STUDIES OF PROTOPLASMIC STRUCTURE IN SPIROGYRA

I. ELASTICITY[1]

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With 3 Textfigures

Received for publication March 10, 1938

The structure of protoplasm may be inferred from studies for determining protoplasmic elasticity. If protoplasm is elastic it is probably built up of intermeshed, linear units. Hence, a method for measuring the elasticity of protoplasm would facilitate studies on protoplasmic structure. The present investigation is an attempt to measure the elasticity of protoplasm quantitatively in *Spirogyra* cells and to determine an equation which will govern the rate with which the chloroplasts move in response to different centrifugal accelerations.

The rate that a spherical body will move through a true fluid is governed by Stokes' law. According to this law:

$$(1) \quad V = c\, 2\, g\, (D—d)\, a^2/9\eta$$

in which V is the velocity, g is the acceleration due to gravity, $(D—d)$ is the difference in specific gravity between the moving particle and the surrounding medium, a is the radius of the particle, η is the viscosity of the medium, and c is a constant which may be determined from the following equation:

$$(2) \quad c = (2\pi n)^2 r/g$$

in which r is the radius of the centrifuge in centimeters, g is taken at 980 cm. sec.$^{-2}$, and n the number of revolutions per second. In equation (1) for any given system $2\, g\, (D—d)\, a^2/9\eta$ is constant and can be designated by k. Hence equation (1) may be written as follows:

$$(3) \quad V = kc.$$

HEILBRUNN (1926) found that equation (3) governed the rate of movement of granules in *Cumingia* eggs when the centrifugal acceleration was varied from 311 x gravity to 4936 x gravity and concluded that protoplasm in *Cumingia* eggs behaved like a true fluid. HOWARD (1932) studied *Arbacia* eggs which were centrifuged with accelerations varying from 100 x g. to 5000 x g. She found that when the product of force and time was constant the granules moved farther with high accelerations than they did with low accelerations. She attributed the slower rate at low accelerations to the counter effect which diffusion

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 162.

The author takes pleasure in expressing his gratitude to Mr. ROY KEUHNER who measured most of the cells.

and streaming might have. After making corrections for the effects of streaming and diffusion she found that the apparent viscosity was constant and hence concluded that the protoplasm in *Arbacia* was a true fluid.

In contrast to the reported results of HEILBRUNN and HOWARD, NORTHEN (1936) found varying viscosity values for the protoplasm of the alga, *Zygnema*, and hence concluded that the protoplasm in *Zygnema* was an anomalous fluid.

The data presented in this paper indicate that the protoplasm in *Spirogyra* cells behaves as an anomalous fluid. The velocity of movement of the chloroplasts in *Spirogyra* cells follows the general equation:

$$(4) \quad V = k\,(c - c_o)$$

in which c_o is the initial starting centrifugal acceleration and the other letters have the same meaning as in equation (3). Equation (4) is a modification of the equation which expresses the velocity of plastic flow. According to this equation:

$$V = k\,(P - P_o)$$

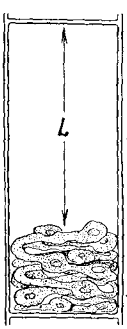
in which V is the velocity of plastic flow, k a constant, P is the normal driving pressure, and P_o is the initial starting pressure (GETMAN and DANIELS, 1931).

From equation (4), it is evident that if c, the acceleration x g. used, is the same as or less than the initial starting acceleration, c_o, the chloroplasts will not move regardless of how long the cells are centrifuged.

In the present experiment approximate values for c_o have been determined for the protoplasm of *Spirogyra*. When an average value of c_o was used, equation (4) approximately governed the rate with which the chloroplasts moved in response to different centrifugal accelerations.

Materials and methods. — Three species of *Spirogyra* were used. One species had two chloroplasts in each cell. The other two had one chloroplast in each cell.

The filaments were centrifuged with different centrifugal accelerations for calculated periods of time. Following centrifugation the cells were fixed in a 1 % chromo-acetic solution and the distances the chloroplasts had moved were determined as illustrated by the following diagram.



In all tables the distances are expressed in relative units, the units on the ocular micrometer. The relative distances may be converted into microns by multiplying by 140.

In order to calculate the number of minutes which would be required to cause the same displacement of the chloroplasts regardless of the acceleration it was necessary to obtain an average value for c_o. The experiments with the *Spirogyra* which had two chloroplasts in each cell will be used to illustrate the determination of c_o and the number of minutes that the cells would have to be centrifuged.

It was observed that when filaments were centrifuged for one hour with an acceleration of 54.5 x gravity the chloroplasts did not move in any of the cells. However, when the filaments were centrifuged for one hour with an acceleration of 60.4 x gravity a decided displacement was observed in about 30 % of the cells. Furthermore, when the cells were centrifuged with an acceleration of 75.6 x gravity for 20.5 minutes about 50 % of the cells showed a decided displacement of the chloroplasts and 50 % showed no displacement.

Considering only the species with two chloroplasts in each cell and with the exception of the experiment reported in table 1, a value of 75.6 x gravity was used for c_o. If 75.6 x gravity be the correct value, the time necessary with each force to cause the same displacement can be calculated as follows:

1. With an acceleration of 680 x gravity 0.5 minutes caused a decided displacement. For convenience assume k equals 1. According to equation (4):

$$V = L/t = k\,(c—c_o)$$

Then: $L = k\,(c—c_o)\,t$

Hence: $L = 1\,(680—75.6)\,0.5 = 302.2$

2. With an acceleration of 382.5 x g. the time necessary for the chloroplasts to move a relative distance of 302.2 should be:

$$L = 1\,(382.5—75.6)\,t = 302.2$$
$$t = 302.2/(382.5—75.6) = 0.99 \text{ min.}$$

3. With an acceleration of 170 x g.

$$t = 302.2/(170—75.6) = 3.2 \text{ min.}$$

4. With an acceleration of 108.8 x g.

$$t = 302.2/(108.8—75.6) = 9.1 \text{ min.}$$

5. With an acceleration of 75.6 x g. it should be

$$t = 302.2/(75.6—75.6) = \infty.$$

DATA. — *Spirogyra with two chloroplasts in each cell.* — In table 1 are given the distances the chloroplasts had moved, the observed relative velocities, the relative velocities as calculated from the equation, $V = k\,(c—c_o)$, and the relative velocities as calculated from the equation, $V = kc$. V_t and V_a, in table 1 and subsequent tables, are relative to the observed velocity when an acceleration of 680 x gravity was used. In other words, the observed velocities with acceleration of 680 x g. have been arbitrarily used as bases. For example, V_a for an acceleration of 170 x g. should be:

$$V_a = k\,(170—60.4)$$
$$k = .798/(680—60.4)$$
$$V_a = (.798)\,(170—60.4)/(680—60.4) = .141.$$

V_t for an acceleration of 170 x g. should be:

$$V_t = \text{k}\,(170)$$
$$k = .798/680$$
$$V_t = (.798)\,(170)/(680) = .201.$$

With the exception of the high value obtained with a force of 108.8 x g., which may be the result of a non-typical sample, the table shows that the higher the force the farther the chloroplasts moved. This suggests that the average value of c_o is greater than 60.4 x gravity. However, it will be noted that V_o is in much closer agreement with V_a than it is with V_t. This indicates that protoplasm is an anomalous fluid.

Table 1

Distances the chloroplasts moved, observed velocities, and calculated velocities with different centrifugal accelerations. C_o equals 60.4 x gravity

c Centrifugal acceleration used	t Minutes centri-fuged	Number of cells measured	L Distances moved	V_o Observed velocity	V_a Velocity calculated from V = k (c—60.4) Base = V_o at 680 x g.	V_t Velocity calculated from V = kc Base = V_o at 680 x g.
680.0 x g.	0.50	117	.399	.798	.798	.798
382.5 x g.	0.98	100	.327	.330	.416	.451
170.0 x g.	2.83	105	.265	.095	.141	.201
108.8 x g.	6.40	102	.309	.048	.062	.128
75.6 x g.	40.50	101	.017	.0008	.020	.089

Table 2, the average of three experiments, records the distances the chloroplasts had moved, the observed relative velocities, the relative velocities as calculated from the equation $V = k\ (c—c_o)$, and the relative velocities as calculated from the equation $V = kc$ when a value of 75.6 x g. was used for c_o.

Table 2

Distances the chloroplasts moved and velocities of movement with different centrifugal accelerations. c_o equals 75.6 x gravity

c Centrifugal acceleration used	t Minutes centri-fuged	Number of cells measured	L Relative distances moved	V_o Observed relative velocity	V_a Relative velocity calculated from V = k (c—c_0) Base = V_0 at 680 x g.	Relative velocity calculated from V = kc Base = V_o at 680 x g.
680.0 x g.	.50	528	.382	.764	.764	.764
382.5 x g.	.99	556	.426	.430	.344	.428
170.0 x g.	3.20	640	.441	.128	.108	.190
108.8 x g.	9.10	307	.369	.041	.037	.122
75.6 x g.	40.00	453	.247	.006	.000	.085

It will be noted, table 2, that with the three lower accelerations there is a close agreement between the observed velocities and the velocities as calculated from the proposed equation for elastic fluids.

If 75.6 x gravity be the correct value for c_o and if equation (4) be valid there should have been no movement of the chloroplasts when a force of 75.6 x gravity was used, but table 2 shows that some movement did occur with that force. However, it must be remembered that the observed velocities are average velocities. Actually, in 51 % of the cells examined the chloroplasts did not move at all when an acceleration of 75.6 x gravity was used. The percentages of cells in which the chloroplasts moved different units with the five accelerations used are expressed in figure 1 which shows the frequency curves.

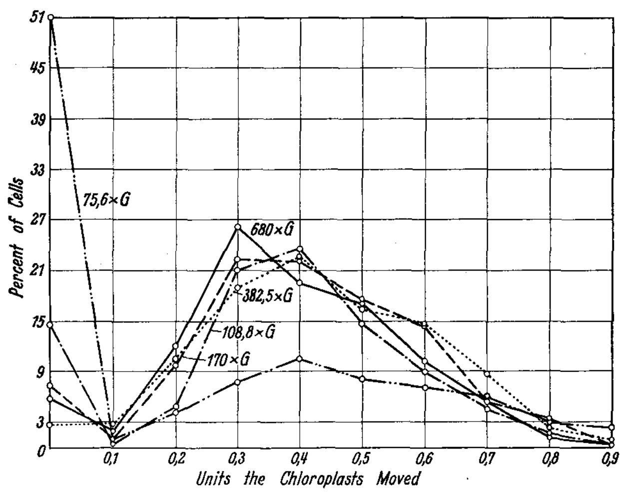


Fig. 1. Percentages of cells in which the chloroplasts moved different distances with accelerations of 680 x g., 382.5 x g., 170 x g., 108.8 x g., and 75.6 x g.

It will be remembered that the time of centrifugation for the four higher accelerations was so calculated that the chloroplasts should move the same distances if the proposed equation be valid and if 75.6 x gravity be the correct value for c_o. In figure 1 there is a decided similarity in the curves for the filaments which were centrifuged with the four higher forces. Discrepancies between the four curves are, in part, due to the number of cells in which the chloroplasts did not move. For instance, in table 2 the lowest value for L, considering only the four higher forces, is recorded for the material centrifuged with a force of 108.8 x gravity. Figure 1 reveals that in this material the chloroplasts moved in fewer cells than in those which were centrifuged with the three higher forces. Experimental difficulties, such as rotating the hand centrifuge with the proper speed and measuring the distances the chloroplasts had moved, may also account for some of the discrepancies. Moreover, the value of c_o is not the same for all of the cells. For 51 % of the cells c_o is equal to or less than 75.6 x gravity and for 49 % of the cells c_o is greater than 75.6 x gravity. For about 6 % of the cells it is even greater than 680 x gravity.

Spirogyra with one chloroplast in each cell, species no. 1. — The cells were centrifuged with a force of 680 x gravity for two minutes. The results are recorded below.

Units the chloroplasts moved	Percentage of cells %	Units the chloroplasts moved (continued)	Percentage of cells (continued) %
.00	40	.50	24
.10	00	.60	10
.20	00	.70	6
.30	5	.80	8
.40	7		

The data indicate that in this species a value of 680 x gravity for c_o is too low for 40 % of the cells. In other words, in this species the initial starting force is considerably greater than it was for the species with two chloroplasts in each cell.

Spirogyra with one chloroplast in each cell, species no. 2. — This species gave results somewhat comparable to the species with two chloroplasts in each cell. The proposed equation was used and t was calculated using a value of 75. 6 x gravity for c_o. The results are recorded in table 3.

Table 3

Movement of the chloroplasts when centrifuged with different accelerations for periods of time calculated with c_o equal to 75.6 x gravity

Distances the Chloroplasts moved	Percentages of cells in which the chloroplasts moved different distances with forces of:				
	680 x g. (0.50 min.) %	382.5 x g. (0.99 min.) %	170 x g. (3.20 min.) %	108.8 x g. (9.10 min.) %	75.6 x g. (40.00 min.) %
0.00	8.6	2.9	18.6	33.3	42.7
.10	2.8	0.0	0.0	0.0	1.9
.20	4.8	5.7	7.9	8.3	3.8
.30	20.9	26.7	28.4	22.5	17.5
.40	31.4	33.3	29.4	15.8	21.4
.50	19.0	22.9	9.8	16.7	12.6
.60	12.3	3.8	3.9	4.2	0.0
.70	0.0	4.8	2.0	1.6	0.0
Average distances chloroplasts moved	.371	.395	.305	.266	.217
V_o	.742	.399	.095	.029	.005
V_a	.742	.377	.116	.041	.000
V_t	.742	.417	.185	.119	.082

If the proposed equation is valid and if 75.6 x gravity is the correct average value for c_o the average distances the chloroplasts moved with the four higher forces should be the same. This is not true but the discrepancies are largely due to the variability of c_o and to a concomitant variation in the number of cells in which no movement occurred. However, it is true that with the three lower forces the observed velocities agree more closely with the values as calculated from the proposed equation than they do with the values as calculated from the general equation for true fluids.

In figure 2, graphs 1, 2 and 3 have been obtained by plotting the observed velocities (as recorded in tables 1, 2, and 3 respectively) against the centrifugal accelerations. Graph 4 has been obtained by averaging the velocities of the three tables.

As the graphs do not pass through the origin, they are graphs of anomalous fluids, because if protoplasm were a true fluid the velocity would be proportional to the acceleration at all points.

Discussion and conclusions. — At or below a definite centrifugal acceleration, c_o, the chloroplasts in *Spirogyra* will not move in response to centrifugation regardless of the time that the acceleration is allowed to act. However, with forces above c_o, the velocity of chloroplastic movement can be approximately calculated from the equation

$$V = k\ (c - c_o)$$

in which V is the velocity, c is the centrifugal acceleration used, and c_o is the initial starting acceleration.

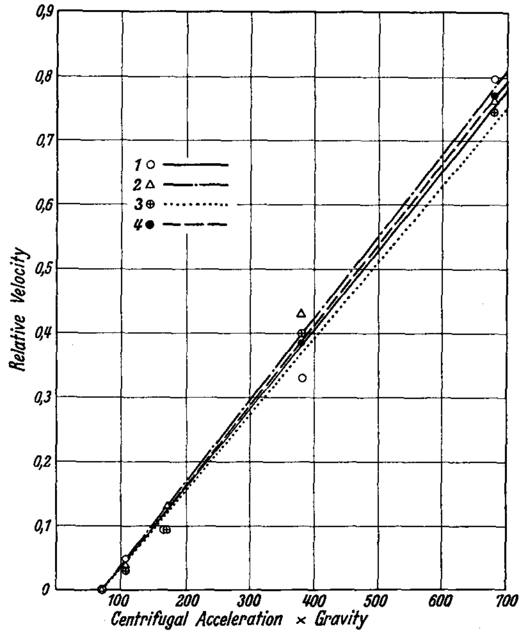


Fig. 2. Relative velocity vs. centrifugal acceleration. The curves do not pass through the origin. Such curves are typical of anomalous fluids.

The above behavior is characteristic of elastic or non-Newtonian fluids. This suggests that the protoplasm of *Spirogyra* may be an elastic fluid bulit up of intermeshed linear units. However, it does not necessarily follow that the protoplasm is of high consistency. On the contrary, the fact that the chloroplasts can be almost completely displaced by thirty seconds of centrifugation with an acceleration of 680 x gravity indicates that the consistency of the protoplasm is relatively low. It must be emphasized that studies of protoplasmic viscosity with the centrifuge method are likely to be very misleading. For instance, if a force of 60 x gravity were used in the experiments with *Spirogyra* one might be led to believe that protoplasm is extremely viscous whereas if a force of 680 x gravity were used one could conclude that the viscosity of protoplasm was quite low.

How may my results be reconciled with the previously quoted results of Heilbrunn and Howard on the nature of the endoplasm? Although Heilbrunn (1937) believes that the endoplasm behaves like a true fluid he recognizes that the cortical cytoplasm in invertebrate cells is elastic. In *Spirogyra* the cytoplasm occurs as a narrow layer inside of the cell wall and in the form of strands stretching across the vacuole. When *Spirogyra* cells are centrifuged the chloroplasts move only through the peripheral layer. Is it not possible that all of the cytoplasm in a *Spirogyra* cell is comparable to the cortical cytoplasm in invertebrate cells?

SUMMARY. — Filaments of *Spirogyra* were centrifuged with different accelerations and with periods of time such that the chloroplasts would move the same distances when accelerations greater than 75.6 x gravity were used. It was found that the velocity of movement of the chloroplasts could be approximately calculated from the following equation:

$$V = k\ (c—c_o)$$

in which V is the velocity, k is a constant, c is the centrifugal acceleration used, and c_o is the initial starting acceleration.

The value of c_o is not the same for all species of *Spirogyra* and likewise it is not the same for all of the filaments of one species. For a species of *Spirogyra* with two chloroplasts in each cell the average value of c_o was approximately 75.6 x gravity. For another species which had one chloroplast in each cell the the average value of c_o was much higher.

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