Studies of Protoplasmic Structure in *Spirogyra*. VI Effects of Sound and Electricity on Elasticity ¹⁾

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Previous investigations from this laboratory have demonstrated that protoplasm in cells of *Spirogyra* is an elastic fluid and probably has a net-like ultra-microscopic structure (Northen 2, Northen and Northen 3). Furthermore, Pfeiffer (7) through investigations on the rate of movement of protoplasts into tubes has also demonstrated that protoplasm is an elastic fluid. All stimulating agents which we have previously investigated, cutting (Northen and Northen 3), anesthetics (Northen 4), heat (Northen 5), uneven pressure (Northen 6), and hypertonic solutions (Northen 6), caused a decrease in the elasticity of protoplasm in cells of *Spirogyra*. The present investigation will demonstrate that sound, direct currents, and alternating currents also cause a decrease in the elasticity of protoplasm. Hence it is hypothesized that stimulating agents initially decrease protoplasmic elasticity probably through loosening the structural network.

Effect of Sound on Elasticity

Method. Three species of *Spirogyra* were used. *Spirogyra 1* was used in experiment one, *Spirogyra 2* in experiment two, and *Spirogyra 3* in experiments three and four. Filaments of *Spirogyra* were placed in a Petri dish and a tuning fork was suspended in the water to a depth of 0.5 cm. In experiment one the filaments were placed about 1 cm. from the fork, whereas in the other experiments they were about 4 cm. from the fork. The fork was then vibrated by striking the part above the water every five seconds. After the filaments had been exposed to the vibrations for the desired time they were laid between two strips of moist cotton and centrifuged with the maximum centrifugal acceleration which did not move the chloroplasts in most cells of control filaments. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced (moved to the centrifugal end) were determined for

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experimental and control groups. For each experimental group about 200 filaments were used.

Data and Discussion. The data are summarized in table 1.

It will be noted that in all instances the chloroplasts have been displaced in more filaments of those exposed to sound (experimental group) than in those which were not so exposed. The chloroplasts

Table 1. Effect of sound on protoplasmic elasticity

Experi- ment number	Centrifugal acceleration	Minutes centri- fuged	Pitch of fork	Minutes exposed	Percentages of filaments in the cells of which the chloroplasts were displaced	
					In experi- mental groups	In control groups
1	42.5 × G	8	512	8	78%	0%
	42.5 × G	8	384	8	88	6
	42.5 × G	8	320	8	61	6
2	75.6 × G	6	512	8	20%	7%
3	265.5 × G	2	512	6	74%	17%
4	1062.5 × G	1	512	5	39%	16%
	1062.5 × G	1	512	8	47	24

moved more readily in the experimental than in the control groups because the vibrations decreased the value of co in the equation, $V = k(c - co)$, which approximately governs the velocity of chloroplastic movement in response to different centrifugal accelerations, (Norden 2). In the above equation V is the velocity of chloroplastic movement, k is a constant, c is the centrifugal acceleration used, and co is the initial starting acceleration at which or below which the chloroplasts will not move regardless of how long the filaments are centrifuged. For example, in experiment 1 the chloroplasts were displaced in 78% of the filaments exposed to sound (pitch = 512) and in 0% of the control filaments. At the time that experiment was performed the average value of co in control filaments was equal to or greater than $42.5 \times$ gravity. Hence when the control filaments were centrifuged with an acceleration of $42.5 \times$ gravity the velocity of chloroplastic movement would be: $V = k(42.5 - 42.5) = 0$ or $V = k(42.5 - > 42.5) = < 0$. A velocity less than 0 is impossible. In those exposed to sound the vibrations decreased the value of co and in 78% of the filaments it was decreased to a value less than $42.5 \times$ gravity. Hence for such filaments the velocity of chloroplastic movement would be: $V = k(42.5 - < 42.5) = > 0$. A decrease in co has been interpreted as a decrease in elasticity and a decrease in elasticity indicates that the protoplasmic network has been loosened.

Effect of Direct Current on Protoplasmic Elasticity

Method. Platinum electrodes connected through an ammeter and a rheostat to a source of current were placed 1 cm. apart on a strip of wet cotton which was partially immersed in water in a Petri dish. Portions of filaments of *Spirogyra* were then placed over the electrodes so that a current of uniform density passed through the filaments. In each experiment about two hundred filaments were used. After the desired intensity of current had flowed for the desired time the filaments were centrifuged with an acceleration of $170 \times$ gravity for three minutes. Following centrifugation the percentages of filaments in the cells of which the chloroplasts were displaced in experimental and control groups were determined.

Data and Discussion. The data are summarized in table 2.

It will be noted that in all instances the chloroplasts have been displaced in more experimental filaments (exposed to current) than in the control filaments. Hence, it is concluded that with the inten-

Table 2. Effect of direct current on protoplasmic elasticity

Milli-amps	Percentages of filaments in the cells of which the chloroplasts were displaced after exposures of										Range of controls
	0 secs.	5 secs.	10 secs.	20 secs.	40 secs.	60 secs.	90 secs.	180 secs.	360 secs.	600 secs.	
0.15	2%	—	—	—	—	—	—	—	—	65%	—
0.25	1	—	—	—	—	—	—	—	—	78	—
0.50	0	—	—	—	23%	12%	—	56%	—	87	—
1.00	5*	—	—	58%	77	63	75%	78	82%	64	0%-10%
2.00	2*	—	84%	84	76	99	—	—	—	—	0%-3%
4.00	4*	—	92	85	82	—	93	—	97%	—	0%-14%
6.00	0	55%	95	81	32	92	59	—	—	—	—

* Controls were run with each duration. The average percentage displacement for the controls is recorded in column 2, whereas the range of the controls is recorded in the last column on the right.

sities and durations used direct current decreases protoplasmic elasticity in cells of *Spirogyra* and it is believed that a decrease in elasticity results from a loosening of the protoplasmic network.

Effect of Alternating Currents on Protoplasmic Elasticity

Method. The method was essentially the same as that used with the direct current except that a twelve volt alternating current was used.

Data and Discussion. The data are summarized in table 3.

Table 3. Effect of alternating currents on protoplasmic elasticity

Milli-amps	Percentages of filaments in the cells of which the chloroplasts were displaced after exposures of					
	0 min.	12.0 min.	15.6 min.	21.3 min.	30.6 min.	48.0 min.
1.0	4%	—	—	—	—	26%
1.25	15	—	—	—	29%	—
1.50	4	—	—	49%	—	—
1.75	7	—	63%	—	—	—
2.00	5	74%	—	—	—	—

In the above experiments the Nernst equation (Heilbrunn 1), $I\sqrt{t} = k$, was tested and apparently did not hold for the intensities used. In the above equation I is the intensity, t is the duration, and k is a constant. It will be noted that when a current of 2 milliamps was allowed to flow for twelve minutes the chloroplasts were displaced by the centrifugal acceleration in the cells of 74% of the filaments. If the law be valid and if the intensities were above the threshold the chloroplasts should have been displaced in 74% of the filaments which were exposed to intensities of less than 2 milliamps because the durations with such intensities were calculated from the Nernst equation. For example, the calculated duration necessary to get a 74% displacement with a current of 1 milliamp would be: $1\sqrt{t} = 2\sqrt{12}$. $t = (2\sqrt{12})^2 = 48$. However, it will be noted that the chloroplasts were displaced in only 26% of the filaments which were exposed to a current of one milliamp. Hence, with the intensities used the Nernst equation did not hold.

However, it will be noted that in all instances the chloroplasts were displaced by the centrifugal acceleration in more cells of experimental filaments than in those of control filaments, thus indicating that the alternating current also decreases the elasticity of protoplasm in cells of *Spirogyra*.

Summary

Vibrations from tuning forks, direct current of intensities from 0.15 milliamps to 6.00 milliamps, and alternating currents of intensities from 1 to 2 milliamps decrease the elasticity of protoplasm in cells of *Spirogyra* as evidenced by the observations that centrifugal accelerations displaced the chloroplasts more readily in cells exposed to the above stimulants than in cells which were not so exposed.

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