References and Notes

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2. Supported by grants from the National Institutes of Health (NB-02484), and from the Alfred P. Sloan Foundation, Inc.

15 November 1962

Behavior in Hydra: Contraction Responses of Hydra pirardi to Mechanical and Light Stimuli

Abstract. Hydra pirardi *contracts in response to light and mechanical agitation. The animals show a reduction in the number of contractions in response to mechanical agitation on repeated testing but continue to contract in response to a light stimulus. Excision of all the tentacles of the animal completely inhibits contraction in response to mechanical agitation but does not affect contraction in response to light. The results of these experiments suggest that* H. pirardi *has different receptors for light and for mechanical agitation and that the control mechanisms for the contraction responses to these two stimuli are independent.*

Trembley (*1*) described the normal movements of hydra in detail, observing that the animals contract and expand spontaneously. More recently, Reiss (*2*) studied rhythmic spatial patterns in the spontaneous contractions of hydra. The experiments described in this report are concerned with the contraction rates for *Hydra pirardi* (*3*) in response to two external stimuli: light and mechanical agitation. The rates of stimulated contraction are much greater than the rate of spontaneous contraction.

Trembley reported that the movements of *Hydra viridis* show a definite relation to a light source (heliotropism). Wilson (*4*) extended this early work and observed that the animals collect on the side of the aquarium having the greatest illumination and appear to be most sensitive to blue light. We observed that when strong sunlight fell on a culture of *H. pirardi*, the animals contracted within 15 to 30 seconds. Artificial lights of various sorts had a similar effect, but stimulation for longer periods (1 to 2 minutes) was required. Doubtless this phenomenon has been observed before, but we have found no reference to it in the literature.

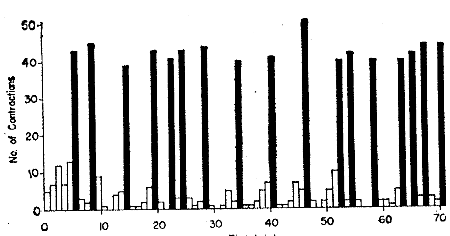

Fig. 1. Number of contractions induced in 50 *Hydra pirardi* by light. (Solid columns) Number of hydra that contracted during 1-minute periods of exposure to strong light; (open columns) number of hydra that contracted during 1-minute periods in which the animals were not exposed to strong light.

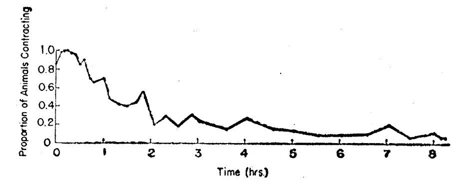

Fig. 2. Curve for the contraction response of *Hydra pirardi* to mechanical agitation. Each point represents the average proportion of animals that contracted within the 2-second period that followed a 2-second shaking period and is based on observations for five animals during ten successive periods.

Experiments were carried out to measure the contraction rates for *H. pirardi* in response to various light sources. The contraction rate is defined as the proportion of animals that contract during a specified time. The *H. pirardi* were cultured by the methods of Loomis and Lenhoff (*5*). In one series of studies the light source was a Mercury vapor arc lamp (*6*) with an emission spectrum that corresponds approximately to that of mercury vapor superimposed upon a low-level continuous spectrum. Unfiltered light from this source was reflected by means of a mirror onto a dish of *H. pirardi* that had been starved for 24 hours at 21°C. The total distance traveled by the light was 40 cm. Four groups of either 12 or 13 hydra were exposed to the light for 1-minute periods at intervals ranging from 1 to 5 minutes. The sequence of these intervals was chosen by a random process for the first group; the same sequence was used for the three groups subsequently tested. During the intervals the animals were exposed to the diffuse light of the laboratory (*7*). Figure 1 shows the number of hydra that contracted during each 1-minute period of exposure. The solid columns of the histogram correspond to the 1-minute periods during which the animals were exposed to the strong light. The open columns indicate spontaneous contractions of the animals in 1-minute periods during which they were not exposed to strong light. As Fig. 1 shows, *H. pirardi* contracts in response to a light stimulus.

In a second series of experiments the effect of mechanical agitation on contraction was investigated. Wagner (*8*) showed that specimens of *Hydra viridis* contract when the outsides of their culture dishes are tapped. He noted that if the animals are stimulated in this way at 1-second intervals, they contract completely. Soon afterward they begin to expand, and they remain extended. Thus it appears that *H. viridis* becomes accustomed to a slight, recurrent, nonlocalized mechanical stimulus and no longer responds to it. Wagner found, however, that if the interval between the stimuli is increased, to allow the animals to expand fully after each contraction, no habituation occurs and the animal always contracts. In contrast to this result we have repeatedly demonstrated that *H. pirardi* becomes accustomed to nonlocalized mechanical stimulation even when the interval between the stimuli is long enough to allow the animal to expand completely. This was demonstrated in more than 98 percent of more than 1000 *H. pirardi* tested. In a typical experiment, five animals which had not been fed for 24 hours were placed in 50 ml of water in a Stender preparation dish. After the animals had attached themselves to the

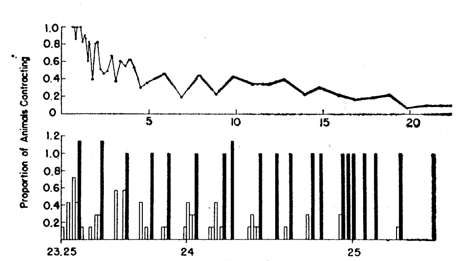

Fig. 3. The effectiveness of light in inducing contraction in *Hydra pirardi* previously habituated to shaking. (Top) Curve for the contraction response of the animals to shaking. Each point represents the average proportion of animals that contracted within the 2-second period that followed a 2-second shaking period and is based on observations for five animals during ten successive periods. (Bottom, solid columns) Proportion of animals that contracted during subsequent 1-minute periods of exposure to strong light; (open columns) proportion of animals that contracted spontaneously or in response to shaking alone.

bottom of the dish they were shaken, at 105 rotations/min, on a variable-speed rotator (*9*) for periods of 2 seconds every 16 seconds. At first the animals contracted in response to each 2-second shaking period. As shaking continued, the proportion of animals that contracted decreased to an asymptote value (Fig. 2). Thus, *H. pirardi* becomes accustomed to intermittent nonlocalized mechanical stimulation.

Since *H. pirardi* was observed to contract in response to strong light and to become accustomed to mechanical agitation, an investigation was made to determine whether a light stimulus would still elicit the contraction response from animals habituated to shaking. In one experiment, five animals that had been starved for 24 hours were placed in 100 ml of water in a shallow dish of 300-ml capacity at 21°C. The dish was shaken at 50 rotations/min for 1-second periods every 19 seconds. The proportions of animals that contracted in response to shaking for 1 second were recorded (Fig. 3, top).

The contraction rate reached the asymptotic value after approximately 20 hours of shaking. When the animals had become accustomed to the shaking, the strong light was directed on them for 1-minute periods, while shaking was continued. In Fig. 3, bottom, the solid columns of the histogram show the proportion of animals that contracted during the 1-minute periods of light; the open columns indicate the proportion of animals that contracted spontaneously or in response to the shaking alone. In 20 trials of response to light, all the animals contracted on each exposure to light. Occasionally an animal contracted, expanded, and contracted again during the 1-minute period. This uniformly high rate of contraction in response to light demonstrates that *H. pirardi* contracts in response to this stimulus even though it no longer contracts on being shaken.

When this experiment was repeated with a lamp of much lower intensity (*10*) than the original light source, similar results were obtained. These results also show that the time between the onset of the light and the contraction response is inversely related to the intensity of the light. In addition, there is a wavelength specificity for the contraction of *H. pirardi* in response to light. The time between the onset of the light and the beginning of a contraction is shortest for blue light (400 to 450 mμ) (*11*). After these experiments had been completed we learned of the recent report of Passano and McCullough (*12*) that *H. pirardi* display rhythmically reoccurring electrical potentials. Their results show that blue light changes both the frequency and the point of origin of these potentials. Since the potentials are related to the contraction response, our behavioral observations support this finding.

It appears that *H. pirardi* does not become accustomed to a light stimulus but does become accustomed to mechanical stimulation. Indeed, in more recent experiments we found that the contraction rate was not reduced after 200 hours of intermittent exposure to light. We have also discovered that excision of all tentacles completely inhibits the contraction response to mechanical agitation. Such removal of tentacles does not affect the contraction response to light. However, removal of tentacles and hypostome inhibits the response to both stimuli (*11*). These results, together with those described earlier in this report (*13*) suggest that *H. pirardi* has different receptors for light and for mechanical agitation. In addition, the fact that an animal showing a reduced rate of contraction in response to mechanical stimulation still contracts in response to light suggests that the control mechanisms for the contraction responses to these two stimuli are independent of each other.

NORMAN B. RUSHFORTH
ALLISON L. BURNETT
RICHARD MAYNARD
Developmental Biology Center, Western Reserve University, Cleveland, Ohio

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7. The intensity of this light was approximately 20 ft-ca.
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10. The lamp of lower intensity was American Optical Company Illuminator model 353, which housed a General Electric bulb model 1493.
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13. This research was supported in part by a grant from the National Science Foundation.

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Human Lipoproteins: Role in Transport of Thyroid Hormones

Abstract. *Lipoproteins from patients receiving therapeutic doses of iodine-131 were isolated by density-gradient techniques. The binding of circulating thyroid hormones by beta lipoproteins (those of low density) was negligible. Alpha lipoproteins (those of high density) bound appreciable amounts. The bulk, however, was bound by proteins of density higher than 1.23 g/ml.*

Although it is generally accepted that various circulating plasma proteins are capable of transporting thyroid hormones, there is no general agreement about the nature of the linkage of thyroxine and triiodothyronine to these (*1*). The different methods used have yielded somewhat different results (*2*). The thyroid hormones appear to be bound mainly to an alpha globulin migrating between the alpha-1 and alpha-2 globulins (thyroxine-binding globulin, TBG) when the human serum containing physiologic concentrations of thyroid hormones is subjected to paper electrophoresis in barbital buffer (*p*H 8.6), but no detailed analysis of the chemical nature of thyroxine-binding globulin has been performed. There is evidence that TBG may be a glycoprotein (*3*).

J. R. Tata has indicated that there are some lipids in addition to carbohydrates in isolated TBG (*4*). Clausen and Munkner, using a combination of immunoelectrophoresis of human serum incubated with radiothyroxine and radiotriiodothyronine and autoradiographic techniques, concluded that the only proteins in human serum which bind hormones are the three known lipoproteins, the alpha-2 lipoproteins (the slow moving lipoprotein fraction), alpha-1 lipoprotein (the faster moving lipoprotein), and lipalbumin (*5*).

If this is so, separation of lipoproteins from sera which have been incubated with radiothyroxine or triiodothyronine or removal of lipoproteins from sera of patients who have received doses of I^{131} would contain practically all the radioactivity bound in organic materials.

This separation can be accomplished without too much difficulty by density gradient techniques by taking advantage of the difference in density between the proteins containing lipids and the remaining proteins (*6*). Of course, these lipoproteins must be isolated without too much handling to obtain them