References and Notes

1. J. A. Campbell, *J. Physiol.* **90**, 91P (1937).
2. C. W. Smith, J. W. Bean, R. Bauer, *Am. J. Physiol.* **199**, 883 (1960).
3. J. W. Bean and R. Bauer, *Proc. Soc. Exptl. Biol. Med.* **81**, 693 (1952).
4. P. Felig and W. L. Lee, Jr., *Ann. N.Y. Acad. Sci.*, in press.
5. J. J. Moran, *Anal. Chem.* **24**, 378 (1952).
6. J. W. Bean, *Physiol. Rev.* **25**, 1 (1945).

7. Supported in part by contract R-87 of the NASA. We thank Dr. B. Katchman and the Clinical Laboratories, Miami Valley Hospital, Dayton, Ohio, for the determinations of protein-bound iodine.

27 March 1964

Behavior in Hydra: Inhibition of the Contraction Responses of Hydra pirardi

Abstract. Hydra pirardi *contracts spontaneously and in response to external stimuli of light and mechanical agitation. Inhibition of these contractions occurs when the animal feeds on* Artemia salina *or when reduced glutathione is present in the environment. Such inhibition demonstrates the control of one receptor-effector system by another in hydra.*

There has been renewed interest in the feeding reflex of hydra, stemming from the contention of Loomis (*1*) that reduced glutathione (GSH) specifically controls the feeding reflex of *Hydra littoralis*. Lenhoff (*2*) has systematically studied the role played by GSH in activating the feeding response in this species, and proposed a quantitative assay for the reflex based on the time of mouth opening of the animal. The uniqueness of GSH as an initiator of feeding in hydra has been questioned by Forrest (*3*) and Burnett *et al.* (*4*). Indeed, Forrest maintains not only that a large class of apparently unrelated substrates induce feeding in hydra, but also that the GSH-induced reaction is not a true feeding response of the animal.

In studies of the contraction responses of *Hydra pirardi* to stimuli of light and mechanical agitation, we observed that the characteristic contractions of the body column which occur in response to these stimuli (*5*) were inhibited while the animal was feeding on *Artemia salina*. The normal, spontaneous contractions of the animal were also temporarily eliminated during feeding. In this report we describe experiments on the inhibition of these contraction responses by feeding *H. pirardi* on *A. salina* and by adding reduced glutathione to the culture medium.

The animals we used in this study were from a clone of *H. pirardi*. The hydra were cultured by the method of Loomis and Lenhoff (*6*), except that distilled water was substituted for tap water in the culture medium. *Artemia salina* larvae were fed to the animals daily. The experimental animals were starved for 24 hours before being tested at 21°C. In experiments to determine the inhibition of the contraction response to light, two groups of ten hydra were exposed to a regime of 75 seconds of light followed by 75 seconds of dark (*7*). Each group of animals was placed in a petri dish containing 9 ml of culture water. Before each test, there was a control period of 15 minutes during which the numbers of animals in each dish contracting to five successive light periods were recorded. Substances were then pipetted into the dishes and the numbers of animals contracting to subsequent light periods were recorded. By this means, the effect of GSH and other chemicals of known concentrations were tested in addition to live *A. salina* or homogenates of *A. salina* (*3*).

Almost all animals contracted in response to the light stimulus in the control period before each test (Fig. 1). In marked contrast *A. salina*, both alive and in homogenate form, and $10^{-5}M$ GSH initially inhibited these contractions almost completely. The proportion of animals contracting to light gradually increased, however, until it became comparable to that of control animals. Thus, the original light response was restored after 50 to 65 minutes.

The behavior of the animal when exposed to light alone is markedly different from the response to light stimulation in the presence of *A. salina* or GSH. The usual contraction response to light alone consists of a successive series of partial body contractions culminating in the animal's forming a tight ball with contracted tentacles. The time between onset of the light and completed contraction of the animal (the reaction time) is a function of the intensity of the light and its spectral composition (*9*). The reaction time is inversely proportional to temperature (*10*). Under the present experimental conditions, the mean reaction time was 42.5 ± 10.2 seconds, based on 50 animals. In contrast to this light-induced contraction response *H. pirardi* did not exhibit the total contraction to light in the presence of *A. salina* or GSH. In this situation the tentacles writhed and twisted toward the mouth and the mouth itself opened widely (*11*). The movements of the animal were similar to those described by Ewer (*12*) and Loomis (*1*). After ingestion of *A. salina* circular contractions of the distal portion of the body column forcing the food down into the gastrovascular cavity of the animal, described by Forrest (see *3*) were frequently observed.

A second series of experiments was devised to demonstrate the inhibitory effect of *A. salina* and GSH on the contractional response to mechanical agitation. Two groups of five animals were placed in 48 ml of culture fluid in Stender preparation dishes. After the animals had attached themselves to the bottom of the dishes, they were shaken (*13*) for periods of 2 seconds every minute. During a control period before each test, the numbers of animals in the two groups contracting to the pulses of 2-second shaking were recorded for five trials. In experiments with live *A. salina* larvae, the hydra were provided with excess larvae and allowed to feed for 5 minutes. Then, the numbers of animals contracting to successive shaking periods were recorded. A similar procedure was used to study the effect of GSH. Figure 2 shows the results of inhibition of the contraction response to such mechanical agitation (*14*), both by live *A. salina* and by $10^{-5}M$ GSH. An effect similar to that with light stimulation was observed here, although inhibition was shorter, lasting for 25 to 30 minutes.

Live *Artemia salina* and GSH also inhibited the rhythmic spontaneous contractions of the animal. In a typical experiment, the numbers of total body contractions per 30-minute period for groups of ten animals in (i) culture fluid alone, (ii) live *A. salina*, and (iii) $10^{-5}M$ GSH were recorded. The mean number of completed contractions of hydra after providing excess numbers

of *A. salina* (mean, 0.10; standard error, 0.10) was significantly less ($p < .001$) than that of controls (mean, 7.7; standard error, 0.62). The mean number of total body contractions was significantly smaller ($p < .001$) for the group treated with GSH (mean, 0.3, standard error, 0.15) compared with controls (mean, 7.3; standard error, 0.27). Thus, when hydra undergo the characteristic tentacular movements and mouth opening when feeding on *A. salina* or when under the influence of $10^{-5}M$ GSH, interruption of rhythmic contraction activity occurs during the first 30 minutes of exposure.

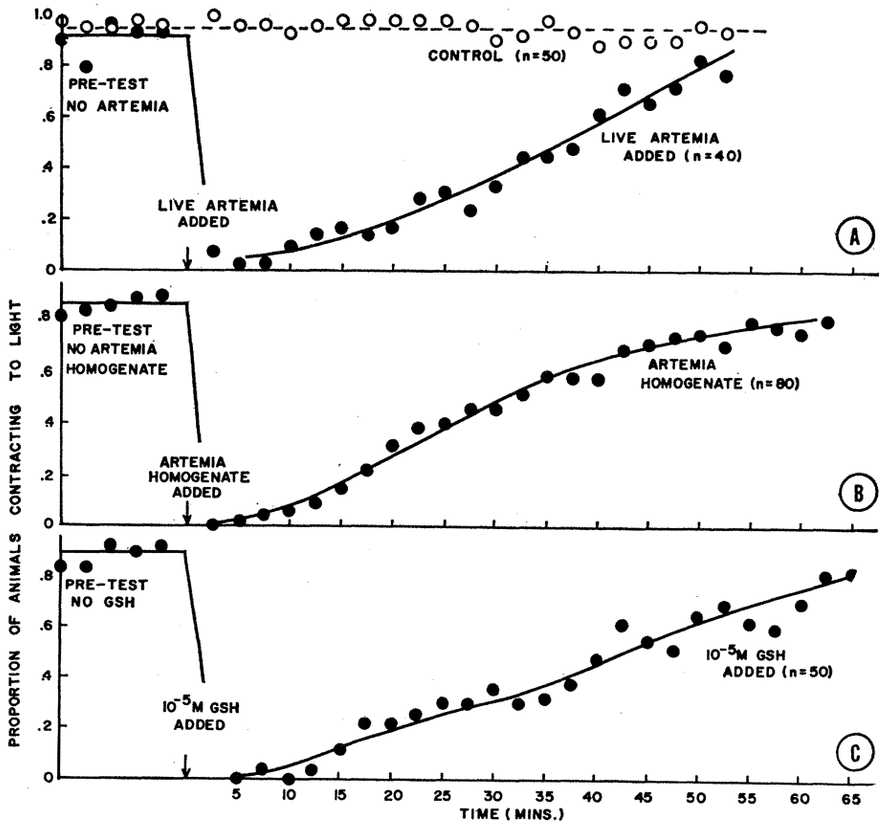

Fig. 1. Inhibition of the light response of *Hydra pirardi*. *A*, The proportions of animals contracting to 75-second periods of light when feeding on live *Artemia salina* (closed circles). *B*, The proportions when hydra are exposed to a homogenate of *A. salina* (pH = 7.7). *C*, The proportions when animals are exposed to $10^{-5}M$ GSH (pH = 7.6). The open circles are the proportions of control animals contracting to light alone.

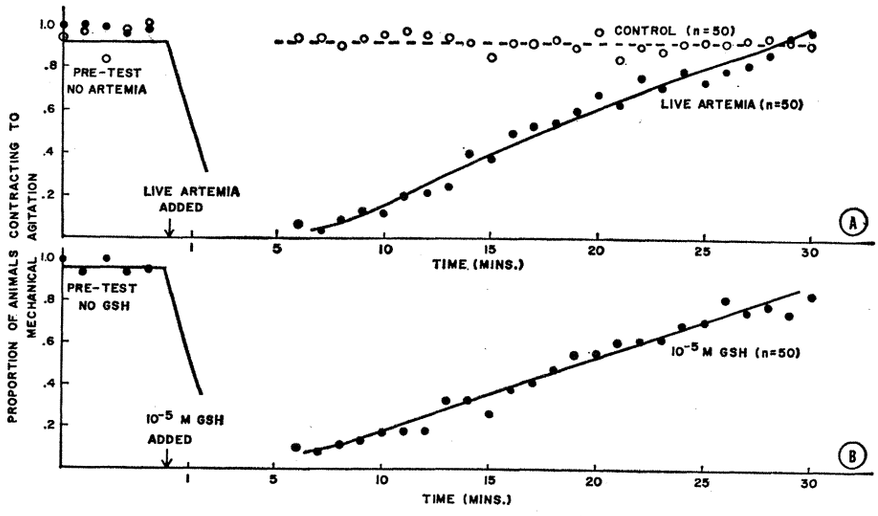

Fig. 2. Inhibition of the contraction response of *Hydra pirardi* to mechanical agitation. *A*, The proportions of hydra contracting to 2-second periods of shaking when feeding on live *Artemia salina* (pH = 7.7). *B*, The proportions when animals are exposed to $10^{-5}M$ GHS (pH = 7.5). The open circles are the proportions of control animals contracting to shaking alone.

The action of GSH in inhibiting the light response of *H. pirardi* (*15*) has some similarities to that observed by Lenhoff (*2*) with *H. littoralis* when the mouth-opening response was used. Inhibition is stopped in the presence of excess $10^{-5}M$ GSH and animals are unaffected by addition of more GSH at that time. Addition of $10^{-5}M$ GSH 12 hours later blocks the light response only slightly, but after 24 hours $10^{-5}M$ GSH again causes maximum inhibition. The length of inhibition, as measured by the time in GSH before 50 percent of the animals recontract to light increases with concentration over the range 10^{-7} to $10^{-5}M$ GSH (approximate times for 10^{-7}, 10^{-6}, and $10^{-5}M$ GSH are 10, 31, and 43 minutes, respectively). Removal of GSH removes inhibition, hence GSH must be present constantly for inhibition to occur. Removal of the tentacles by surgery markedly reduces inhibition of the light response by $10^{-5}M$ GSH, but has little effect on the light response in the absence of GSH. This result suggests that GSH receptors are predominantly located in the tentacles. The sulfhydryl moiety is not essential for inhibition since the S-methyl analog of gluthathione is active. However, S-acetyl gluthathione and oxidized glutathione do not block contractions to light. Thus the substitution of large groups for the sulfhydryl moiety leads to analogs which do not have the right configuration to inhibit the light response.

Previous experiments (*5*) showed that *H. pirardi* has different receptors for light and for mechanical agitation even though the final effectors for both are the longitudinal muscles of the animal. The mechanism of inhibition of contractions responses to these stimuli by GSH is not known. Inhibition could occur at the level of the receptors, or the effectors, or in any nervous elements which participate in these induced contraction responses.

NORMAN B. RUSHFORTH
IVAN T. KROHN
LOMA K. BROWN
Developmental Biology Center,
Western Reserve University,
Cleveland 6, Ohio

References and Notes

1. W. F. Loomis, *Ann. N.Y. Acad. Sci.* **62**, 209 (1955).
2. H. M. Lenhoff, *J. Gen. Physiol.* **45**, 33 (1961); *Biology of Hydra* (Univ. of Miami Press, Coral Gables, Fla., 1961), pp. 203–232.
3. H. Forrest, *Biol. Bull.* **122**, 343 (1962).
4. A. L. Burnett, R. Davidson, M. Warren, *ibid.* **125**, 226 (1963).
5. N. B. Rushforth, A. L. Burnett, R. Maynard, *Science* **139**, 760 (1963).
6. W. F. Loomis and H. M. Lenhoff, *J. Exptl. Zool.* **132**, 555 (1956).
7. Identical cardboard disks from which appropriate portions had been cut and removed were placed above and below two petri dishes. The disks were attached to a rotatory motor producing one revolution every five minutes. Unfiltered light from two American Optical illuminators (both housing a General Electric bulb model 1594 operated at 7.5 v) provided the illumination for each dish of animals during the 75-second light period. The light sources were placed 20 cm above and below each of the dishes. The dishes were placed so that the cut-out disks exposed one dish to the light leaving the other in relative darkness (approx. 55 lumen/m^2).
8. The homogenate of *A. salina* was prepared from a dense 1-ml suspension of *A. salina* in 5 ml of distilled water. The solution was treated for 1 minute with sonic oscillations and centrifuged at 3000 rev/min for 5 minutes. Samples of 1 ml of the supernatants having a total protein content of 5.8 mg/ml were diluted tenfold and samples of 1 ml of this solution were administered to the hydra.
9. R. H. Singer, N. B. Rushforth, A. L. Burnett, *J. Exptl. Zool.* **154**, 169 (1963).
10. N. B. Rushforth, unpublished observations.
11. In *H. pirardi* the mouth intermittently opens and closes in the presence of $10^{-5}M$ GSH. The duration of mouth opening in this species may be used as a reliable measurement of the feeding reflex (Lenhoff, personal communication). However, it provides a more tedious assay than with *H. littoralis*.
12. R. F. Ewer, *Proc. Zool. Soc. London*, **117**, 365 (1947).
13. The animals were shaken at 105 rotations per minute on a Clay Adams Model A 2273 Yankee Variable Speed Rotator.
14. Inhibition of contractions to mechanical agitation also occurs if the stimulus consists of poking the hydra with a dissecting needle. Inhibition of contractions is thus not specific to the mechanical stimulation used, although that applied by means of a rotator provides a more uniformly reproducible stimulus.
15. N. B. Rushforth, in preparation.

16. This research was supported in part by grants from the National Science Foundation and National Institutes of Health.

26 May 1964

Intercellular Electrical Coupling at a Forming Membrane Junction in a Dividing Cell

Abstract. *Ion communication between the halves (blastomere) of a dividing cell* (Asterias forbesi *egg), as determined electrically, diminishes progressively during division as a cell membrane junction forms at the plane of cleavage. Virtually all communication is lost towards the end of division by the time the first intercellular space becomes continuous with the exterior. Resting membrane potentials in both cell halves are essentially equal and constant throughout division.*

During division of egg cells, a cell junction forms at the plane of cleavage. The present study concerns the electrical intercellular coupling as a membrane barrier to ions develops at the junction.

We selected for this study the dividing egg cell of the starfish (*Asterias forbesi*) which offers several advantages. Its plane of cleavage can be predicted at an early stage of cell division; its plasma membrane can be impaled without apparent injury with microelectrodes; and it is large and transparent, so that the position of a microelectrode inside the cell can be clearly viewed and controlled under a microscope (Fig. 1).

The cell was fertilized in vitro, bathed for about 5 minutes in an isotonic urea solution (1M in urea, 0.1M in phosphate buffer, pH 8) to soften and to induce swelling of its fertilization membrane, and impaled with three microelectrodes. Square pulses of current could thus be passed through the cell membrane, and the resulting changes in membrane voltage (time $\rightarrow \infty$) could be measured across the cell membrane on either side of the plane of cleavage (Fig. 2). This arrangement gives continuous information on the intercellular electrical coupling, that is, on the fraction of the current that passes from cell to cell across the plane of cleavage. Moreover, it gives the electrical resistance of the mother cell and, after completion of cell division, the resistance of the daughter cells. The resistance of the urea-treated fertilization membrane is negligible. This resistance, measured directly with microelectrodes placed in the space between fertilization membrane and cell membrane, was in all cases three orders of magnitude lower than the resistance of the cell membrane. Both membranes presented no appreciable rectification nor signs of excitation over the entire range of current used. Microelectrodes of very small tip diameters were employed (*1*). With these electrodes, there were no signs of current leakage, and some of the cells continued division well into the blastula stage. The results reported here are from such cells.

Figure 2 illustrates the results of a typical experiment in which measurements were made throughout the process of cell division, from the earliest stages of cleavage to its completion. At the onset of cleavage, the changes in membrane voltage are about equal in the two cell halves. As cleavage proceeds, the voltage decreases progressively in the half opposite to the current electrode, falling rather abruptly

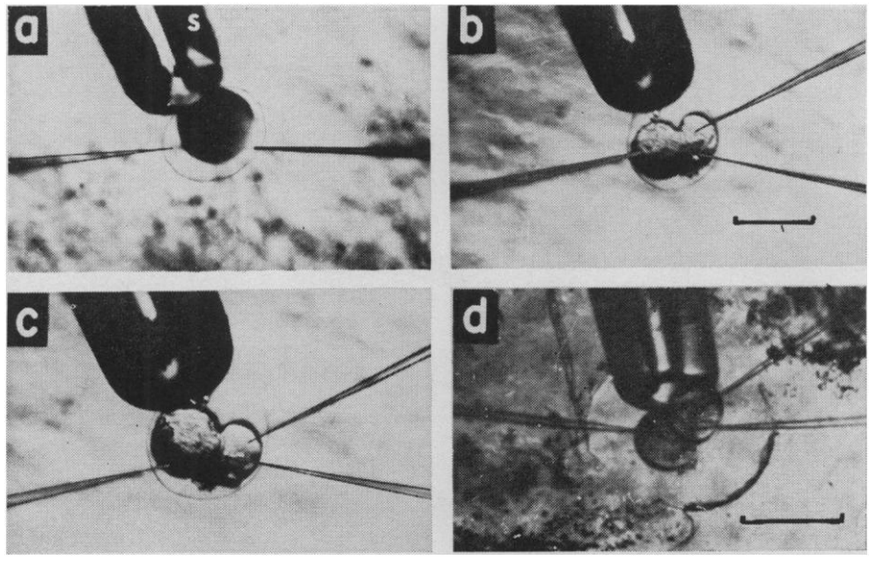

Fig. 1. A dividing egg cell. (*a*) The cell about 95 minutes after fertilization (16°C) in the process of being impaled by two microelectrodes. Its swollen fertilization membrane is sucked onto a glass capillary (*S*) (*5*) to hold the preparation. (*b, c, d*) The preparation at various stages of cleavage with three microelectrodes in intracellular position. Calibration lines for upper and lower rows are 100 μ.