showed a slight initial reduction in the photosynthetic rate, possibly because of damage to the fragile upper portions at the time of cutting. Localization of the nucleus in the rhizoidal part of the plant is well established for other species of *Acetabularia* (*A. mediterranea* and *A. crenulata*) (*11*). A similar position for the nucleus in *A. major* was indicated in specimens stained with Feulgen's reagent or methyl green-pyronin (*12*).

A marked diurnal periodicity in photosynthesis was observed in both juvenile and capped plants maintained in natural light. In both cases the maximum rates occurred at about local noon and were approximately five times greater than the night values. Representative data are presented in Fig. 1 without correction for respiration. Measurements of respiratory rates (in the presence of potassium hydroxide) at noon and at night, however, gave values of only 6 and 8 percent, respectively, of the noon rates of net photosynthetic oxygen production.

The endogenous nature of this rhythm was established by observations that, at constant temperature, the photosynthetic rhythm persisted for at least several days in plants maintained in continuous light of low intensity (Fig. 1) and for one cycle when the plants were maintained in darkness. After enucleation, the rhythm in photosynthesis continued unaltered both in juvenile plants and in those with caps (Fig. 1). This was true of plants maintained either in alternating light and darkness or in constant light.

In a recent extension of these studies to *Acetabularia crenulata*, collected from the Florida Keys, a similar situation was observed. Enucleated plants showed a persistence of the endogenous photosynthetic rhythm characteristic of intact plants. In addition, it was found that the rhythm of enucleated, as well as intact, plants could be reset by inversion of an artificial light-dark schedule (Fig. 2).

These results lead to the conclusion that the nucleus is not required for the immediate maintenance of time-keeping in *Acetabularia*. In this organism, processes in the cytoplasm are able to control both the period and the phase of the endogenous diurnal rhythm in photosynthesis (*13*).

BEATRICE M. SWEENEY
FRANCIS T. HAXO
Scripps Institution of Oceanography, La Jolla, California

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Effect of Ribonuclease on Retention of Conditioned Response in Regenerated Planarians

Abstract. Conditioned planarians were transected and allowed to regenerate in a ribonuclease solution or in pond water. Heads which had regenerated in ribonuclease displayed a retention level equal to that of head and tail sections which had regenerated in pond water. However, tails regenerated in ribonuclease performed randomly although they could be retrained to criterion.

Various data suggest that the neurophysiological mechanism of memory consists of two classes of process: (i) a short-term process, perhaps consisting of reverberatory electrical activity, and (ii) a long-term process, by which neural excitability patterns are maintained by some sort of structural alteration. As radio isotope exchange data on brain compounds have accumulated, it has become apparent that these compounds seem to be characterized by rather rapid rates of turnover. In order to reconcile the persistence of memory with this lability of brain chemistry, it seems logical to search for a substance capable of maintaining a structural modification by imposing an experientially specified configuration on molecules being built in neural tissue. Imposition of the additional requirement that this substance be cytoplasmic in locus directs attention to ribonucleic acid (RNA). Essentially similar conclusions have been suggested in theoretical speculations by von Foerster (*1*) and Hydén (*2*).

Some experimental data seem compatible with this suggestion. Brattgård (*3*) has demonstrated a relationship between RNA synthesis and stimulation in retinal ganglion cells. Morrell (*4*) has demonstrated histochemically an increase in RNA concentration which is a result of prior excitation. Kreps (*5*) has reported differentially increased turnover of RNA in the cortical receiving area of the conditioned stimulus after elaboration of conditioned responses in the dog. In earlier work, John, Wenzel and Tschirgi (*6*) observed that injection of ribonuclease solution into the lateral ventricle of cats interfered with performance of pattern discrimination for food but not with a conditioned avoidance response to visual or auditory stimuli. The anatomical and chemical complexity of the preparation posed formidable obstacles to the gathering of control data necessary for unambiguous interpretation of these results. Rather than attempt to cope with these complexities, it seems desirable to devise a simpler preparation.

Recent research on the planarian has demonstrated that this comparatively simple organism is capable of learning both a classical conditioned response and a T-maze (*7–9*). Of particular relevance to our present concern was the finding that when cut in half and allowed to regenerate, both the head and tail sections display equal savings scores in both types of situations (*8*). Since there is cephalad dominance in these animals, the tail sections have in some manner apparently transmitted the effects of learning experience to the regenerated anterior portion. Experiments in our own laboratory, with the classical conditioned response, have confirmed the above findings (*10*). These various considerations suggested to us the possibility that RNA might play a role in the transmission of an acquired structural configuration from the trained portion to the regenerating tissue. Conditioned tails, regenerating in the presence of ribonuclease, might be expected to produce anterior portions with a depleted or altered RNA structure, perhaps due to influences exerted at the regenerating interface. Such an organism might then have a naive dominant head. Conversely, since trained heads have only a nondominant tail to re-

Table 1. Response to light and training scores.

Item	Group									
	I	II	III	IV	V	VI	VII	VIII	IX	X
N	12	13	6	6	6	6	6	6	6	6
Base (%)	15.4	16.7	17.5	11.9	19.2	19.1	23.9	16.1	14.5	15.2
Training trials (No.)	500.8	512.4	None	None	None	None	441.3	546.2	412.3	311.0
Cut	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Treatment*	E	PW	E	PW	E	PW	E	PW	E	PW
Retention I (%)†										
Day 1										
Heads	46.6	42.8	15.0	13.3					54.5	48.8
Tails	16.2	37.4	18.2	14.8						
Day 2										
Heads	50.9	36.8	17.7	11.3					53.0	48.5
Tails	20.2	34.2	15.8	12.2						
Day 3										
Heads	42.0	28.7	13.8	9.8					46.8	33.2
Tails	20.9	30.2	14.3	10.2						
Retraining trials (No.)										
Heads	258.6	185.0	420.8	377.7					223.0	241.2
Tails	336.4	253.4	457.5	403.7						
Retention II (%)										
Day 1										
Heads	39.0	64.0	70.3	66.8	13.5	15.3	33.3	40.5	69.3	45.6
Tails	45.5	58.3	73.8	71.3	15.0	14.8	27.3	34.0		
Day 2										
Heads	41.0	54.2	61.5	48.8	13.6	16.0	36.0	30.2	55.6	50.0
Tails	40.5	48.6	51.3	44.1	15.2	18.8	14.0	31.0		
Day 3										
Heads	33.0	51.5	42.8	31.7	9.5	19.7	26.0	19.8	32.8	36.7
Tails	21.5	35.0	34.3	35.8	9.7	10.8	15.7	19.5		

*Treatment: E, enzyme (ribonuclease); PW, pond water. †Retention: Heads, regenerated heads; Tails, regenerated tails.

grow, they should demonstrate a greater degree of retention. Histological data provided by Chow (*11*) indicate that planarian tails contain nerve somata, a fact clearly relevant to the aforementioned hypotheses.

In pilot work, we found that planarian tails could regenerate heads in pond water containing ribonuclease in concentrations of 0.1 mg/ml (*12*). The visible structural anomalies invariably obtained at this concentration indicated clear effects of the enzyme. In the experiments here reported, ribonuclease concentrations ranged from 0.07 to 0.1 mg/ml. Structural anomalies were seldom observed at the lower concentrations.

As reported elsewhere (*7*), paired presentation of light and shock to planarians results in the consistent appearance of a conditioned contraction and head movement to light alone. Specimens of *Dugesia dorotocephala* (*13*) were divided into ten groups. In accordance with the design apparent from Table 1, which summarizes the results, base rates of response to light alone were determined. Worms were trained to the criterion of 34 conditioned responses per daily session of 40 trials; then they were transected into equal portions, which were allowed to regenerate for 14 to 18 days in either pond water alone or pond water containing ribonuclease. The regenerated portions were tested for 3 consecutive days for retention of the conditioned response to light alone, retrained to previous criterion, and then again tested for their response to light alone.

The contrast between the retention of the conditioned response by worms regenerated from heads and tails in the presence of ribonuclease and the retention of worms regenerated in pond water alone is shown by groups I and II. Control for possible sensitization due to the effects of transection or of regeneration in ribonuclease, as well as control for the possible effects of ribonuclease on structures necessary for acquisition of the conditioned response, was provided by groups III and IV. Control for the effects of the time period necessary for the total procedure was provided by groups V and VI. A control to ascertain whether the retraining procedure or merely the lapse of time accounted for the reappearance of the conditioned response in groups I and II was provided by groups VII and VIII. Groups IX and X provide a basis for evaluating whether ribonuclease affects intact tissue or acts only where regeneration occurs.

The results of all significant *t*-tests obtained are shown in Table 2. Conditioned tails regenerated in ribonuclease do not retain the conditioned response. Conditioned heads similarly treated do not differ significantly from the controls. That ribonuclease does not affect intact tissue, as confirmed by results with groups IX and X, suggests that the effect occurs at the regenerating interface. Regeneration in ribonuclease does not interfere with acquisition as evidenced by groups III and IV. The configuration of the remaining control data permits us to rule out effects of transection alone, as well as temporal effects, in these results.

The savings scores obtained for retraining on groups I and II suggest that the tails treated with ribonuclease may retain some residual effects of the prior experience, although they are unable to transmit the effects to the regenerating tissue. A clearer understanding of these findings seems to require a more intimate understanding of the mechanism of information transfer, which we hope to obtain from electrophysiological and grafting experiments

Table 2. Significant *t*-tests for the data given in Table 1.* Some animals died during the experiments. The *N* on which these statistics were computed can be inferred from the degrees of freedom (df).

Group†	Condition	*t*	*p*	df
I_h vs. I_t	Retention I	6.5	.001	19
I_t vs. II_t	Retention I	6.4	.001	23
I_t vs. VII_t	Retention II	5.5	.02	3
II_t vs. VII_t	Retention II	3.2	.01	10
II_h vs. $VIII_h$	Retention II	2.5	.05	10
VII_h vs. VII_t	Retention II	5.8	.01	4
I_t	Training vs. retraining	3.01	.02	12
I_h	Training vs. retraining	4.1	.01	8
I_h	Base rate vs. retention I	3.5	.01	12
II_t	Training vs. retraining	4.9	.001	12
II_h	Training vs. retraining	5.5	.001	12
II_t	Base rate vs. retention I	5.6	.001	17
II_h	Base rate vs. retention I	3.6	.01	17
IX	Training vs. retraining	3.1	.02	17

*t-Tests on retention data have been reported only for the first day on which significant differences were obtained. †Roman numerals denote groups; subscripts: h, regenerated heads; t, regenerated tails.

now in progress. While the results of this investigation do not establish the identity of the chemical substance responsible for the conditioned behavior of the regenerated animal, they appear entirely compatible with the assumption that this substance may be RNA. Further histological and biochemical explorations of this preparation are needed to evaluate this possibility adequately (*14*).

W. C. CORNING
E. R. JOHN
Center for Brain Research and
Department of Psychology, University of Rochester, Rochester, New York

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Latent Period of Relaxation

Abstract. The latent period of relaxation of molluscan myocardium due to anodal current is much longer than that of contraction. Although the rate and the grade of relaxation are intimately related to both the stimulus condition and the muscle tension, the latent period of relaxation remains constant, except when the temperature of the bathing fluid is changed.

It is well known that a muscle exerts its tension after a short latent period. Hill (*1*) stated that this latent period of a muscle after stimulation is an extremely sensitive indicator of slack between the fixed support at one end of the muscle and the mechano-transducer at the other. Sandow (*2*) considered the latent period of a muscle to be intimately related to chemical reactions prior to muscle contraction. There appears to be much information concerning the latency before muscle contraction, but we do not have much information about the latent period of relaxation. The probable reason for this lack of information is that we have not been able to induce relaxation by easily controlled electrical techniques.

It has been demonstrated that relaxation can be produced by applying an electrical stimulus to a tonic smooth muscle preparation (*3*) and to a skeletal muscle in a state of contracture (*4*). The relaxation is probably due to the electrotonic effect of the current applied on the mechanical system. Recently we have observed in oyster myocardium that relaxation can be produced by anodal stimulus and contraction by cathodal stimulus (*5*).

In the present study, we isolated an oyster heart in an artificial sea-water bath; the atria were ligated and connected to a bonded strain gauge. The other end of the heart was left joined to the aorta in order to record the isometric tension. A 500-μ silver–silver chloride electrode rested on an atrium and the other end was connected to an isolator circuit of the electronic stimulator. A large wick electrode in the sea-water bath served as the source of polarity and as the stimulating electrode. Because the current-applying electrodes were the surface electrodes, the passage of the current through the membrane is not simple and is difficult to analyze, but the effect of reversal of polarity is very definite, as is demonstrated by the illustration.

A typical example of cathodal contraction and anodal relaxation is shown in Fig. 1*A*, where the effects of stimulation during the relaxation phase of a heart cycle are shown superimposed on a rhythmical heart beat. In Fig. 1, *A-1* represents the normal cycle and *A-2* and *A-3* represent cathodal and anodal stimulation, respectively. The latency of cathodal contraction is estimated to be 90 msec, whereas the anodal relaxation latency is 300 msec. The difference in latency is more than threefold. The difference in latency of relaxation and contraction can be demonstrated even more clearly when the myocardium is arrested in a state of tonic contraction by means of potassium chloride depolarization (Fig. 1*B*). In 58 instances of anodal polarization the latent period of relaxation averages 291 msec; this value is very similar to the latent period of relaxation from spontaneous contraction. We believe that the latent period for relaxation is independent of the tonic state of the myocardium, and independent of the intensity of stimulus, although the speed and the grade of relaxation are both intimately related to the strength of stimulus.

The administration of narcotics, such as urethane and ether, or metabolic inhibitors such as monoiodoacetic acid and 2,4-dinitrophenol, causes a remarkable inhibition of relaxation, but the latent period remains constant before and after an administration of these drugs. The temperature change of the external fluid appears to be the only condition that will vary the latent period. The higher the external temperature, the shorter the latent period. The average temperature coefficient is 1.98. The latent period of relaxation appears to have some relation to the chemical process. This relationship is supported by the observation of Weber (*6*) who suggested that the adenosine triphosphate splitting is a prerequisite for contraction, and that the relaxation phase of muscle is not an active process, but simply the end of the adenosine triphosphate splitting.

Still another theory for the explanation of the latent period is that there

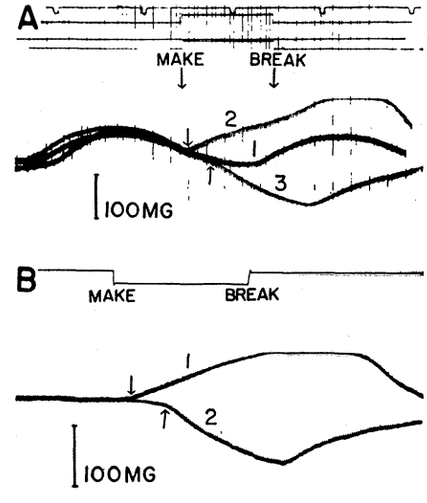

Fig. 1. Tension curves showing latent periods of contraction and of relaxation. Stimulation was applied at the stimulus mark; duration, 1 sec. Time signal, 1 sec. *A*, Latent periods in a spontaneously contracting muscle: 1) control, 2) cathodal contraction, 3) anodal relaxation. *B*, Latent periods in a tonic muscle with 1.25-percent KCl solution applied: 1) cathodal contraction, 2) anodal relaxation. A downward arrow indicates the point at which contraction starts and an upward arrow shows the point at which relaxation starts.