Activation of the Feeding Reflex in *Hydra littoralis*

I. *Role played by reduced glutathione, and quantitative assay of the feeding reflex*

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ABSTRACT A simple and accurate quantitative assay of the glutathione-activated feeding reflex in *Hydra* is described. The results show: (*a*) There are a limited number of receptor-effector systems, probably localized in the area immediately around the mouth and on the tentacles. (*b*) Concentrations of glutathione greater than 5×10^{-6} M activate all these systems; 10^{-6} M glutathione elicits a half-maximum response. (*c*) Glutathione must be constantly present at the receptor site in order for a response to occur. (*d*) The response stops in the presence of excess glutathione because of some change within the *Hydra*, and not as a result of any alteration to the glutathione. The present state of knowledge concerning the mechanism by which glutathione combines with and activates the glutathione receptor to elicit the feeding reflex is summarized.

Many biological responses are initiated as the result of a specific excitatory compound combining with its cellular receptor. In order to gain some insight into the action of excitatory compounds, we are investigating the mechanism by which glutathione combines with the glutathione receptor of *Hydra* to elicit the coordinated feeding reflex.

The glutathione-*Hydra* system offers advantages for investigating the action of excitatory substances. For one, the action can be studied on a structurally intact live organism (1). *Hydra*, furthermore, can be grown in a relatively simple, rigidly controlled, fluid environment (*Hydra* require only traces of calcium (2) and sodium (3) in distilled water). The response is initiated by a specific compound, the activity of glutathione residing in the specific structural characteristics of its peptide backbone (1, 4, 5); the response is not dependent upon the generally known reducing properties of this molecule. In

addition, glutathione is readily available in the pure form, and has many known analogues. And, finally, the response to the excitatory compound can be determined simply and accurately by visually measuring the duration of the mechanical process of mouth opening.

The present investigation was undertaken with the purpose of determining: first, a quantitative and reliable assay of the feeding reflex; second, the role played by glutathione once the response is initiated; third, the location of the receptor; and fourth, the quantitative aspects of the saturation of the receptor by glutathione.

MATERIALS

Growth and Maintenance of Hydra Meaningful measurements of the feeding reflex require large numbers of *Hydra* that respond to glutathione in a quantitatively reproducible manner. We obtained such experimental animals by starving for 1 or 2 days mass cultures of *Hydra littoralis* (6) that had been reproducing asexually in 10^{-3} M $CaCl_2$ and 10^{-4} M $NaHCO_3$ in deionized water. Special care was taken to remove most of the organic waste products from the cultures twice daily (6). The animals in each tray were not allowed to reach a density of over two or three thousand hydranths per 1500 ml of culture solution.

METHODS

Description of the Normal Feeding Reflex Before measuring the feeding reflex, the observer should be familiar with the characteristic coordinated movements of the *Hydra* that are elicited by glutathione. These movements have been described earlier by Ewer (7) and Loomis (1). The drawings in Fig. 1 illustrate each of these steps.

A *Hydra* in the absence of the glutathione is shown in Fig. 1A; the mouth is closed, and the tentacles are outstretched and relatively motionless. After the addition of glutathione, the tentacles first begin to writhe and sweep inwards toward the central vertical axis of the animal (Fig. 1B). Next, the tentacles bend toward the mouth, and the mouth opens (Fig. 1C). Shown in this composite drawing (Fig. 1C) are the various positions that a tentacle takes before contracting. These movements, culminating in mouth opening, usually all take place within half a minute. Fig. 1D shows how a *Hydra* looks during the greater portion of the feeding reflex, its mouth open wide and the tentacles in various phases of contraction. Frequently, the tips of the tentacles are observed within the *Hydra's* mouth, as shown in Figs. 1C and 1D.

It should be emphasized that in the presence of added glutathione, *Hydra* can be made to manipulate and ingest non-living material devoid of endogenous glutathione. This ability to ingest inert food as a result of chemical stimulation, as pointed out by Loomis (1), is the most important characteristic of the feeding reflex. Caution should be taken not to confuse the normal feeding reflex with any abnormal mouth openings which occur in response to non-specific agents. Many deleterious compounds, such as acids, cause the *Hydra* to open their mouths. However, we have observed that

these gaping *Hydra* do not carry out the characteristic feeding reflex, cannot be made to ingest dead food, and usually die within a few hours.

Quantitative Measurement of the Feeding Reflex Five starved *Hydra*, obtained from the mass cultures, were rinsed three times in 30 ml portions of a solution lacking glutathione and consisting of 10^{-3} M $CaCl_2$, 10^{-4} M NaCl, and 10^{-3} M histidine chloride buffer, pH 6.2. The five *Hydra* were then transferred in one drop of the solution into 2 ml of the same solution containing glutathione (Sigma Chemical Co., St. Louis).

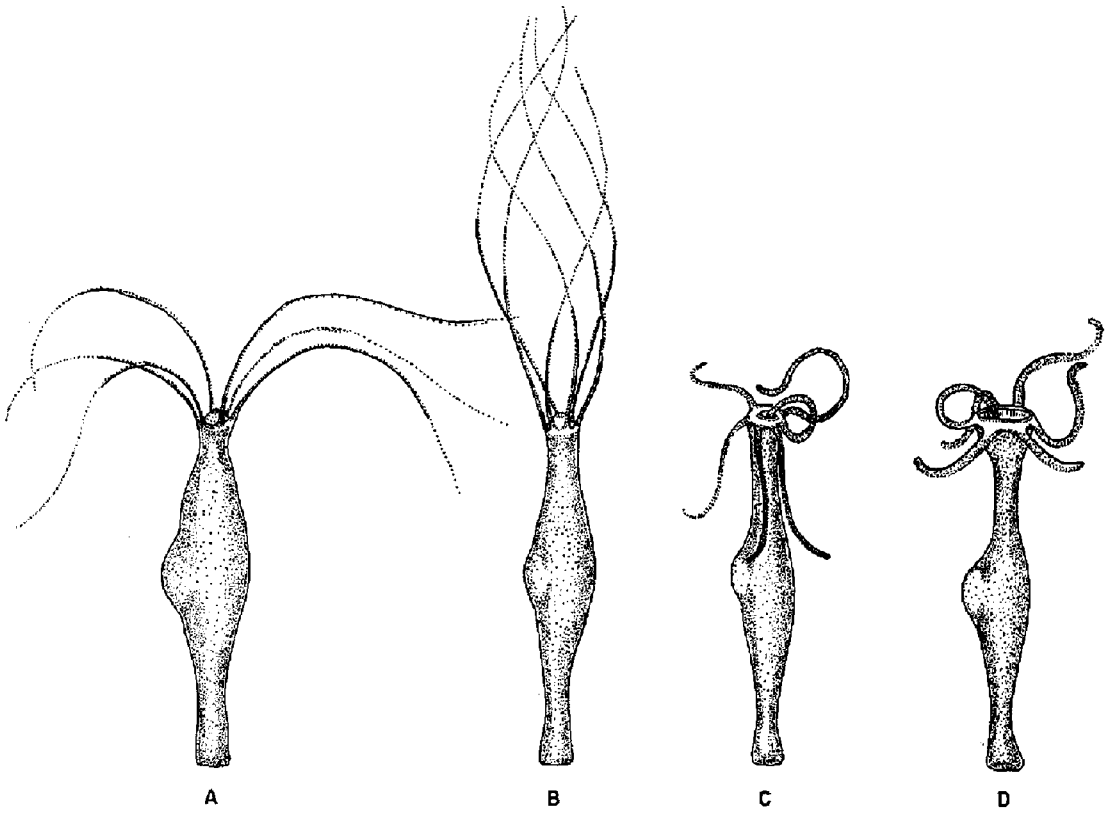

FIGURE 1. Stages of the normal feeding reflex (see text).

(Reduced glutathione is not readily oxidizable at pH 6.2.) This glutathione solution was in the spherical concavity (36 mm diameter × 5 mm deep) of a Maximov tissue culture slide. The *Hydra* were immediately observed through a binocular dissecting microscope set at a magnification of 19.5. The time intervals between the moment the *Hydra* were placed in the glutathione solution and the initial and final times (t_i and t_f) that the mouth of each animal was open were recorded. The magnitude of the feeding reflex is expressed as the average time ($t_f - t_i$) during which the mouths of the five *Hydra* remained open in response to glutathione.

RESULTS

In Table I are shown the results of five different experiments (a–e) which were carried out in excess glutathione. In these experiments the individual *Hydra* opened their mouths within 0.4 to 1.0 minutes (t_i) after being placed in the glutathione solution. Under optimal conditions, the variations observed in the opening time t_i were small when compared to t_f, and did not significantly alter the over-all time during which the mouth was open ($t_f - t_i$).

TABLE I
METHOD OF EXPRESSING THE DURATION OF THE FEEDING REFLEX

Experiment	Glutathione concentration	t_i	t_f	$t_f - t_i$ Mean ± S.D.
		min.	*min.*	*min.*
a	10^{-5} M	0.43, 0.46, 0.60, 0.78, 1.33	33.00, 35.36, 38.08, 39.71, 41.00	36.71±2.95
b	10^{-6} M	0.50, 0.53, 0.71, 0.88, 0.91	32.80, 33.16, 36.25, 36.43, 38.11	34.64±2.10
c	7.5×10^{-6} M	0.38, 0.86, 0.91, 0.95, 1.00	29.88, 32.00, 32.25, 33.91, 41.91	33.17±4.51
d	7.5×10^{-6} M	0.43, 0.46, 0.58, 0.68, 0.96	28.21, 36.50, 36.50, 38.30, 43.45	35.97±5.30
e	5×10^{-6} M	0.48, 0.50, 0.61, 0.78, 1.05	26.88, 35.25, 37.41, 42.00, 43.41	36.31±6.36
f	5×10^{-7} M	0.68, 2.33, 2.63, 4.75, ∞	5.08, 16.25, 22.50, 25.60, —	11.81±9.29
g	2×10^{-6} M (+10^{-4} M glutamine)	2.45, 3.75, 6.00, ∞, ∞	6.41, 13.45, 22.91, —, —	6.11±7.23

Hydra starved for 2 days were used in all experiments.

The closing time (t_f) for the individual *Hydra* in each experiment (Table I, Experiments a through e) was about 35 minutes. Because the standard deviations were small in comparison to the total length of the response, they were not routinely calculated.

At suboptimal concentrations of glutathione (Table I, Experiment f), or in the presence of a compound known to compete with glutathione for the glutathione receptor (5) (Table I, Experiment g), some *Hydra* took as long as 6 minutes to open their mouths, while others did not carry out the feeding reflex at all. In these cases, the standard deviation is large relative to $t_f - t_i$. Data of this type are similarly expressed as the average time ($t_f - t_i$) during which the mouths of the *five Hydra* tested remained open regardless of the number that responded positively. In addition, appropriate superscripts and symbols are used in tables and figures to indicate the number of animals responding (see legends, Table IV and Fig. 2).

The values for $t_f - t_i$ at excess glutathione concentrations are usually between 25 and 35 minutes, depending upon whether the *Hydra* were starved 1 or 2 days respectively preceding the experiment. This fact should be borne in mind when comparing data from different sets of experiments (see Discussion).

Effects of Removing Glutathione during the Feeding Reflex Groups of *Hydra* were incubated in 10^{-5} M glutathione for periods varying from 5 to 25 minutes (Table II). At the end of each incubation period, the *Hydra*, in one drop of glutathione solution, were placed in 30 ml of a solution of the same composition but lacking glutathione. In all cases the mouths closed in less than 1 minute (Table II). It was observed that the longer the *Hydra* were exposed to the glutathione, the sooner the mouths closed when the glutathione was removed. These experiments demonstrate that the *Hydra* require the continuous presence of glutathione in the medium in order to carry out the feeding reflex.

TABLE II

TIME REQUIRED FOR MOUTHS TO CLOSE ON REMOVAL OF GLUTATHIONE

Time in glutathione	Time to close
min.	*min.*
5	0.94
10	0.92
15	0.76
20	0.72
25	0.41

All experiments were carried out using *Hydra* starved for 1 day, and at excess glutathione (10^{-5} M).

Effect of Using the Same Glutathione Solution Repeatedly on Different Groups of Hydra The following experiments were carried out in order to determine whether mouth closure results from some intrinsic change within the *Hydra*, or from the oxidation or alteration of glutathione in the culture solution: A group of five *Hydra* was exposed to 2 ml of 10^{-5} M glutathione until their mouths closed (Table III). The same glutathione solution was then transferred to another group of five *Hydra*; this latter group of *Hydra* opened their mouths for 27 minutes, indicating that sufficient glutathione remained to elicit a near maximum response. This transfer process was repeated, and again the *Hydra* responded positively, although for a somewhat shorter time. Using the *p*-mercuribenzoate procedure of Boyer (8), parallel experiments were run in which the respective solutions were assayed for sulfhydryls after the *Hydra* had closed their mouths. No perceptible decrease in the sulfhydryl content of the solutions occurred.

Similar experiments were carried out using the glutathione analogues ophthalmic acid and S-methyl glutathione, compounds that activate the feeding reflex, and are not autooxidizable (4, 5). As shown in Table III, these analogues, like glutathione, retain much of their activity after several exposures to *Hydra*. It can be concluded from all the experiments summarized in Table III that the feeding reflex normally ends for some reason other than the oxidation, disappearance, or alteration of the glutathione molecule; also it does not end because of the accumulation of inhibitors in the culture solution.

TABLE III

RESPONSE OF DIFFERENT GROUPS OF *HYDRA* EXPOSED TO THE SAME SOLUTION OF EXCITATORY COMPOUND USED THREE TIMES

Group of *Hydra*	Duration of feeding reflex $t_f - t_i$, min.		
	Glutathione 10^{-4} M	Ophthalmic acid 10^{-6} M	*S*-Methyl glutathione 10^{-5} M
1	28.06	27.60	29.54
2	27.06	23.08	26.20
3	19.80	23.96	21.72

All *Hydra* were starved for 1 day.

Effect of Removing Hydra from Glutathione Solution and Returning the Same Animals to a Fresh Glutathione Solution The results in Tables I and III show that the feeding reflex stops after about 30 minutes even in the presence of excess glutathione. The questions arise as to (*a*) whether the response, once prematurely interrupted (as in Table II), can resume when the *Hydra* are returned to a glutathione solution, and (*b*) whether the total duration of these responses is independent of the interruptions.

Groups of five *Hydra* (starved for 1 day) were placed in 10^{-5} M glutathione for periods of 5 to 25 minutes. At the end of each exposure the *Hydra* were rinsed in 30 ml of culture medium containing no glutathione; during this rinsing, the mouths closed. Within 2 minutes, each group of animals was placed in 2 ml of fresh solutions of 10^{-5} M glutathione, and most of the mouths reopened. The average length of time that the mouths of the five *Hydra* remained open during this second exposure to glutathione was recorded. The data shown in Table IV demonstrate that total duration of the two combined responses was independent of the time of the initial exposure to glutathione and was not affected by the interruption. The duration of the response on the second exposure was inversely related to the length of the first exposure. In these experiments the total time that each *Hydra* responded to glutathione averaged around 25 minutes.

Effect of Glutathione on Hydra Having Different Numbers of Tentacles and on Isolated Parts of the Animal In order to obtain information concerning the localization and nature of the glutathione receptors, glutathione was added (*a*) to *Hydra* having different numbers of their tentacles removed, (*b*) to *Hydra* with supernumerary tentacles, and (*c*) to various dissected parts of the animal. The results are shown in Table V.

Experiment 1 gives the duration of the feeding reflex of *Hydra* having different numbers of tentacles. Normally *Hydra littoralis* have five to six tentacles. Those with less than five had their tentacles removed by dissection either 15 minutes or 3 hours before the assay. (We waited 3 hours in order to allow any damaged contractile elements time to heal.) The data show that

TABLE IV

SUM OF DURATION OF RESPONSE FROM TWO EXPOSURES TO GLUTATHIONE

Initial exposure	Second exposure	Total time open
$t_f - t_i$, *min.*	$t_f - t_i$, *min.*	*min.*
5.66	18.92	24.52
10.66	17.16[1]	27.82
15.22	8.12[2]	23.36
20.44	3.14[3]	23.58
22.40	2.12[3]	25.02
25.76	0.22[4]	25.98
Average		25.06

The superscripts 1, 2, 3, 4 indicate the number of *Hydra* that did not respond to the second exposure to glutathione. The *Hydra* used in these experiments were starved for 1 day. The glutathione concentration was 10^{-5} M.

the duration of the feeding reflex did not decrease in proportion to the number of tentacles removed although some decrease was observed. Some response occurred even in *Hydra* with all their tentacles removed.

Experiment 2 gives the duration of the feeding reflex elicited by glutathione added to *Hydra* having tentacles ranging from six to nine in number. (These *Hydra* were starved 2 days and thus responded longer to glutathione than did the *Hydra* used in Experiment 1, which were starved for only 1 day.) Supernumerary tentacles were induced by cutting the hypostome (H. D. Heath and E. Wangersky, unpublished). The results show that the duration of the feeding reflex did not lengthen in proportion to the increased number of tentacles; in fact, there was a decrease as the tentacle number approached nine.

Experiment 3 gives the duration of the feeding reflex performed by parts of the *Hydra*. In Experiment 3a, *Hydra* having the distal half of their tentacles

removed responded maximally. The head area alone (the hypostome and tentacles), when removed 1 hour before the glutathione was added, also reacted maximally (Experiment 3b). The remaining portion of the *Hydra* (the body tube) from Experiment 3b did not contract or respond to added glutathione (Experiment 3c). The isolated tentacles contracted immediately on the addition of glutathione and remained in this contracted condition for as long as 20 minutes (Experiment 3d).

TABLE V

DURATION OF THE RESPONSE BY *HYDRA* HAVING VARYING NUMBERS OF TENTACLES AND BY ISOLATED PARTS OF THE HYDRA

Experiment 1	No. of tentacles	$t_f - t_i$, min. Immediately after cutting	3 hrs. after cutting
a	6	25.15	30.92
b	5	28.70	23.54
c	4	25.76	25.80
d	3	15.63	28.04
e	2	23.50	20.98
f	1	22.22	20.92
g	0	7.40*	7.34*
Experiment 2	No. of tentacles		
a	6	37.76	
b	7	35.52	
c	8	35.78	
d	9	31.42	
Experiment 3	Part of *Hydra*		
a	*Hydra* having one-half of each tentacle removed	35.31	
b	Hypostome (with tentacles) alone	38.07	
c	Body tube alone	0	
d	Tentacles alone	18–20	

The *Hydra* used in Experiment 1 were starved for 1 day, while those used in Experiments 2 and 3 were starved for 2 days. All experiments were carried out in excess glutathione (10^{-5} M).
* Only three *Hydra* responded.

Effect of Glutathione Concentration on the Duration of the Feeding Reflex The duration of the feeding reflex was measured at different concentrations of glutathione. For each concentration of glutathione, duplicate groups of five animals were used. In experiments employing *Hydra* starved for 2 days (solid curve), a maximum response was observed at concentrations of 5×10^{-6} M and greater. No further increase in the duration of the feeding reflex occurred at higher glutathione concentrations. At lower glutathione concentrations, the duration of the feeding reflex increased in nearly direct proportion to the amount of glutathione added. However, at these smaller values there was

greater variation in the response of the individual *Hydra*, some not responding at all, as indicated by the symbols used in Fig. 2.

The concentration of glutathione eliciting a half-maximal response was about 10^{-6} M regardless of whether the *Hydra* had been starved for 1 or 2 days. The maximal responses to high concentrations of glutathione shown by these two groups of *Hydra* differed by about 10 minutes. Nonetheless, the shapes of the curves were nearly alike.

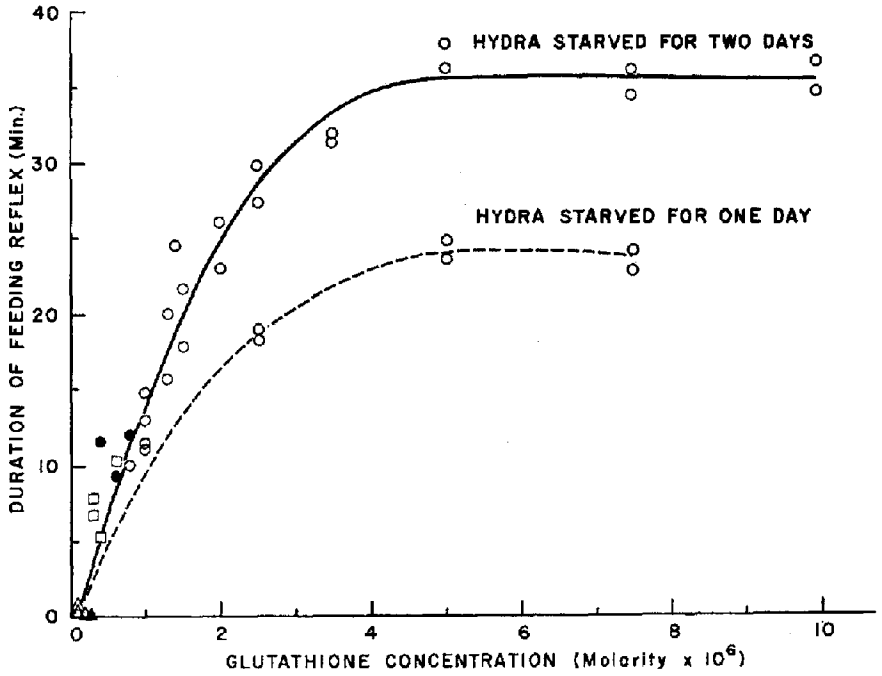

FIGURE 2. Effect of glutathione concentration on the duration of the feeding reflex. Each point represents the mean for five *Hydra*. The type of symbol used indicates the number of *Hydra* in the group of five responding to glutathione; *i.e.*, ○, five; ●, four; □, three; △, two; and, ▲, one.

DISCUSSION

The feeding reflex of *Hydra littoralis* is initiated by the glutathione in the water surrounding the animal. During normal feeding, the following processes are believed to occur: A small organism, such as *Daphnia*, swims into the *Hydra's* tentacles and causes eversion of the nematocysts which then lasso and puncture the prey. Among the substances present in the fluids oozing from the wounded prey is glutathione. The glutathione causes the tentacles holding the prey to contract and bend towards the mouth. Finally, the mouth, stimulated in turn by the glutathione being emitted from the prey, opens and swallows the captured organism.

The data presented in Table I, as well as providing a basis for the assay, give insight into central problems concerning the mechanism by which glutathione elicits the feeding reflex. The values given for t_i must include the time required for at least two major processes to occur: (*a*) the union of glutathione with its receptor, which in Experiments a–e is probably rapid (*i.e.*, within a few seconds), and (*b*) all the subsequent events leading to mouth opening. The values for t_i (0.4 to 1.0 minutes) may represent, for the most part, the latter events.

Large values of t_i (those greater than 2.0 minutes) indicate that the experimental conditions for the feeding reflex are not optimal. For example, it takes longer for the mouth to open at low glutathione concentrations (Table I, Experiment f) or in the presence of a competitive inhibitor (Table I, Experiment g) than at excess glutathione concentrations under optimum conditions. Similarly, cellular poisons, such as *N*-ethyl maleimide or heavy metals, also cause an increase in t_i (Lenhoff, unpublished). Further, *Hydra* in distilled water take longer to respond than do *Hydra* in distilled water containing added calcium ion (9). In the cases mentioned here, it would appear that the large values of t_i result from the interference with the activation of a sufficient number of functional receptor sites needed to elicit an optimally rapid response. Another cause of a delay in mouth opening might stem from interference with some of the cellular events initiated by the combination of glutathione with its receptor.

At suboptimal concentrations of glutathione (Table I, Experiment f, and Fig. 2 at concentrations less than 5×10^{-6} M glutathione) the t_f values were small in comparison to those obtained at higher glutathione concentrations. These results show that mouth opening is not an all-or-none response, and that graded responses can occur when conditions are not optimal. In addition, it is generally observed that the larger the value of t_i, the smaller the value of $t_f - t_i$.

The experiment described in Table II indicates that glutathione had to be constantly present during the total time of the feeding reflex in order for the response to continue. In addition, since the mouths close rapidly on removal of glutathione it is concluded that the equilibrium between glutathione and the receptor is rapidly attained. In Table II, the times given for mouth closure after glutathione was removed probably represent the time required both for the dissociation of the glutathione and for the cessation of the cellular events involved in the receptor-effector system. The observations that mouth closure was more rapid the longer the *Hydra* had been exposed to glutathione may imply that bound metabolic intermediates or cofactors, postulated to be released by and to take part in this system (10), become depleted as the feeding reflex nears completion.

The evidence that t_f, at optimum conditions, is limited by some property

of the *Hydra* is twofold: (*a*) the mouths did not close because glutathione was oxidized, altered, or consumed (Table III), and (*b*) the combined t_f values for a single group of *Hydra* were not affected by the discontinuous application of glutathione (Table IV). Thus we might postulate as one result of glutathione activation, the consumption of some substance in the receptor-effector system the concentration of which limits the duration of the feeding reflex to 25 to 35 minutes. In fact, once *Hydra* have carried out a maximum response, they will not respond fully again to excess glutathione for another 24 to 48 hours (11). The 24 to 48 hour recovery period between glutathione exposures may represent the time required for the postulated consumed substance to be resynthesized.

Further examination of Table III, however, does indicate some shortening of t_f–t_i by the time the third group of *Hydra* had been exposed to the same glutathione solution. Thus, it appears that either the glutathione concentration was in some manner lowered, or that some inhibitory factor stimulated by glutathione was secreted into the environment and was accumulated there.

The results summarized in Table V do not allow us to determine the exact sites of the receptors on the *Hydra*. Interpretation is difficult because we do not know the effects on the contractile elements involved in the feeding reflex caused by either the cellular damage or the altered structure of the animal resulting from the operations (as in Experiments 1 and 2). Nevertheless, it is evident that there are sufficient receptors on the isolated head area (hypostome and tentacles) (Experiment 3b) to give a maximum response. In fact, removal of the distal half of the tentacles did not lower the response in the remaining animal (Experiment 3a).

The plot of the duration of the feeding reflex against glutathione concentration (Fig. 2) is similar to the Langmuir adsorption isotherm (12), and to a curve illustrating the saturation of an enzyme by its substrate (13). The results (Fig. 2) are interpreted as indicating that at glutathione concentrations greater than 5×10^{-6} M all the glutathione receptors are saturated. In these experiments, in contrast to an enzyme saturation, the glutathione does not appear to be metabolized, but rather continues to activate all the receptor-effector systems until the response ceases. At subsaturation levels of glutathione, the animal does not respond to its fullest capacity (see also Table I, Experiment f).

Analogous to the Michaelis constant, K_M, used in enzymology, is the concentration of glutathione eliciting a half-maximum response. For the glutathione-*Hydra* system this value, *ca.* 10^{-6} M, probably closely approximates a true dissociation constant because of the apparent absence of glutathione products. A rough mass law treatment using the method of Scatchard (14) indicates that we can measure this constant within a factor of 2. The significance of this constant is threefold: First, its smallness indicates that the

receptor has a high affinity for glutathione. Second, the value of 10^{-6} M is within the physiologically active range expected to occur under natural conditions of feeding. And third, this number provides us with a means of characterizing the receptor; that is, the glutathione receptor can be said to have a constant of 10^{-6} M. This constant has been found to be characteristic of the receptor and to be nearly the same no matter what the condition of the *Hydra*. For example, Fig. 2 demonstrates that *Hydra* starved for 2 days respond to high concentrations of glutathione for a greater period of time than do *Hydra* starved for 1 day. Nonetheless, the concentration of glutathione eliciting a half-maximal response on both sets of *Hydra* was 10^{-6} M.

The differences in the maximum response observed in *Hydra* starved 1 or 2 days (Fig. 2) become understandable if we make another comparison with enzyme systems. Just as the maximum activity of an enzyme reaction is dependent on the quantity of enzyme present and is not a specific property of the enzyme, in a similar manner the duration of the reflex at concentrations eliciting the maximum response is dependent upon the quantity of completed receptor-effector systems of the *Hydra*. The maximum response is not an intrinsic property of the receptor or of the *Hydra* as is the K_M. Thus, *Hydra* starved for 1 day are interpreted as having fewer completed receptor-effector systems than *Hydra* starved for 2 days.

As emphasized in the above comparison, it is imperative in experiments using excess glutathione concentrations (10^{-5} M) that each *Hydra* possess approximately the same number of active receptor-effector systems. Since it is impossible to know beforehand the number of such systems per *Hydra*, our only criterion for obtaining quantitatively reproducible results is to select *Hydra* reared under nearly identical laboratory conditions. We repeatedly find that the standard deviation of the response of *Hydra* to excess glutathione is low if these animals come from the same mass culture (Table I, Experiments a–e). Therefore, one should not compare experiments employing *Hydra* taken from different mass cultures; variation might result either from differences in the time elapsed since the previous exposure to glutathione (11), in environmental cations or anions (9), in temperature, or from some presently unidentified factors.

One practical aspect suggested by the data shown in Fig. 2 is a quantitative microbioassay for reduced glutathione. Such an assay, having as the reference standard the linear portion of the saturation curve (Fig. 2), has been used with success in this laboratory.

Previous results and those presented in this paper allow us to summarize the present state of knowledge concerning the mechanism by which glutathione combines with and activates the glutathione receptor of *Hydra* to elicit the feeding reflex: The activity of the glutathione resides in the size and

shape of the γ-glutamylalanylglycine backbone of the tripeptide, and not in the reducing properties of the molecule (1, 4, 5). The presence of small amounts of calcium ion in the medium surrounding *Hydra* is required in order that a response may occur (9). There are a limited number of receptor-effector systems, probably localized in the area immediately around the mouth and on the tentacles (Table V). Concentrations of glutathione greater than 5×10^{-6} M activate all these systems; the concentration of glutathione eliciting a half-maximum response is 10^{-6} M (Fig. 2). In order for a response to take place, the glutathione must be constantly present at the receptor site (Table II). The association of glutathione with the receptor is rapidly attained (Table II); the affinity of the receptor for glutathione is high (Fig. 2). After glutathione combines with the receptor, it takes about 0.5 minute for all the events necessary for mouth opening to occur (Table I). Once the reflex begins, it will continue for 25 to 35 minutes (Table I, III, IV, V). The response does not stop because of any alteration in the glutathione molecule (Table III), but because of some property of the *Hydra*. The duration of the response is probably limited by the consumption of some limiting substance (Tables I, III, IV, V). The response can be stimulated in the absence of glutathione by certain proteases (10).

The receptor is visualized as an inactive protein on the surface of certain *Hydra* cells. When that protein combines with glutathione, its tertiary structure is altered, rendering the receptor protein physiologically active. One of the processes stimulated by this active protein is the utilization of a substance that limits the duration of the feeding reflex to about 30 minutes.

The results discussed in this paper are concerned with a single biological system in which a specific excitatory compound combines with its receptor to activate a coordinated response. Activations by an excitatory compound comprise the common step in many basic biological phenomena such as chemoreception, olfaction, and hormone action. Some of the results described here on the interaction of glutathione with the *Hydra* receptor may bear a relation to the functioning of some of these other systems.

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