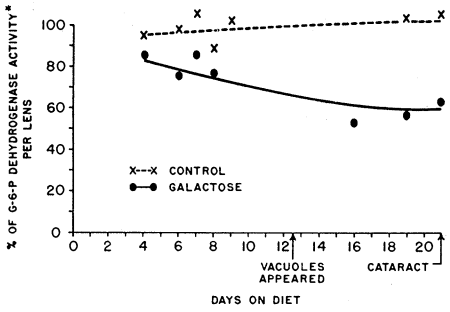

Fig. 1. Percentage of G-6-P dehydrogenase activity per lens (ordinate) as a function of days on diet (abscissa). The enzyme activity (assayed at *p*H 7.4) is expressed as μgr of TPN$^+$ reduced per hour per milligram of soluble lens protein (100 percent G-6-P dehydrogenase activity equals 21.93 units).

uolization), which occurred at the 12th to 14th day after the commencement of the galactose diet, both the control and experimental groups of animals were sacrificed. Their lenses were immediately assayed for G-6-P dehydrogenase and 6-PG dehydrogenase activities according to the method of Glock and McLean (*6*).

In the lenses derived from the experimental (galactose) animals, there was a marked inhibition of G-6-P dehydrogenase activity. The average level of activity in these animals was 13.8 units (*7*) (range 7.9 to 18.8) which was approximately 45 percent lower than the value obtained for G-6-P dehydrogenase in the control group, 25.2 units (range 23.7 to 26.9). However, the 6-PG dehydrogenase activity was essentially the same for both groups of animals. The average value in the experimental (galactose) group was 6.25 units (*7*) (range 4.71 to 9.15), while the control group showed an average of

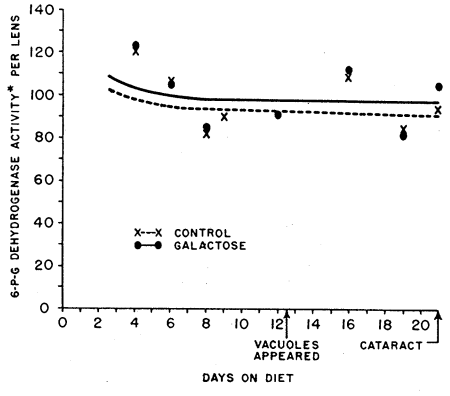

Fig. 2. 6-Phosphogluconate dehydrogenase activity per lens (ordinate) as a function of days on diet (abscissa). The enzyme activity (assayed at *p*H 7.4) is expressed as μgr of TPN$^+$ reduced per hour per milligram of soluble lens protein (100 percent 6-P-G dehydrogenase activity equals 6.9 units).

6.16 units (range 5.10 to 7.41) of 6-PG dehydrogenase activity.

This experiment was repeated with 28 rats of the same strain and weight. Half of these animals were fed the galactose diet, while the remaining half were fed the dextrose diet. Two animals in each group were sacrificed at the 4th, 6th, 7th, and 8th days, respectively, after commencing on their respective diets. The remaining animals were sacrificed at varying periods of time, as shown in Figs. 1 and 2.

The activity of G-6-P dehydrogenase showed a significant decline at the 4th day of galactose feeding and continued to fall till the 14th day, where it leveled off at approximately 60 percent of normal, as shown in Fig. 1.

The level of 6-PG dehydrogenase showed no change in the lenses of the galactose-fed rats as compared to those of the control animals (Fig. 2).

The soluble protein fractions of all these lenses were measured by the micro-Kjeldahl method. The results are generally in agreement with Dische's findings (*8*), although the apparent inhibition of soluble protein synthesis did not occur until the 8th day of galactose feeding.

In an effort to reproduce this enzymatic inhibition in vitro, paired lenses from five normal rats were employed. Each pair of lenses was homogenized (in an ice-water bath) in 2 ml of distilled water, and the insoluble protein was removed by centrifugation. Half of each soluble protein fraction was incubated with an excess of gal-1-P to G-6-P in the media (*9*), while the remaining half was incubated with G-6-P as the sole substrate. After 3 hours of incubation at 37°C, an aliquot (0.6 ml) was removed and assayed for combined G-6-P and 6-PG dehydrogenase activities. The results of this experiment showed an average inhibition of 21 percent (range 16 to 35 percent).

Since all the aliquots contained some G-6-P, it was not possible to derive the activity of each dehydrogenase separately. This experiment was therefore repeated with four sets of paired normal lenses in which half of the incubates contained 15 μmole of gal-1-P while a similar amount of sucrose was added to the other incubates.

The results of these in vitro experiments showed that an average of 20-percent inhibition (15.6 to 34 percent) of G-6-P dehydrogenase occurred, while the 6-PG dehydrogenase activity remained unimpaired.

The foregoing experiments have demonstrated that gal-1-P is capable of specifically inhibiting G-6-P dehydrogenase in vitro and that it may act in a similar manner in vitro. This inhibition apparently occurs prior to any observed inhibition of soluble protein synthesis. An inhibition of the hexose monophosphate shunt may therefore be implicated in the pathogenesis of experimental galactose cataract. (*10*).

SIDNEY LERMAN

Division of Ophthalmology, University of Rochester School of Medicine and Dentistry, Rochester, New York

References and Notes

1. H. M. Kalckar, *Science* **125**, 105 (1957); E. P. Anderson, H. M. Kalckar, K. J. Isselbacher, *ibid.* **125**, 113 (1957).
2. S. Lerman, *A.M.A. Arch. Ophthalmol.* **61**, 88 (1959).
3. V. Schwarz and L. Golberg, *Biochim. et Biophys. Acta* **18**, 310 (1955).
4. J. H. Kinoshita, *A.M.A. Arch. Ophthalmol.* **54**, 360 (1955).
5. A. M. Yodkin and C. H. Arnold, *ibid.* **14**, 960 (1936).
6. G. E. Glock and P. McLean, *Biochem. J.* **55**, 400 (1953).
7. The enzyme activity is expressed as micrograms of TPN$^+$ reduced per hour per milligram of soluble lens protein. Both G-6-P and 6-PG dehydrogenase activities were assayed at *p*H 7.4.
8. Z. Dische, G. Zelmenis, J. Youlos, *Am. J. Ophthalmol.* **44**, 332 (1957).
9. The incubation media consisted of Krebs-Ringer phosphate buffer, 0.1*M* at *p*H 7.4. The control group contained 1.5 μmole of G-6-P Na$^+$ salt, while the experimental group contained 1.5 μmole of G-6-P Na$^+$ salt and 15 μmole of gal-1-P K$^+$ salt.
10. This investigation was supported by research funds granted by the Rochester Eye Bank and Research Society.

30 June 1959

Requirement of Bound Calcium for the Action of Surface Chemoreceptors

Abstract. The ability of *Hydra* to carry out the feeding reflex in response to reduced glutathione was inhibited by either (i) standing in distilled water, (ii) the presence of ethylenediamine tetraacetic acid, or (iii) the presence of magnesium ions. These three types of inhibition were reversed instantaneously by the addition of calcium ions.

Chemoreceptors are known to exist on the surface of some cnidarians, as shown by the effects of meat juice in the environment on eliciting a feeding reflex in *Hydra* (*1*) and in other species (*2*). It was not until the quantitative chemical studies of Loomis that the substance which stimulated the *Hydra* chemoreceptor was positively identified as reduced glutathione (GSH). In a lucid study Loomis demonstrated that this tripeptide is among the numerous compounds present in the fluids oozing from the wound of the prey punctured by the harpoon action of the *Hydra's* deadly nematocysts, and that GSH induces the *Hydra* to open its mouth in an attempt to swallow that prey. Loomis colorfully describes GSH as an "environmental hormone," released from a specific source—the damaged prey; passing through a fluid environment—the water between the prey and the

Hydra; and after stimulating a chemoreceptor, eliciting a specific response—the feeding reflex (*3*).

The existence of surface receptors which respond almost instantaneously to a specific molecule in a fluid environment of known chemical composition (*4*, *5*) seemed to be ideally suited for studies of the mechanism of chemoreception. In this report we demonstrate that GSH-receptors require the presence of calcium ions in order to function.

The feeding reflex is expressed numerically by averaging the lengths of time (in minutes) during which the mouths of all the *Hydra* tested remained open in response to GSH. Asexual *Hydra littoralis*, grown in laboratory mass cultures (*6*), were used in all the experiments. The assay consisted of the following procedure: Five *Hydra*, which had been starved for 1 day, were rinsed in 15 ml of the solution to be tested ("test solution") for 5 seconds and placed in 30 ml of fresh test solution for 1 minute. [In experiments 1*b* and 1*c* (Table 1) the *Hydra* were kept in the test solution—here, deionized water—for 35 minutes.] The *Hydra* were then placed in a spherical cavity (38 mm in diameter) of a 3- by 2-in. depression slide, and the test solution carried over with the *Hydra* was removed by means of a fine-tipped medicine dropper. To the *Hydra* were added 2 ml of the test solution containing $10^{-5}M$ GSH, freshly diluted from $10^{-3}M$ GSH. The *Hydra* were observed through a binocular dissecting microscope set at magnification 19.5. The lengths of time that the mouths of the *Hydra* remained open were recorded, and the average was taken.

The data are presented in Table 1. As shown in experiment 1*a*, the *Hydra* placed in deionized water for 1 minute opened their mouths for periods lasting over 20 minutes in the presence of GSH. However, after being placed for 35 minutes in deionized water prior to the addition of GSH, they lost some of their ability to respond (experiment 1*b*); otherwise, the *Hydra* appeared to be normal. These experiments suggested that some compound, which was perhaps bound to the surface of the cells, slowly dissociated from the membrane when the animals were kept in deionized water. Since calcium is known to be required in the *Hydra's* fluid environment for normal growth (*4*), and for many processes of excitable tissues (*7*, *8*), experiments were carried out in order to determine whether calcium is involved in the GSH-induced feeding reflex. Experiment 1*c* demonstrates that the addition of $10^{-3}M$ $CaCl_2$ to the *Hydra* which had been standing in deionized water for 35 minutes restored their ability to respond to GSH.

Table 1. Requirement of calcium for the action of GSH-receptors of *Hydra*. In addition to the ions dissolved in the different test solutions, $10^{-5}M$ GSH was present in all solutions after the 1-minute (or 35-minute) incubation periods (see text). The *Hydra* usually opened their mouths within 30 seconds after the GSH was added; the only exception was in experiment 1*b*, when the *Hydra* took about 5 minutes to open their mouths. The *p*H in experiments 1*a*, 1*b*, and 1*c* was around 5.5, and in the other experiments, around 7.8.

Expt. No.	Test solution (time in water in parentheses)	Average time *Hydra's* mouth was open (minutes)
1a	Deionized water (1′)	23.6
1b	Deionized water (35′)	12.7
1c	Deionized water (35′); $10^{-3}M$ $CaCl_2$(1′)	27.2
1d	$10^{-4}M$ $NaHCO_3$	22.2
2a	$1.34 \times 10^{-5}M$ EDTA + $10^{-3}M$ $NaHCO_3$	0.0
2b	$1.34 \times 10^{-5}M$ EDTA + $10^{-3}M$ $NaHCO_3$ + $10^{-3}M$ $CaCl_2$	28.5
3	$1.34 \times 10^{-5}M$ EDTA + $10^{-3}M$ $NaHCO_3$ + $10^{-3}M$ $SrCl_2$	6.8
4a	$10^{-5}M$ $CaCl_2$ + $10^{-4}M$ $NaHCO_3$	21.2
4b	$10^{-5}M$ $CaCl_2$ + $10^{-4}M$ $NaHCO_3$ + $10^{-3}M$ $MgCl_2$	1.4
4c	$10^{-3}M$ $CaCl_2$ + $10^{-4}M$ $NaHCO_3$ + $10^{-3}M$ $MgCl_2$	23.9

More conclusive experiments demonstrating the involvement of calcium in eliciting the feeding reflex were carried out with disodium ethylenediamine tetraacetic acid (EDTA). This chelating agent binds calcium as well as other metallic ions. As shown in experiment 2*a*, after 1 minute of exposure to EDTA, the *Hydra* lost completely their ability to carry out the feeding reflex. This inhibition was reversed in a correspondingly instantaneous manner by the addition of $10^{-3}M$ $CaCl_2$ (experiment 2*b*), the *Hydra* responding maximally (see experiment 1*c*). In all the experiments involving EDTA, $10^{-3}M$ $NaHCO_3$ was added to keep the solutions at a slightly alkaline *p*H. The addition of $NaHCO_3$ alone to deionized water (experiment 1*d*) did not significantly affect the response of the *Hydra*, as compared to their response in the slightly acid deionized water (experiment 1*a*). In addition, experiment 2*a* also demonstrates that sodium ions, recently shown to be required in the *Hydra's* fluid environment for normal growth (*5*), would not replace calcium. Similarly, sodium chloride also did not replace calcium chloride.

Other metals were tested for their ability to reverse the EDTA inhibition of the feeding reflex. The chlorides of barium, magnesium, cadmium, potassium, lithium, rubidium, and cesium were without effect. Strontium, known to be chemically very similar to calcium, partially replaced calcium in effecting the GSH-induced feeding reflex (experiment 3).

Magnesium ions are well known for their anesthetic properties (*7*). The term *anesthesia* is used here to signify "a reversible loss of the power to respond to a stimulus," according to a definition by Heilbrunn (*7*). Since calcium has been reported to reverse the effects of magnesium anesthesia (*7*, *8*), it seemed of interest to carry out the opposite experiment—that is, the inhibition of a response requiring calcium, the feeding reflex, by the addition of magnesium. As shown in experiment 4*b*, the presence of $10^{-3}M$ $MgCl_2$ in the test solution containing $10^{-5}M$ $CaCl_2$ decreased the activity of the feeding response to about 1/15 of the activity shown in experiment 4*a*. This magnesium anesthesia was reversed by increasing the calcium concentration to $10^{-3}M$ (experiment 4*c*). Thus, in these experiments, magnesium ions seem to produce their anesthetic effect by competing with calcium ions for some common sites on the *Hydra*.

Calcium is also required for the response of another receptor of the *Hydra*—the cnidoblast (*9*). This cell contains the explosive coiled nematocyst, which is discharged when the cnidoblast is stimulated by some tactile or tactile-chemical agent. We have found that these receptors respond to calcium, EDTA, and magnesium much as the GSH-receptors do. The ability of GSH to induce a feeding reflex has been demonstrated in the marine hydroids *Physalia* and *Campanularia* (*10*). Calcium is also known to be required for the action of another receptor—the stretch receptor of the crayfish (*11*), and for the action of another excitatory compound—adrenocorticotrophic hormone (*12*).

Calcium is known to have many effects on excitable tissue (*7*, *8*). For example, calcium has been reported to be involved in cell irritability, cell permeability, nerve action, and muscle contraction. The role of calcium in bioelectric events in nerve and muscle has recently been reviewed by Shanes (*13*). The mechanism of action of calcium in the GSH-receptor of *Hydra* has not as yet been determined.

The GSH-receptor of the *Hydra* is highly amenable to studies concerning the action of calcium ions on chemoreceptors. The response is to a specific molecule (GSH), it is nearly instantaneous, and it can be accurately measured by visual observation. In addition,

the fluid environment bathing the receptor can be precisely controlled experimentally and is not complicated by the presence of the many "antagonistic" cations found in Ringer's solution, sea water, or extracellular body fluids. Furthermore, since this fluid environment is of very low ionic strength, cations in the concentrations present are without osmotic effect (*4*); therefore, the amount of calcium added to elicit the feeding response experimentally perhaps closely approximates the actual amount of calcium necessary for the natural functioning of GSH-receptors.

Note added in proof: In addition to the specific cation requirement reported here, anions also were found to influence the feeding reflex. The order of effectiveness of the anions in increasing the duration of the feeding reflex was: $Cl > Br > I = NO_3$. This order seemed to be the reverse of the lyotropic series.

HOWARD M. LENHOFF
JOHN BOVAIRD
Howard Hughes Medical Institute, Miami, Florida

References and Notes

1. R. Beutler, *Z. vergleich. Physiol.* **1**, 1 (1924).
2. L. H. Hyman, *The Invertebrates* (McGraw-Hill, New York, 1940), vol. 1.
3. W. F. Loomis, *Ann. N.Y. Acad. Sci.* **62**, 209 (1955).
4. ———, *J. Exptl. Zool.* **126**, 223 (1954).
5. H. M. Lenhoff and J. B. Bovaird, *Exptl. Cell Research*, in press.
6. W. F. Loomis and H. M. Lenhoff, *J. Exptl. Zool.* **132**, 555 (1956).
7. L. V. Heilbrunn, *The Dynamics of Living Protoplasm* (Academic Press, New York, 1956).
8. F. Brink, *Pharmacol. Revs.* **6**, 243 (1954).
9. A number of investigators have independently observed that calcium plays a role in nematocyst discharge. Among these are D. Slautterback, C. Fulton and W. F. Loomis (personal communications).
10. H. M. Lenhoff and H. A. Schneiderman, *Biol. Bull.* **116**, 452 (1959).
11. Y. Katsuki, S. Yoshino, J. Cheng, *Japan. J. Physiol.* **1**, 264 (1951).
12. M. K. Birmingham, F. H. Elliot, P. H.-L. Valère. *Endocrinology* **53**, 687 (1953).
13. A. M. Shanes, *Pharmacol. Revs.* **10**, 59 (1958).

15 July 1959

Molting of Preadult Nematodes of the Genus Paratylenchus Stimulated by Root Diffusates

Abstract. The nonfeeding preadult larvae of the plant-parasitic nematodes, *Paratylenchus projectus* and *P. dianthus,* survive in moist soil during long periods in the absence of host plants. In water, only small percentages molt to the adult stage. In root diffusates from some but not all plants, nearly all of them molt.

The last larval or preadult stage of certain species of the genus *Paratylenchus* Micoletzky often constitutes a high percentage of the total population in Illinois field soils and in old pot cultures, even in the presence of suitable host plants. Results of recent studies (*1*) indicate that the preadults of *P. projectus* and *P. dianthus* do not feed and that they are capable of long survival in moist soil without feeding; also, that those of *P. projectus,* at least, are more tolerant of desiccation and sudden freezing than are younger stages or adults. The preadult stage of these two species and also of *P. hamatus* and two apparently undescribed species is readily distinguishable from other stages by the greatly reduced stylet and esophagus and by an accumulation of opaque granules in the region of the esophagus, yet this stage seems to have attracted little attention. Reuver (*2*) described it for *P. amblycephalus* after the study reported here was far advanced. Thorne and Allen (*3*) evidently recognized but did not describe it in *P. hamatus,* and Ferris and Bernard (*4*) commented on its abundance in *Paratylenchus* spp. in some samples from Illinois soybean and corn fields.

Table 1. Efficacy of carnation-root diffusate in inducing molting of *P. projectus* and *P. dianthus* preadults.

Treatment	No. of molts among 125 nematodes				
	3 days	6 days	9 days	12 days	15 days
	P. dianthus				
Control*	1	1	2	2	2
Root diffusate	28	73	96	99	104
	P. projectus				
Control*	0	1	8	16	20
Root diffusate	0	87	123	124	125

*Distilled water to which activated carbon (Darco G-60) was added and which was then decanted and filtered. Indistinguishable results were obtained with nontreated distilled water.

In Petri-dish cultures of host plants in agar, preadults of *P. projectus* begin the final molt within 4 or 5 days after emerging from the preceding one and at no time constitute any large percentage of the total population. In pot cultures of this same species with red clover, after 100 days the preadults constituted only 7.7 percent of the total population but increased progressively to 66 percent during the following 85 days. In many older pot cultures and field samples, the proportion of preadults has exceeded 80 percent of the population of a species. The cause of this accumulation is not known, but evidently it operates either by preventing the final molt or by failing to provide the stimulus necessary to initiate it.

Working with preadults of *P. projectus* from field collections and old pot cultures and of *P. dianthus* chiefly from a jar of soil that had been stored moist for over 2 years until all other nematodes were dead, we found that preadults do not molt readily in water but that they are stimulated to molt by some substance or substances that diffuse from roots immersed in water. Leachate from actively growing pot cultures frequently, but not consistently, induces molting.

Consistently effective root diffusate was obtained by immersing the roots of heavily rooted White Sims carnation cuttings in 50 ml of distilled water for 72 hours, with 12 hours of artificial illumination each day. Table 1 presents results of a representative experiment in which preadults, in five lots of 25 each, were placed into small Syracuse dishes containing about 1 ml of carnation-root diffusate, or of water for the controls, and were held at 23° to 26°C in the dark. Fluids were changed at 3-day intervals, diffusate that had been stored at about 7°C being used. Nematodes in which the adult form and size of stylet had developed were counted as having molted.

In a temperature experiment with *P. projectus* no molting occurred at 5°, 10°, or 35°C in carnation-root diffusate or in water during 16 days. The numbers of those that molted among 125 nematodes in diffusate and 125 in water, respectively, were as follows, at four intermediate temperatures: at 15°C, 43 and 0; at 19°C, 124 and 9; at 26°C, 124 and 23; and at 30°C, 125 and 8. Molting occurred at a much more rapid rate at 26° and 30°C than at 15° and 19°C, and more rapidly in diffusate than in water at the two higher temperatures.

Seedlings of nine species of dicotyledonous plants, representing seven families, grown in sand and fertilized with an N-P-K fertilizer (Hyponex), produced root diffusates that markedly increased molting of one or both nematode species. Nine other plant species, six of them monocots, produced diffusates that were without effect on either nematode in a single test, and six additional plant species produced diffusates that were no more than slightly active. There was an imperfect agreement between suitability of plants as hosts and their production of active root diffusate. For example, *Trifolium pratense* L. (Kenland red clover) is a suitable host for both nematodes, yet in repeated tests it has yielded root diffusates that induce molting in *P. projectus* but not in *P. dianthus.*

The activity of certain root diffusates in stimulating hatching or emergence of larvae from cysts of *Heterodera* spp. is a well-known phenomenon, but we are not aware of any prior demonstration that root diffusates may stimulate the molting of plant-parasitic nematodes.

H. L. RHOADES*
M. B. LINFORD
Department of Plant Pathology, University of Illinois, Urbana