HYDROSTATIC PRESSURE AND TEMPERATURE IN RELATION TO STIMULATION AND CYCLOSIS IN NITELLA FLEXILIS

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This study was undertaken for two reasons, (1) to determine if Nitella can be stimulated by a sudden increase or decrease of hydrostatic pressure; (2) to compare the cyclosis velocity under pressure in Nitella with that in Elodea, which Marsland (1939) found to be progressively slowed as the pressure was increased. This slowing was accompanied by a similar progressive decrease in viscosity, as measured by the rate of sedimentation of chloroplasts within the Elodea cell under the influence of centrifugal force.

As is well known, response of Nitella cells is observed after stimulation by touching or bending (see Ewart, 1903; also Osterhout and Hill, 1931), application of solutions such as KCl or chloroform (Osterhout and Hill, 1930), cold or heat (Dutrochet, 1838), electrically (Dutrochet, 1838; Hörmann, 1898) or by flashes of ultraviolet light (Harvey, 1942). The criterion of response may be an action potential or a stopping of the cyclosis, which has been called "shock stoppage."

Nitella does not under all circumstances obey the all-or-none law, since the magnitude of the action potential may vary and conduction with a decrement may occur (Blinks, Harris and Osterhout, 1929). Hill (1941) studied the relation between stimulation and cyclosis in Nitella and concluded that electrical stimulation is accompanied by an action potential only if the stimulus is of sufficient intensity. A week stimulating voltage will cause the cyclosis gradually to slow down and come to a stop, no action potential appearing, while a higher voltage causes an abrupt stop with an action potential.

In addition to the normal action potential, which is propagated at a rate of 2 to 3 cm. per second, electrical variations may occur in Nitella due to mechanical disturbances which travel along the cell with high velocity and stimulate each region as they pass (Osterhout and Hill, 1931), as well as death waves, also travelling with high velocity (Osterhout and Harris, 1928, 1929). The response may thus be decidedly complicated but shock stoppage of cyclosis is a sure sign of stimulation.

Since the pressure chamber used in these experiments contained no electrodes for leading off the action potential, the criterion of stimulation by pressure was a sudden stopping of cyclosis. In addition we may expect pressure to affect cyclosis directly as in the Elodea experiments of Marsland (1939).
As \textit{Nitella} is particularly sensitive to mechanical stimulation, the first experiments were directed to determining if a pressure wave in water at atmospheric pressure would cause shock stoppage of cyclosis. For this purpose a brass chamber (10 cm. high and 5.1 cm. internal diameter) was made, which contained a glass window 6.2 mm. thick in the lower end and in the other a solid brass piston containing a small hole fitted with a lucite plug. The chamber was completely filled with pond water (excluding air bubbles) and a \textit{Nitella} cell, which rested on the glass bottom and could be observed with an inverted microscope by means of a light beam that passed through the small hole fitted with lucite in the piston head. The piston head rested on the water column and could be given a sharp blow with a hammer or with a steel ball falling from a given height. A pressure wave could thus be started, which travelled downward through the water to strike the \textit{Nitella} cell, whose cyclosis was continually observed. It was found that such pressure waves resulting from blows with a hammer or from a steel ball of 534 gm. weight falling 145 cm. (energy = 7.6 joules) did not affect cyclosis, unless a glancing blow, that actually moved the \textit{Nitella} cell, struck the cylinder. A pressure wave like the above in water at atmospheric pressure may therefore be eliminated as a stimulus to stoppage of cyclosis.\footnote{Ewart (1903, p. 72) quotes Hörmann (1898) as finding that rapid changes in pressure up to 2 atmospheres do not cause shock stoppage of cyclosis in \textit{Nitella syncarpa}, but that a smart blow on a piston head connected with the chamber containing \textit{Nitella} will cause temporary cessation of streaming. The conditions of the experiment are evidently important.}

The next experiments were with sudden changes of hydrostatic pressure. These were applied to the \textit{Nitella} cell in a special pressure chamber with glass windows, similar to that used by Marsland (1939), so that the \textit{Nitella} could be observed with an inverted microscope during the increase in pressure. The pump was a 7 ton hydraulic auto truck jack converted to deliver hydrostatic pressures up to 10,000 lbs. per square inch.\footnote{One atmosphere = 14.7 lbs. per square inch = 1033 gm. per sq. cm. 1000 lbs. per square inch = 68 atmospheres = 7.2 kg. per square centimeter.}

The results depended on the \textit{Nitella} cell. In general sudden increases in pressure from 0 to 5000 lbs. per square inch or 5000 to 9000 lbs. or increases in steps of 1000 lbs. each do not stop cyclosis. Some cells, however, will occasionally stop on a sudden increase of pressure and others whose cyclosis does not stop on changes in pressure up to 5000 lbs. will stop on changing from 5000 to 6000 or 6000 to 7000 lbs. or 7000 to 8000 lbs. These are exceptions but nevertheless the stoppage of cyclosis can only be attributed to stimulation by pressure and not to joggling of the pressure chamber or movement of the \textit{Nitella} cell in the chamber. The response to sudden pressure increase is somewhat like the response to sudden ultraviolet light stimulation, as described by Harvey (1942), where an action potential appeared in only about one-third of the cells exposed to the ultraviolet light flash.

When the pressure on \textit{Nitella} was suddenly released from varying high pressures (9000 to 5000 lbs.) to atmospheric pressure, the cyclosis stopped in 50 per cent of twenty-two trials. The behavior depended on the \textit{Nitella} cell used in the study. Stopping of cyclosis did not necessarily occur as a result of a slight movement in the
field and could again be attributed only to stimulation. Perhaps the greater certainty of stimulation on release of pressure is due to the fact that release of pressure can always be attained more suddenly by turning a valve, whereas increase of pressure must be built up by pumping the handle of the hydraulic jack.

In order to complete the picture of pressure effects, experiments were carried out to see if a sudden decrease of the atmospheric pressure over water containing the *Nitella* cells would cause shock stoppage of cyclosis. The cells were in a glass chamber with a plane glass bottom so that observation could be made with an inverted microscope. The chamber could be connected by a two-way stop-cock and pressure tubing to a large evacuated desiccator or to the air. By turning the cock, sudden decreases or increases of pressure (20 mm. to 760 mm. Hg) could be applied to the *Nitella* cells but shock stoppage was never observed to occur.

The direct effect of pressure on rate of cyclosis apart from stimulation is indicated in Fig. 1, in which the cyclosis rate in micra per second is plotted against the pressure in pounds per square inch, as the pressure is raised in steps of 1000 lbs. per square inch. Cyclosis rate was measured at 23°C. by timing the movement of the granules in the protoplasm across the lines of a square ruled micrometer in the eye-piece. Since the individual granules vary considerably in rate the figures are averages of 5 or more readings. 3 to 5 minutes elapsed between the 1000 lb. changes in pressure. Table I gives the data for all successful experiments.

The slowing of cyclosis by pressure in *Nitella* is not as regular as in *Elodea*, and different cells behave differently. Some show a progressive slowing of cyclosis as the pressure is increased in increments of 1000 lbs. per square inch while in others there is little change in rate until 4000 lbs. is reached and then a progressive decrease. Curve A is the average of all readings on three different cells taken on different days, in which there was a progressive slowing of cyclosis rate as the pressure was increased. Curve B is also the average of all readings on three different cells taken on different days where the slowing of cyclosis began at 4000 lbs. At 9000 lbs. the rate is only about $\frac{1}{2}$ of that at atmospheric pressure and cyclosis does not stop completely at 10,000 lbs. per square inch. Marsland (1939) found cyclosis to stop in *Elodea* between 6000 and 7500 lbs.

If the pressure is suddenly released and no stimulation occurs, the rate generally returns to very nearly the original rate at atmospheric pressure. The same is true when the pressure is decreased in steps of 1000 lbs. per square inch. The return of cyclosis velocity is over approximately the same course as when the pressure is increased. Some cells, however, which have been kept at the high pressure for some time (30 minutes) appear to be injured by the pressure treatment and the cyclosis is permanently slowed.

*Nitella* cells thus behave in most respects in the same way that *Elodea* cells do, except that the cyclosis rate is complicated by phenomena connected with stimulation. From analogy with other experiments on pressure, which brings
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Fig. 1. Cyclosis rate in micra per second as a function of hydrostatic pressure. A, average of three experiments on three different cells at 23°C, where the pressure effects are marked from the start. B, average of three experiments on three different cells at 23°C, where the pressure effects begin after 4000 lbs. per square inch. C, experiment with a single cell at 10°C. Cyclosis returned to 18 μ per second at 10°C, when the pressure was removed and to the original value (41 μ per second) at 24°C, when the temperature was raised.

about solation in many cells (see Marsland, 1942), the decreased viscosity of the protoplasm may be the cause of the slowing of cyclosis but a similar argu-
ment applied to the effects of temperature on cyclosis would lead to another conclusion, namely, that decreasing viscosity with increasing temperature should also result in less rapid cyclosis. Actually cyclosis rate is greatly increased by rise of temperature. Moreover, if viscosity changes determine the rate of cyclosis, one might argue that cyclosis which has been slowed (be-

### TABLE I

**Summary of Nitella Pressure Experiments at 23°C**

<table>
<thead>
<tr>
<th>Date</th>
<th>lbs./in²</th>
<th>1000</th>
<th>2000</th>
<th>3000</th>
<th>4000</th>
<th>5000</th>
<th>6000</th>
<th>7000</th>
<th>8000</th>
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<td>41.0</td>
<td>38.5</td>
<td>38.8</td>
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<td>39.1</td>
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<td>25.3</td>
<td>24.7</td>
<td>26.0</td>
<td>23.5</td>
<td>(21.5)</td>
<td>(20.0)</td>
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<td>47.0</td>
<td>44.4</td>
<td>42.4</td>
<td>38.0</td>
<td>37.8</td>
<td>(35.0)</td>
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<td>31.4</td>
<td>31.4</td>
<td>35.0</td>
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<td>30.0</td>
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<td>22.0</td>
<td>16.9</td>
<td>11.6</td>
<td>(07.0)</td>
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<td>31.4</td>
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<tr>
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<td>32.0</td>
<td>32.3</td>
<td>31.3</td>
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<td>Grand average</td>
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<td>37.9</td>
<td>37.1</td>
<td>35.3</td>
<td>32.8</td>
<td>30.6</td>
<td>28.0</td>
<td></td>
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<tr>
<td>Apr. 4 (dec.)</td>
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<td>47.7</td>
<td>44.5</td>
<td>41.7</td>
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<td>41.3</td>
<td>34.5</td>
<td>29.0</td>
<td>28.1</td>
<td>18.8</td>
<td>19.3</td>
</tr>
</tbody>
</table>

The figures give the cyclosis velocity at increasing pressures. Those in parentheses are extrapolations from a graph. An asterisk indicates a second experiment with the same cell. Nitella cells in the first six experiments showed continuously decreasing cyclosis velocity with increasing pressure. Cells in the last seven experiments were little affected until after 3000 lbs. per square inch pressure. The last line gives values for cyclosis in the Apr. 4 experiment as pressures are decreased from 10,000 lbs. per square inch.

cause of viscous protoplasm) at a low temperature could be increased by raising the pressure and hence decreasing the viscosity, but such is not the case, as I have determined by experiment (see below). However, according to Marsland's (1942) theory, Elodea cyclosis is due to a wave of sol $\rightarrow$ gel transformation, accompanied by change in volume, which passes around the cell. The volume changes are responsible for the circulatory movement. Pressure slows the cyclosis by preventing this sol $\rightarrow$ gel
change, by forcing the equilibrium toward the sol state making the protoplasm mere liquid. Pressure thus acts not by continuously decreasing the viscosity in the ordinary sense but by preventing the sol → gel transformation.

The whole question of pressure effects is intimately connected with temperature. Recently Johnson, Brown, and Marsland (1942) have described an important pressure-temperature relationship in luminous bacteria. These forms have a well defined temperature optimum for maximum luminescence intensity, ranging from 20–32°C, depending primarily on the bacterial species. Above this temperature the light intensity decreases reversibly, so that lowering the temperature will restore the original luminescence if the bacteria are not kept too long at the higher temperature. An increase of pressure will also increase the luminescence intensity at temperatures above the optimum. This striking result is explained by the effect of pressure in reversing the equilibrium between active and reversibly heat-denatured luciferase, the enzyme responsible for luminescent oxidations in organisms. Rise in temperature shifts the equilibrium toward the denatured enzyme, while rise in pressure shifts the equilibrium in the opposite direction. At temperatures above the optimum the lowered light intensity is due to lowered active luciferase concentration and pressure restores the luminescence by shifting the equilibrium toward greater concentration of active luciferase. It would be interesting to know if a similar pressure-temperature relationship exists for cyclosis.

The effect of temperature on cyclosis in Characeae is quite well known. It has been studied by Velten (1876), using Chara foetida, who found a maximum rate at 34°C, and by Nägeli (1860), using Nitella syncarpa, where the maximum is at 37°C, as well as by Lambers (1925, 1926) and Romijn (1931). Lambers (1925) gives a curve for cyclosis rate and temperature in Nitella mucronata which is very nearly a straight line between 2°C (8µ per second) and 34°C (100µ per second). Above 34°C the cyclosis rate depends on the time of heating.

The Nitella flexilis used in these pressure experiments had an optimum temperature of cyclosis of about 35–36°C, when heated at a rate of 10° in 30 minutes, with rapid falling off of rate with higher temperatures. At 38.5° the rate was the same as that at 22°C. If kept at the high temperatures there was a progressive falling off in rate which made the experiments difficult to carry out. Therefore the Nitella cells within the pressure chamber at one atmosphere pressure were quickly brought to various temperatures ranging from 32°–39°C. At 32°C the cyclosis rate was greater than at 22°C, whereas at 39°C, the rate was less than at 22°C. When the proper high temperature was attained, the pressure was raised to 3000 lbs. per square inch, where the cyclosis rate was quickly measured, and then to 5000 to 6000 lbs. per square inch where the cyclosis rate was again quickly measured.

In no case did cyclosis rate increase as a result of the increase in pressure.
There was always a decrease in rate, slight at 3000 lbs. per square inch and
great at 6000 lbs. per square inch. If raised only to 32°C, there was a return
of cyclosis to the normal value at 32° when the pressure was released but when
raised to higher temperatures (37° or 38°) the cyclosis was permanently slowed
when the pressure was released and did not completely recover at room tem-
peratures except after a long time.

Because of the rapid decline in cyclosis rate at temperatures above the opti-
mum and the time factor, it is not feasible to work out curves showing the
effect of pressure on cyclosis at various critical temperatures. The material
is not good for pressure-temperature studies, because the optimum tempera-
ture is so near the maximum temperature at which cyclosis can continue. In
luminous bacteria there is a rather long temperature step between the optimum
and the maximum temperature at which these organisms can luminesce. However,
I have convinced myself that increase in pressure never increases the
cyclosis rate of *Nitella* at temperatures near or above the optimum as is the
case for the luminescence intensity of luminous bacteria. Probably the pres-
sure effects on cyclosis, ameboid movement, cell division, and pigment flow in
cromatophores are in another category from pressure effects on the lumini-
scence reaction, which primarily depends on catalytic oxidation. Lumines-
cence of bacteria is more comparable to such activities as muscle contraction,
nerve conduction, and ciliary or flagellar movement, where moderate pressures
may increase the activity.

To complete the picture of temperature effects, 10°C. was selected as a convenient
point for studying the effect of pressure at temperatures well below the optimum and
well below the temperature of the room in which the plants had been growing all
winter. At 10° the rate is about half that at 20°. However, when the pressure is
increased at this low temperature, the rate of cyclosis is always decreased, relatively
little for the first 5000 lbs. per square inch but markedly at 6000 to 10000 lbs. per
square inch. When the pressure was removed, the rate returned to that which is
normal at 10°C. and when the temperature was raised to 20°C. the rate returned to
that characteristic of the higher temperature. A curve is given in Fig. 1, C. There-
fore, if the slowing of cyclosis at low temperatures is due to increased viscosity of the
protoplasm, as previously explained, a solution by increased pressure at this low
temperature cannot counteract the slowing. However, we do not know how tempera-
ture (or pressure) affects the motive force for cyclosis (whatever it may be) apart
from an effect on viscosity, and such knowledge is essential for extended discussion.

There is another reason why temperature studies on cyclosis are difficult.
I can confirm the older observations of Hofmeister (1867), Hörmann (1898),
Lambers (1925), Umrath (1930), Romijn (1931), and Hill (1935) that a sudden
decrease in temperature will stimulate, causing shock stoppage of cyclosis,
and in addition have confirmed Dutrochet (1838), that sudden increases in
temperature will also cause shock stoppage. Time must always be allowed
for recovery from this shock effect.
I take pleasure in acknowledging the aid of my research assistant, Mr. Harold Towne, who made many of the observations on the rate of cyclosis at different pressures.

**SUMMARY**

*Nitella flexilis* cells are not stimulated to “shock stoppage” of cyclosis by suddenly evacuating the air over the water or on sudden readmission of air, or on suddenly striking a piston in the water-filled chamber in which they are kept with a ball whose energy is 7.6 joules, provided the *Nitella* cell is not moved by currents against the side of the chamber.

Sudden increases in hydrostatic pressure from zero to 1000 lbs. or 0 to 5000 lbs. per square inch or 5000 to 9000 lbs. per square inch usually do not stimulate to “shock stoppage” of cyclosis, but some cells are stimulated. Sudden decreases of pressure are more likely to stimulate, again with variation depending on the cell.

In the absence of stimulation, the cyclosis velocity at 23°C. slows as the pressure is increased in steps of 1000 lbs. per square inch. In some cells a regular slowing is observed, in others there is little slowing until 4000 to 6000 lbs. per square inch, when a rapid slowing appears, with only 50 per cent to 30 per cent of the original velocity at 9000 lbs. per square inch. The cyclosis does not completely stop at 10000 lbs. per square inch. The pressure effect is reversible unless the cells have been kept too long at the high pressure.

At low temperatures (10°C.) and at temperatures near and above (32°–38°C.) the optimum temperature for maximum cyclosis (35–36°C.) pressures of 3000 to 6000 lbs. per square inch cause only further slowing of cyclosis, with no reversal of the temperature effect, such as has been observed in pressure-temperature studies on the luminescence of luminous bacteria.

Sudden increase in temperature may cause shock stoppage of cyclosis as well as sudden decrease in temperature.

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