increase with starvation, since both nematocyst discharge and the duration of mouth opening, two other components of coelenterate feeding behavior are enhanced by starvation (Wagner, 1904; Pantin and Pantin, 1943; Lenhoff, 1961b). However there is a reduction in concert frequency as well as tentacle and column contractions with progressive starvation (Table III).

Excision of the hypostome and tentacles suppresses column contractions in *H. pirardi* (Passano and McCullough, 1964). Removal of the tentacles alone

slightly reduces the frequency of spontaneous contraction bursts in this animal (Rushforth, in preparation). If *H. pirardi* is exposed to GSH after removal of the tentacles, the normal inhibitory effects on spontaneous contraction burst activity are observed. Thus, GSH inhibition of column contractions does not take place exclusively by mechanisms requiring input from the tentacle. However, using a more sensitive assay system for the effects of GSH on *H. pirardi*

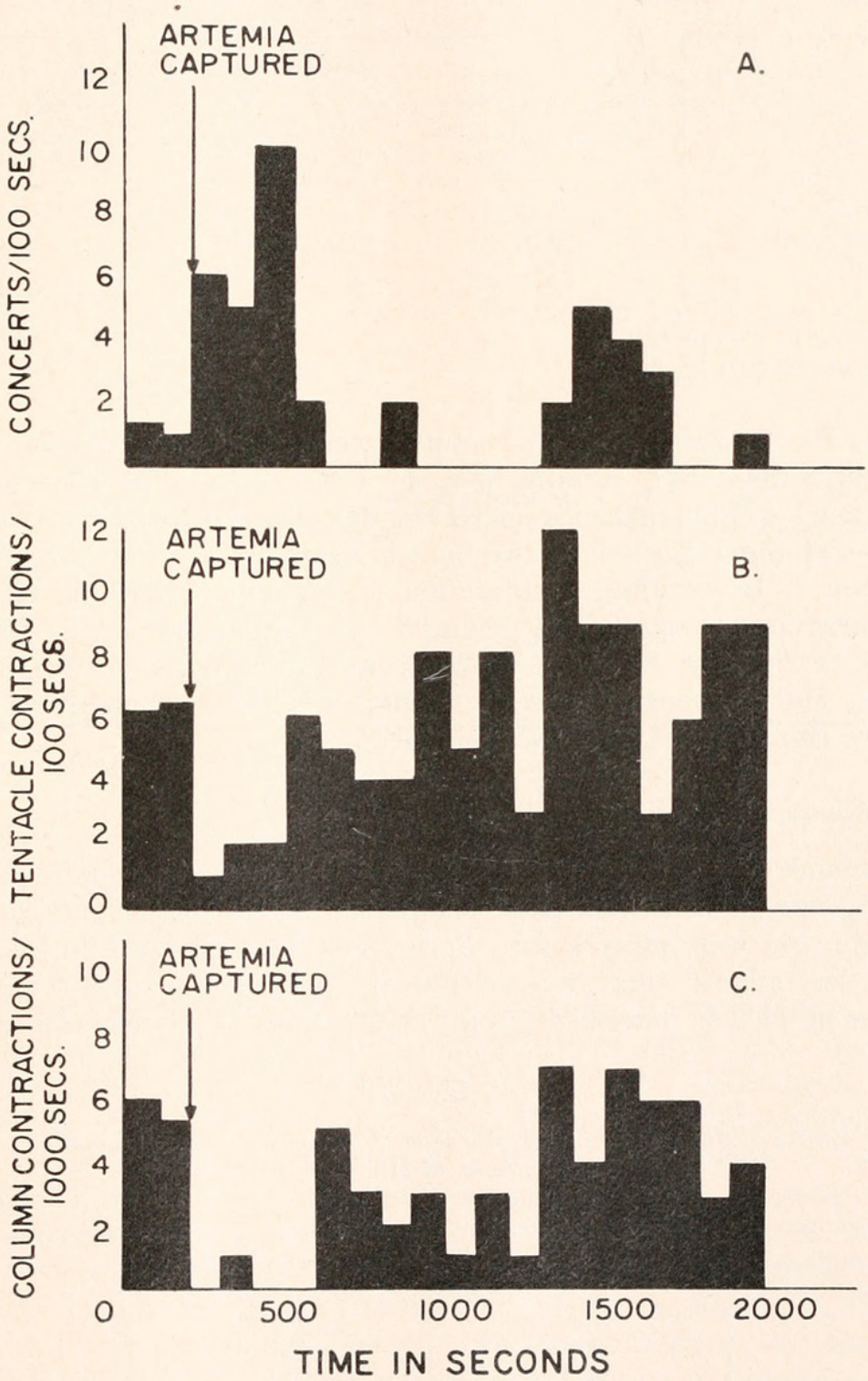

FIGURE 7. Effect of a single *Artemia* on behavioral sequences in *H. littorialis;* A: number of concerts/animal/100 sec before and after *Artemia* capture; B: number of tentacle contractions/animal/100 sec before and after *Artemia* capture; C: number of column contractions/animal/100 sec before and after *Artemia* capture (based on a group of 5 hydra).

TABLE II

Relative frequencies of concerts, column and tentacle contractions in three species of Hydra

	Concerts	Tentacle contractions	Column contractions
H. littoralis	3.1 (0.2)	1.5 (0.2)	4.1 (0.2)
H. pseudoligactis	2.8 (0.3)	1.6 (0.2)	4.4 (0.3)
H. pirardi	0.5 (0.1)	7.9 (0.4)	9.3 (0.4)
	Correlation coefficients		
	Concerts *vs.* tentacle contractions	Concerts *vs.* column contractions	Tentacle contraction *vs.* column contraction
H. littoralis	−0.28*	−0.10	0.29*
H. pseudoligactis	−0.26*	−0.20	0.15
H. pirardi	−0.42**	−0.18	0.53**

* Significant at 0.05 level.
** Significant at 0.01 level.

(Rushforth, 1965), the tentacle structures were shown to play a role in suppressing contractions. *H. pirardi,* with or without tentacles contract to strong light. Removal of the tentacles increases the time between onset of the light stimulation and the initiation of the light-induced contraction burst (Rushforth, in preparation). In addition, the duration of inhibition by 10^{-5} M GSH of light-induced contractions is significantly reduced after tentacle removal (Fig. 8). This result suggests that the tentacles do contribute to GSH suppression of column contractions, and is consistent with Lenhoff's (1961a) hypothesis that GSH receptors are concentrated on these structures.

Electrophysiological correlates of feeding responses

Passano and McCullough (1963) showed that both tentacle and column contractions were preceded by large compound potentials. Electrical recordings of individual hydra were taken before, during and after exposure to feeding stimuli, in order to determine if there were electrical correlates for any of the behavioral modifications previously described. Joint observations of the behavior and electrical

TABLE III

Effects of starvation on the frequencies of concerts, column and tentacle contractions in H. littoralis
Number/15 minutes

Days since feeding	Concerts	Tentacle contractions	Column contractions
0	3.8	1.0	3.5
1	2.0	1.0	3.0
2	1.3	0.5	0.8
3	1.2	0.5	1.2
4	0.5	0.2	0.8
5	0.5	0.2	0.8

activity of several species of *Hydra* have failed to show electrical potentials associated with spontaneous concerted oral flexions. However, Tentacle Contraction Pulses (TCP's) and Column Contraction Pulses (CP's) always associated with tentacle contractions and column contractions respectively, occur spontaneously as single events and in bursts. When *H. pirardi* feeds on *Artemia,* CP bursts and singles are suppressed. TCP bursts are absent although single TCP's occur during this period. Rhythmic pulses are evident throughout feeding, as was first observed by Passano and McCullough (1963). After ingestion of the *Artemia,* CP's return first as single events and then as CP bursts of enhanced frequency. Associated with the return of CP's is a marked increase in TCP firing.

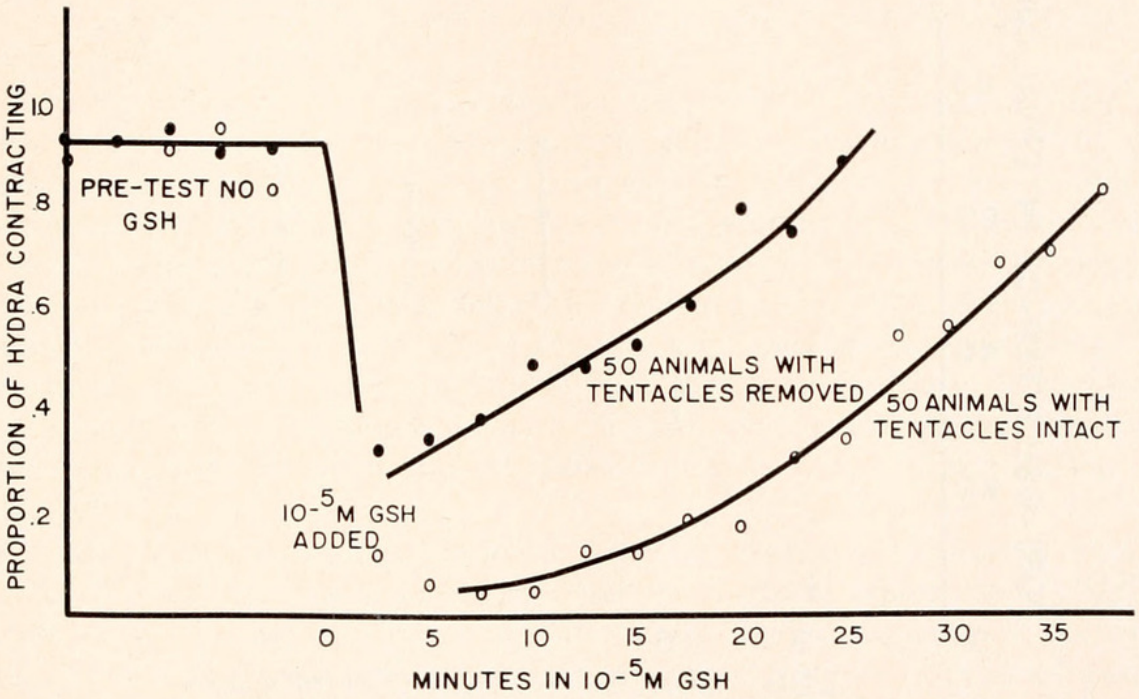

FIGURE 8. Effect of tentacle removal on light-induced contractions in *H. pirardi.* Per cent of a group of 50 hydra with tentacles removed (closed circles) and a group of 50 intact hydra (open circles) contracting in a 75 second period of bright light followed by a 75 second period of ambient illumination.

When a preparation of *H. pirardi* is exposed to 10^{-5} M reduced glutathione, effects similar to those resulting from contact with *Artemia salina* occur in pacemaker activity. Column Contraction Pulses are inhibited, returning first as singles and then as CP bursts. Tentacle contraction bursts are also inhibited but return as TCP bursts. The Rhythmic Potential Pacemaker System is not, however inhibited by GSH. The frequencies of TCP's and CP's are markedly reduced on administration of GSH, but return at higher than prestimulation values, before returning to control levels. As in the case of exposure to *Artemia,* inhibition of CP's is longer than for TCP's. The duration of the blockage of CP's in *H. littoralis* was determined as a function of GSH concentration over the range 10^{-5} to 10^{-9} molar. The length of inhibition of spontaneous CP's increases with GSH concentration from 10^{-9} to 10^{-7} M (Fig. 9).

In the final stages of formation, prior to detachment from the column of the hydra, a bud exhibits CP's independently of the parent's CP's. However, if

feeding stimuli are administered to the preparation, both parent and bud CP's are suppressed. This preparation afforded a suitable method of showing that during a capture of an *Artemia* by the parent hydra sufficient chemical stimulation is released to suppress CP's of the bud. The same effect was observed using two budless specimens of *H. littoralis*. The capture of *Artemia* in one hydra released sufficient chemical feeding stimuli not only to suppress CP's of that animal, but also to inhibit CP's of the other hydra for a short time period. In addition, the concert frequencies were significantly increased in both animals.

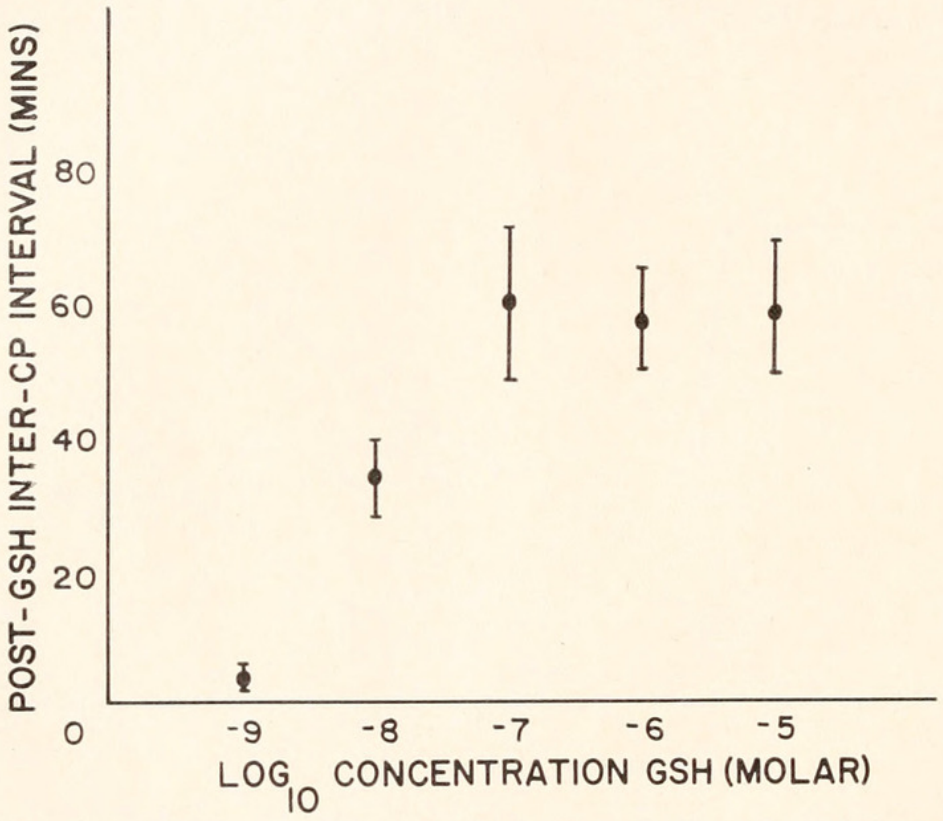

FIGURE 9. Effect of GSH concentration on inhibition of CP's in *H. littoralis,* Post-GSH inter-CP interval plotted against $\log_{10}$ GSH concentration. This interval length measures the time in minutes from a CP immediately preceding GSH administration to the next CP that is followed by another CP within a 10 minute period. Mean values and ranges are based on five hydra.

DISCUSSION

1. Overt behavioral responses of feeding

As previously pointed out by Josephson (1965c, page 34) the mechanisms underlying feeding behavior in *Hydra* can be explained as "linked sequences of local responses, each component being initiated by the results of the preceding one." The first element in feeding behavior is the attachment of the prey to an outstretched tentacle by means of nematocyst discharge. The two types of nematocyst involved in prey capture, stenoteles and desmonemes, both discharge to the mechanical stimulation caused by prodding the tentacles with a clean glass rod. The threshold for such activation is drastically lowered, however, by the presence of food extracts. This has been shown by Pantin (1942) for the anemone *Anemonia,* and for *Hydra* by Ewer (1947). The desmonemes coil around the

prey entrapping it, while the stenoteles pierce its exoskeleton in a harpoon-like fashion. The discharged nematocysts are released from the tentacle, as the prey organism is enclosed by the mouth (Ewer, 1947).

As the prey is attached to the tentacle by nematocyst discharge, after a latent period of a few seconds, the stimulated tentacle moves to bring the prey in contact with the mouth. Josephson (1965c) observed that sometimes this latency lasts up to a minute, while in the hydroid *Pennaria* a delay lasting up to 30 seconds is not uncommon (Fred Delcomyn, University of Oregon, personal communication). Such latencies appear surprisingly long and are difficult to explain. Possibly they occur in cases where the *Artemia* is ensnared so that the initial movements of the prey are not vigorous enough to elicit immediate tentacle movement. However, as the *Artemia* continues to struggle, finally the threshold for tentacle activity is reached.

Distinctly different tentacle movements are produced depending on the position of prey attachment. However, the reaction time appears independent of the type of movement. This result strongly suggests that the times for conduction of impulses, or diffusion of chemical factors from the point of prey attachment to the hypostome, where presumably coordination of tentacle movements takes place, do not play a major role in determining the reaction time. The occurrence of such movements following nematocyst discharge with mechanical stimulation by an inert object, suggests that the stimulus from the prey for these behavioral components is probably mechanical in nature. The contraction of the tentacle below the point of prey attachment, and not above it, implies polarized conduction towards the hypostome.

A third behavioral sequence in the feeding response, concerted oral movements of the tentacles, is primarily a result of chemical stimulation. While such concerts occur spontaneously, usually in bursts, their frequency is increased by dilute extracts of *Artemia* or extremely small concentrations of reduced glutathione or its analogs, (*i.e.*, below 10^{-8} M). Such chemical stimulation may occur in nature by release of GSH from the prey organism, as was proposed for other elements of feeding behavior by Loomis (1955). Alternatively, the release might be from the discharged nematocyst capsule itself as postulated by Burnett *et al.* (1963), although Lenhoff (1968a) has shown this second alternative to be improbable in the case of the mouth opening response (which occurs at a much higher GSH concentration). Chemical activation of repeated concerts at dilute concentrations, and of tentacle writhing at higher concentrations, results from stimulation via the external environment. However, a single concerted oral flexion of the tentacles invariably follows movement of a stimulated tentacle if nematocyst discharge occurs with mechanical stimulation. This suggests that the concert and basal tentacle contraction may be linked internally rather than through the external medium. Tentacle writhing and mouth opening are chemically initiated (Wagner, 1904; Ewer, 1947; Loomis, 1955; Lenhoff, 1961b). Both sequences are induced by GSH at concentrations ($10^{-6} - 10^{-7}$ M) much higher than that giving maximal concert frequencies in *H. littoralis* (about 10^{-8} M). The lower threshold for chemical stimulation of concerts than for mouth opening, suggests why concerts occur earlier in the feeding response. Concerted oral flexions of the tentacles occur when the prey organism is held above the hypostome before

ingestion. Frequently the tentacle opposite to that attached to the *Artemia* pushes the prey towards the mouth region in the concert movement. At higher GSH concentrations the mouth begins to open and creep over the prey, and the concerts give rise to uncoordinated tentacle writhing. Possibly such writhing movements are the result of excess stimulation of mechanisms coordinating the concert activity.

The later stages of feeding behavior in *Hydra* consist of mouth creeping around the prey, closing about it, a reduction in the vigor of tentacle writhing, and a gradual return of coordinated concert frequencies to base-line levels. Throughout the feeding sequences the endogenous contractions of both the tentacles and column are inhibited. They return after *Artemia* ingestion, at enhanced frequencies, before they are restored to prestimulation values. Inhibition of contractions and their associated electrical potentials will be discussed later.

Little is known concerning the mechanisms involved in mouth closing and creeping about the prey. Creeping of the mouth may result from stimuli due to contact of the prey on the inside of the mouth. An inert object, such as a pin, will be swallowed when it is placed in the mouth of hydra, in the absence of added GSH (quoted in Kanaev, 1952). An isolated hypostome will creep slowly up and off the shaft of a pin inserted through the mouth (Rushforth, unpublished observations). The hypostome always moves in an oral direction, at a reduced speed if the tentacles are removed. The present day description of mouth movements in the feeding response of *Hydra* go little further than those of Hartog (1880), given almost a century ago. He pointed out that the tentacles play little role in the feeding response as soon as the mouth comes into contact with the food.

Very little is known of the mechanism responsible for mouth closure and termination of the various behavioral sequences. Burnett, Lentz and Warren (1959) show that fully fed hydra are capable of discharging nematocysts and subduing prey. However, once the gastrovascular cavity of the animal is filled, the hydra does not attempt to ingest the captured prey. The mouth appears incapable of opening, due to the internal pressure exerted on the walls of the gastrovascular cavity, as a result of a large intake of water during the early phases of digestion. However, during an extended period following ingestion of *Artemia* (1–6 hours) the mouth may open to engulf single prey while the hydra forms a tight constriction in the region just below the hypostome (Blanquet and Lenhoff, 1968). This "neck formation" was shown in *H. pirardi* to be caused by a combination of three factors: (a) glutathione on the exterior of the hydra; (b) distension of the wall of the hydra's body column; and (c) the presence of tyrosine within the gut. Tyrosine was found to be highly specific in causing neck formation. No other amino acid, including phenylalanine was active, and tyrosine having either the α-amino or α-carboxyl blocked had no effect (Blanquet and Lenhoff, 1968).

2. Electrical correlates of feeding behavior

Throughout the initial phases of feeding the spontaneous contraction of the tentacles and body column are suppressed. These recurring simple behavioral events which result from pacemaker activity, are supplanted by the linked se-

quences of local responses described above. Similar effects are seen with other coelenterates. Long term modification of the rhythmic contractions of the body wall of *Metridium* have been observed with food extracts (Pantin, 1950). Suppression of the swimming response by food extracts was also reported by Ross and Sutton (1964) for *Stomphia.* The control mechanisms involved in swimming and feeding in this sea anemone appear mutually inhibitory, since there is also suppression of nematocyst discharge during swimming. Such findings suggest that nematocysts do not always act as purely independent effectors. Inhibition of the discharge of atrichous isorhizas, the nematocyst type used in hydra's locomotion has been found to occur with food extracts (Ewer, 1947).

In *Hydra,* feeding stimuli also inhibit the production of electrical potentials which are associated with column contractions (CP's) and contractions of the tentacles (TCP's). After ingestion of *Artemia* both the contractions and their associated potentials return at enhanced frequencies. It was initially thought that the increase of body contractions resulted from the mechanical stimulation to the gut of the hydra produced by the engulfed *Artemia.* However, the same effect was observed with extracts of *Artemia* and 10^{-5} M GSH. The process is reminiscent of post-inhibitory rebound observed in neural systems (Erlanger and Glasser, 1937) and occurs in *Hydra* whenever the endogenous contractions and correlated bioelectric events are inhibited by external stimuli (Passano and McCullough, 1964; Rushforth, 1971).

In another hydroid, *Tubularia,* concerts and associated electrical potentials are suppressed during feeding (Josephson and Mackie, 1965), and are inhibited by the presence of food extracts (Rushforth, 1969). In view of the inhibitory effects on both the behavior and electrical activity of *Hydra* and *Tubularia,* together with the reports of suppression of behavioral events with feeding in sea anemones, one may conjecture as to the generality of the phenomenon. Clearly even in other hydroids, spontaneously recurring behavioral events and large compound electrical potentials are not always present. However, in *Cordylophora, Pennaria* and *Hydractinia* where there is an apparent lack of endogenous behavior and electrical activity, feeding leads to a dramatic electrical awakening in such polyps (Mackie, 1968, Fred Delcomyn, personal communication). As a working hypothesis, it is proposed that in hydroids and possibly other coelenterates, feeding stimuli, in addition to initiating reflexly linked local responses either (1) inhibit spontaneous movements and correlated potentials or (2) induce electrical activity in normally quiescent animals. In addition, such feeding stimuli may inhibit responses to external stimuli: light and mechanically induced contraction in *Hydra* (Rushforth, 1965); swimming responses in *Stomphia* initiated by inert objects previously rubbed on the aboral surface of the starfish *Demasteras* (Ross and Sutton, 1964); and gastrozooid contraction following a strong mechanical stimulus to the basal mat of the colony of *Hydractinia* (Darrell Stokes, personal communication).

The mechanisms by which feeding stimuli simultaneously elicit concert activity, and suppress tentacle and column contractions in *Hydra,* are unknown. Sustained behavioral observations together with electrical recordings have failed to reveal electrical correlates of concert activity. Even in recordings of isolated tentacles no pulses were observed to correspond with sweeping movements pre-

sumably the same as concert activity in the whole animal. In this preparation, when tentacle writhing and asymmetric movements occurred in response to feeding stimuli, characteristic single monophasic potentials supplant the normal bursts of pulses (TCP's) associated with symmetric contractions of the longitudinal muscles (Rushforth and Burke, 1971). However, there is insufficient evidence to indicate that the monophasic pulses are produced by a discrete pacemaker system separate from the Tentacle Contraction Pacemaker System.

The mode of inhibition of column contractions and the associated CP's is also unknown. It is unlikely to result from direct effects on the transepithelial potential (Josephson and Macklin, 1969) since GSH while drastically modifying CP activity has no apparent effect on this potential (Rushforth and Burke, in preparation). In *Tubularia,* inhibition of concerts and associated HP's with live *Artemia* or *Artemia* extracts occurs by activating a specific conducting system, the Distal Opening System (Rushforth, 1969). This system produces small potentials (DOSP's) on electrical stimulation, and was previously shown to be inhibitory to pacemaker systems, and to suppress concerts, as it initiated aboral flaring of the distal tentacles (Josephson and Uhrich, 1969). Possibly in *Hydra* a similar mechanism occurs, but no conducting system similar to the Distal Opening System has been discovered. It should be recalled that in *Hydra* not all of the pacemaker systems are inhibited during feeding, since RP's occur throughout the sequences of the feeding behavior (Passano and McCullough, 1965). In *Sarsia,* feeding produces "swallowing pulses" which are conducted from the manubrium to the tentacle bases; these pulses seem to inhibit swimming pulses by the latter's pacemakers (Passano and Hernandez-Nicaise, 1967).

In the past decade considerable effort has been invested in studies of the "physiochemical aspects of the macro- and micro-environments surrounding *Hydra* during activation of their feeding behavior" (Lenhoff, 1965). However, our knowledge of mechanisms within the animal, controlling its sequential feeding responses is quite rudimentary. Clearly both excitatory and inhibitory processes are involved, and sequences appear to consist mainly of local responses, linked by stimuli from the external environment. In some coelenterates coordination of contiguous effectors via externally mediated stimuli may be associated with inhibition of pacemaker activity. The possible interrelationships between the two types of behavior nevertheless provide challenges for future study.

Summary

The capture and engulfment of a single *Artemia* nauplius by *Hydra* consists of a series of complex behavioral sequences: (1) nematocyst discharge; (2) tentacular movements; (3) mouth opening, creeping over the prey and closure; and (4) inhibition of endogenous tentacle and body column contractions. The present study focused on two of these sequences: tentacular movements and inhibition of tentacle and column contractions.

On attachment of the prey to a tentacle by nematocysts, there is a latent period (1–3 sec in *H. littoralis*). Then the portion of the tentacle proximal to the prey contracts, sometimes accompanied by oral bending or inward spiralling when the prey attaches to the basal or distal tentacle regions, respectively. The latency is independent of the position of attachment or the type of tentacle movement. This

implies that neither conduction time nor the time for the diffusion of chemical factors to the hypostome is a predominant component of the latent period.

As the prey nears the mouth on tentacle contraction, the surrounding tentacles concertedly flex orally (a concert). Concerts are frequent during and following the engulfment of prey. They are highly coordinated movements, unlike tentacle writhing, which is also frequently observed when hydra feeds. Concert frequency is markedly enhanced by exposure of the hydra to homogenates of *Artemia,* reduced glutathione (GSH) or analogs of GSH. In *H. littoralis* concert frequency increases with GSH concentration starting at 1×10^{-10} M GSH and levelling off to a plateau value at about 8×10^{-9} M GSH, above this concentration tentacle writhing is induced. No electrical correlates of concerts have been observed.

Column and tentacle contractions are inhibited during feeding. Such inhibition is also observed with extracts of *Artemia* and GSH. Concerts are negatively correlated both with tentacle and column contractions in several species of *Hydra.* However, the spontaneous frequencies of all three behavioral events decrease with starvation over a period of several days. GSH inhibits endogenous column contractions in *H. pirardi* without tentacles, but removal of tentacles significantly reduces inhibition of light-induced contractions in this animal.

Electrical potentials associated with tentacle contractions (TCP's) and column contractions (CP's) are suppressed when hydra feed on *Artemia* or are exposed to extracts of *Artemia,* or GSH. After such inhibition both types of potentials return together with contractions, at enhanced frequencies. The duration of the blockage of spontaneous CP's increases with GSH concentration over the range 1×10^{-9} M to 10^{-7} M, above which the length of inhibition is constant. A single *Artemia* fed to a hydra releases sufficient chemical factors into the medium to increase concert frequencies and suppress CP's in an attached bud of the fed animal, or in a second hydra some centimeters away from it.

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