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The Density of the Cell Sap and Endoplasm of *Nitellopsis* and *Chara*

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We measured the densities of the cell sap, endoplasm and cell wall of *Nitellopsis obtusa* and *Chara corallina* using interference microscopy, refractometry, immersion refractometry, equilibrium sedimentation and chemical microanalysis techniques. These values are important for the determination of many rheological properties of the cytoplasm as well as for understanding buoyancy regulation, dispersal mechanisms and how cells respond to gravity. The average densities of the cell sap, endoplasm and cell wall are 1,006.9, 1,016.7 and 1,371 kg m^{-3} for *Nitellopsis* and 1,005.0, 1,013.9 and 1,355.3 kg m^{-3} for *Chara*.

Key words: Cell sap — *Chara corallina* — Density — Endoplasm — Interference microscopy — *Nitellopsis obtusa*.

We have proposed a novel model for the way plant cells sense gravity in which the plant cell protoplast brings a pressure to bear against the cell wall, providing a directional signal for gravitational responses. By this model, all the cell contents, including the amyloplasts, act as ballast (Wayne et al. 1990, Staves et al. 1991). The falling of the protoplast consequently causes a compression of the plasma membrane of the lower part of the cell and a tension on the plasma membrane of the upper part of the cell. The difference between these forces is sufficient to activate the ion channels necessary for plant cells to respond to gravity (Wayne et al. 1990). The magnitude of the graviresponse depends on the volume of the protoplast as well as the density of the protoplast relative to the density of the solution in the wall. Since the densities of the endoplasm and cell sap now take on added importance in gravitational work, we have measured the densities of the endoplasm and cell sap of *Nitellopsis obtusa* and *Chara corallina*. These data extend the early measurements of the densities of the endoplasm and the cell sap done by Kamiya and Kuroda (1957b) on *Nitella flexilis* and Collander (1930) on *Chara ceratophylla*, which heretofore provided the basic knowledge on protoplasmic components which may be needed to understand how cells respond to gravitational and centrifugal accelerations. The densities of the endoplasm and cell sap determined by Kamiya and Kuroda (1957b) have been used previously to calculate the motive force of streaming (Kamiya and Kuroda 1958b, Wayne et al. 1990), the viscosity of the cytoplasm (Kamitsubo et al. 1988), the tension at the surface of a protoplasmic droplet (Kamiya and Kuroda 1958a, 1965) and other rheological properties of cytoplasm (Kamitsubo et al. 1989, Kamiya and Kuroda 1957a). A knowledge of the densities of the endoplasm and cell sap is also important in the understanding of buoyancy regulation (Raven 1984, Walsby 1975) and dispersal mechanisms (Gregory 1961).

Abbreviations: a, specific refractive increment; ACS, artificial cell sap; D_{cs}, density of cell sap; D_{en}, density of endoplasm; D_o, concentration of organic dry matter; D_s, concentration of the salt solution, OPD, optical path length; S, total solids; t, diameter of endoplasmic drop; W, percentage of water; W_s, percentage of salt solution.

Materials and Methods

Culture conditions—Nitellopsis obtusa (Devs. in Lois.) J. Gr. and *Chara corallina* Klein ex Willd., em. R.D.W. (= *Chara australis* R. Brown) were grown in a soil/water medium under continuous white fluorescent light at 26°C. Under these conditions, $CaCO_3$ does not deposit on the cell wall and there is not any visible banding pattern. The soil water medium contained 3.75 mM Ca, 1.71 mM Mg, 1.37 mM S, 0.98 mM Cl^-, 637 μM K, 508 μM Na, 111 μM Si, 277 μM Mn, 12.9 μM P, 6.51 μM B, 2.47 μM Al, 1.96 μM V, 1.13 μM Ni, 756 nM Fe, 465 nM Ag, 356 nM Co, 223 nM Cr, 152 nM Mo, 112 nM As, 76.5 nM Zn, and 69.7 nM Y. The density of the medium is 1,000.5 kg m^{-3} as determined by refractometry and gravimetrically. Experiments were carried out at 25°C.

Measurement of the density of the cell sap

Refractometry method—The density of the cell sap was determined by measuring the total solids (S in g (100 ml)$^{-1}$) of the cell sap with a hand held refractometer (Reichert-Jung, Model 10441, Cambridge Instruments, Buffalo, NY, U.S.A.) and assuming that KCl, with a specific volume of 0.5040 (which is derived from the reciprocal of the specific gravity), made up the majority of the solute. The total density of the cell sap (D_{cs} in g (100 ml)$^{-1}$) is then obtained with the following equation:

$$D_{cs}=W+S \quad (1)$$

where the percentage of water (W in g 100 ml^{-1}) is given by the following equation:

$$W=100-0.5040(S) \quad (2)$$

Equilibrium sedimentation method—In order to minimize the importance of the assumption that the specific volume of the solute of the cell sap is 0.5040, we also measured the density of the cell sap using an equilibrium method where the cell sap was expressed, collected with a plastic pipette tip (ca. 3 μl) and released in solutions of various sucrose concentrations that were all made isotonic (0.617 MPa for *Chara*; 0.702 MPa for *Nitellopsis*) with ethylene glycol. We then determined whether the drop floated, sank or did not move. The density of the solution in which the drops neither sank nor floated was considered to be equal to the density of the cell sap. This method is similar to that used by Kamiya and Kuroda (1957b).

Chemical analysis method—Lastly, in order to apply an independent check on the density of the cell sap, we analyzed chemically the individual constituents in order to find the concentration of the individual solutes (S in g (100 ml)$^{-1}$). The elements (except Cl^-), were measured with an inductively coupled argon plasma atomic emission spectrometer (Model ICAP 61, Thermo Jarrel Ash Corp., Franklin, MA. U.S.A.) and the total and free amino acids were measured with an amino acid analyzer (Pico-tag System, Waters Chromatography, Milford, MA, U.S.A.).

Cl^- was measured with a Ag/AgCl electrode using a glass microcapillary electrode filled with 100 mol m^{-3} KNO_3 as a reference electrode. The electrodes were pulled with a microcapillary pipette puller (Model PP-83, Narashige Scientific Instrument Lab., Tokyo, Japan). The reference electrode was either connected directly to a Ag/AgCl pellet or first passed through a 3 M KCl bridge. Both methods worked equally well. The electrodes were placed in 5 μl of cell sap 1.45 mm apart using micromanipulators (Narashige) on a Zeiss IM 35 inverted microscope (Opti-Systems Inc., Walden, NY, U.S.A.) and the signal was amplified with an electrometer (Model S7071A, World Precision Instruments, Sarasota, FL) and the voltage was recorded on a strip chart recorder (Model 2125M, Allen Datagraph, Inc. Salem, NH, U.S.A.).

In order to calculate the density of the cell sap, we assumed that the average specific volume of the cell sap constituents was 0.5040. The total density of the cell sap (D_{cs} in g (100 ml)$^{-1}$) is then obtained using equation 1 and the percent of water (W in g (100 ml)$^{-1}$) is obtained by using equation 2.

Measurement of the density of the endoplasm

Quantitative interference microscopy method—We used an AO-Baker interference microscope with a 40× shearing type objective lens (NA=0.7) and a Zeiss Jamin-Lebedeff interference microscope with a 40× objective lens (NA=0.65) to determine the density of the endoplasm (Beneke 1966, Davies et al. 1954, Hale 1958). We expressed the endoplasm from internodal cells of *Chara* into a solution of artificial cell sap (ACS_c) containing 80 mM KCl, 10 mM $CaCl_2$, 30 mM NaCl and 10 mM $MgCl_2$. Using the specific volumes of these salts (0.5040, 0.4651, 0.4619 and 0.4304, respectively), we calculated that the density of the artificial cell sap was 1,004.23 kg m^{-3}. Likewise, we expressed the endoplasm from internodal cells of *Nitellopsis* into a solution of artificial cell sap (ACS_n) containing 90 mM KCl, 10 mM $CaCl_2$, 30 mM NaCl and 10 mM $MgCl_2$. Again, we calculated that the density of the artificial cell sap was 1,006.22 kg m^{-3}. In both cases, the endoplasmic drops were spherical and the chloroplasts retained their natural shapes, indicating that the osmotic strengths of the artificial cell saps were appropriate for the endoplasmic drops of each species (Wayne and Tazawa 1988).

Quantitative phase-contrast method—Endoplasmic drops were effused into drops of ACS, appropriate for each species, to which various amounts of Bovine Serum Albumin (BSA, Fraction V, Sigma, St. Louis, MO, U.S.A.) had been added. We then determined the percentage of droplets that were darker (using negative phase contrast) or lighter (using positive phase contrast) than the medium. This gave us the percentage of drops that had a lower refractive index than the medium. We plotted this percentage against the concentration of BSA in the medium. The density of the drops was calculated by assuming that the concentration (in percent) of organic dry matter in the drop was equivalent to the percentage of BSA when 50% of the droplets had a higher refractive index than the medium and 50% of the droplets had a lower refractive index than the medium. The concentration of BSA (in g (100 ml)$^{-1}$) is the concentration (in kg m^{-3}) divided by 10. We then assumed that the organic dry matter is mostly protein and had a specific volume of 0.75 (Barer and Joseph 1954, 1955, 1955a, 1958). We then calculated the percent salt solution using the following formula:

$$W_s=100-0.75(D_o) \quad (3)$$

where W_s is the percentage (v/v) of the salt solution in the endoplasmic drop (in percent), D_o is the concentration of organic dry matter (in g $(100\ ml)^{-1}$ = percent), 0.75 is the specific volume of the organic matter (protein).

We can then calculate the density of the endoplasm that results from the salt solution (D_s in g $(100\ ml)^{-1}$) by dividing the percentage of the endoplasmic drop that is taken up by the salt solution by 100 to get the volume fraction of the salt solution. Then we multiply the volume fraction of the salt solution by the density of the salt solution. The density of the salt solutions for *Nitellopsis* and *Chara* are 100.622 g 100 ml^{-1} (1,006.22 kg m^{-3}) and 100.423 g 100 ml^{-1} (1,004.23 kg m^{-3}), respectively. The formulae used to calculate the contribution of the salt solution to the density of the endoplasm are given below:

$$D_s=(W_s/100)\ 100.622\ g\ (100\ ml)^{-1} \text{ for } \textit{Nitellopsis} \quad (4)$$

and

$$D_s=(W_s/100)\ 100.42\ g\ (100\ ml)^{-1} \text{ for } \textit{Chara} \quad (5)$$

We can then calculate the density of the endoplasmic drops (D_{en}, in g $(100\ ml)^{-1}$) using the following formula:

$$D_{en}=D_s+D_o \quad (6)$$

We used both positive low phase-contrast (A40PL) and negative high phase-contrast (A40NH) objective lenses to observe the endoplasmic drops on upright microscopes (Model BH-2 or CH-2, Olympus Optical Company Ltd., Tokyo, Japan). We scored 11,300 individual droplets with this method.

Measurement of the density of the cell wall

The density of the cell wall was determined by cutting an internodal cell and expelling the protoplasm with pressure. The walls were then placed in various solutions of OPTIRAY (Mallinckrodt Medical Inc., St. Louis, MO, U.S.A., density = 1,371 kg m^{-3}) and the solutions were placed under a gentle vacuum. We determined the equilibrium density by observing whether the wall rose or sank. The equilibrium density is equivalent to the density of the solution in which the wall neither rose nor sank.

Results and Discussion

Density of the cell sap—Using the refraction method, the equilibrium method and the chemical analysis method we have determined that the densities of the cell sap of *Nitellopsis* and *Chara* are 1,006.9±0.8 and 1,005.0±0.5 kg m^{-3}, respectively (Table 1). Even though each method depends on different assumptions, they each yield approximately the same answer for the density, providing confidence that we have a reliable estimate of the density of the cell sap in these two genera. The density measured by refractometry is probably an underestimate due to the low specific refractive increment of salt solutions (Hale 1958). Our estimate of the density of the cell sap in the two genera is less than the estimate made by Kamiya and Kuroda (1957b) for *Nitella flexilis* and Collander (1930) for *Chara ceratophylla*. Kamiya and Kuroda (1957b) estimated that the density of the cell sap of *Nitella flexilis* is between 1,010 and 1,011 kg m^{-3} and Collander (1930) estimated that the density of the cell sap of *Chara ceratophylla* is 1,010.6 kg m^{-3}.

The specific results of the chemical analyses are shown in Table 2. Our results are similar to those reported previously (Kikuyama et al. 1984, Kishimoto and Tazawa 1965a, b, Sakano and Tazawa 1984, Tazawa et al. 1974, Tazawa and Shimmen 1987). We were unable to resolve isoasparagine, a major component and marker amino acid in characean cells of these species (Sakano et al. 1989). Under our conditions, it co-elutes with aspartic acid and asparagine to form the large ASX peak. In both *Nitellopsis* and *Chara*, K^+ and Cl^- are the solutes which contribute the most to the density of the cell sap, thus justifying the assumption of using the specific volume of KCl to determine the percent water in the cell sap.

We find that the concentration of solids in the cell sap

Table 1 The density of the cell sap of *Nitellopsis* and *Chara*

Method	*Nitellopsis obtusa* kg m^{-3}	*Chara corallina* kg m^{-3}
Refractometry	1,005.2±0.7 (45)	1,004.1±0.6 (29)
Equilibrium method	1,007.8±0.3 (2)	1,005.3 (10)
Chemical analysis	1,007.6	1,005.6
Average of three methods	1,006.9	1,005.0
SD	1.45	0.79
SE	0.84	0.57

In order to obtain D_{cs} in units of kg m^{-3}, the value in g $(100\ ml)^{-1}$ is multiplied by 10.

Table 2 The concentration and density of various elements, amino acids and water in the cell sap of *Nitellopsis* and *Chara*

Element	*Nitellopsis obtusa*[a]		*Chara corallina*[b]	
	mol m^{-3}	kg m^{-3}	mol m^{-3}	kg m^{-3}
Cl^-	190 ±9 (25)	6.738	140 ±5 (30)	4.961
K	136 ±8	5.320	70 ±5	2.749
Na	24 ±5	0.543	71 ±2	1.643
Mg	17 ±1	0.526	4.2 ±0.4	0.102
Ca	9.4 ±0.4	0.375	3.2 ±0.2	0.127
S	30.6 ±4.4	0.982	8.3 ±1.4	0.265
P	8.9 ±0.9	0.276	16.8 ±1.1	0.522
Zn	0.12 ±0.02	0.008	0.2 ±0.0	0.010
Mn	0.030±0.020	0.0017	0.092±0.012	0.0051
Fe	0.009±0.003	0.0005	0.005±0.001	0.0003
Cu	0.008±0.002	0.0005	0.007±0.001	0.0004
B	0.009±0.004	0.0001	0.014±0.003	0.0001
Mo	0.011±0.005	0.0011	0.004±0.001	0.0004
Al	0.036±0.009	0.0010	0.027±0.018	0.0007
Co	0.006±0.002	0.0004	0.014±0.006	0.0008
Cd	0.003±0.000	0.0003	0.003±0.001	0.0003
Cr	0.009±0.001	0.0004	0.009±0.002	0.0005
Ni	0.006±0.003	0.0004	0.013±0.004	0.0008
Pb	0.001±0.001	0.0003	0.003±0.002	0.0007
Si	0.103±0.029	0.0029	0.055±0.002	0.0016
V	0.004±0.000	0.0002	0.006±0.001	0.0003
As	0.015±0.004	0.0011	0.015±0.007	0.0011
Se	0.017±0.004	0.0013	0.006±0.005	0.0005
Y	0.001±0.000	0.0001	0.002±0.000	0.0002
Ag	0.002±0.002	0.0002	0.005±0.002	0.0006
ASX	1.196±0.496	0.1587	2.624±0.155	0.3477
GLX	0.923±0.351	0.1354	0.725±0.011	0.1062
SER	0.275±0.049	0.0289	0.317±0.006	0.0333
GLY	0.408±0.094	0.0307	0.573±0.155	0.0430
HIS	0.067±0.016	0.0105	0.007±0.003	0.0120
ARG	0.196±0.040	0.0342	0.248±0.024	0.0431
THR	0.130±0.032	0.0135	0.179±0.007	0.0213
ALA	0.429±0.089	0.0382	0.364±0.019	0.0324
PRO	0.154±0.036	0.0178	0.213±0.056	0.0245
TYR	0.075±0.016	0.0137	0.132±0.006	0.0133
VAL	0.225±0.051	0.0263	0.266±0.008	0.0311
MET	0.052±0.011	0.0078	0.066±0.005	0.0099
CYS	0.250±0.128	0.0304	0.214±0.011	0.0259
ILE	0.096±0.022	0.0127	0.128±0.006	0.0168
LEU	0.157±0.039	0.0206	0.257±0.024	0.0337
PHE	0.092±0.019	0.0152	0.109±0.005	0.0179
LYS	0.139±0.036	0.0204	0.202±0.006	0.0295
H_2O	55,124.4	992.24	55,240.9	994.34
Total density (kg m^{-3})		1,007.64		1,005.58

[a] n=5 for plasma emission analysis; 3 for amino acid analysis.
[b] n=3 for plasma emission analysis; 3 for amino acid analysis.
mol m^{-3} is equivalent to mM

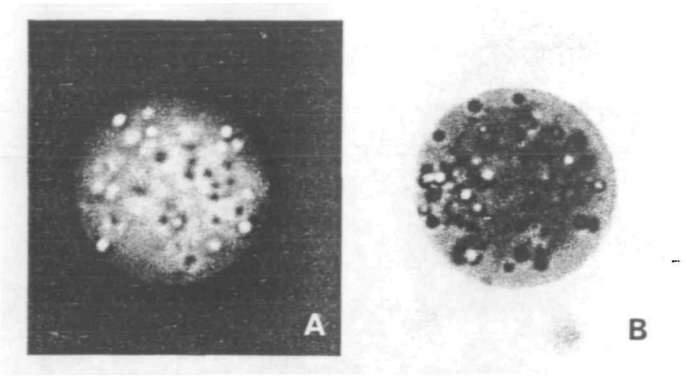

Fig. 1 Micrographs of a living endoplasmic drop containing motile organnelles taken with an interference microscope. (a) Background extinction; (b) Specimen extinction. The magnifications of both photomicrographs are 217×.

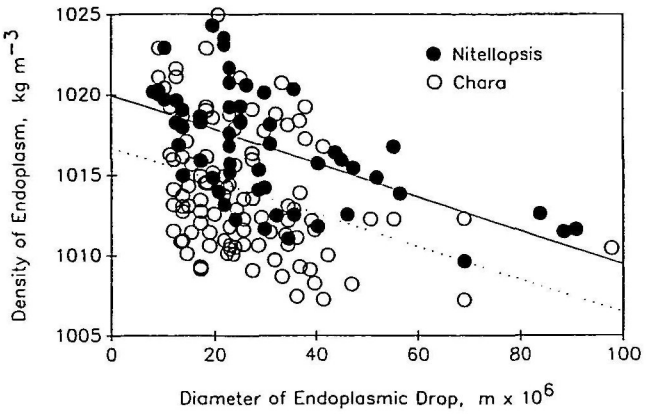

Fig. 2 The density of endoplasmic drops vs. droplet size using interference microscopy. (●) *Nitellopsis obtusa*. (○) *Chara corallina*.

as measured with refractometry is 1.0533 g (100 ml)$^{-1}$ and 0.8172 g (100 ml)$^{-1}$ in *Nitellopsis* and *Chara*, respectively. The concentration of water in the cell sap is thus 99.47 g (100 ml)$^{-1}$ for *Nitellopsis* and 99.59 g (100 ml)$^{-1}$ for *Chara*.

Density of the endoplasm—Using the interference microscopes, we observed endoplasmic drops that were free of nuclei and chloroplasts (Figure 1). Using monochromatic light of 550 nm, we determined the OPD between the center of the endoplasmic drop and the ACS prepared for each species. Since the salt concentration of the ACS is similar to the salt concentration of the endoplasmic drop, we assume that the measured OPD represents the concentration of organic matter in the endoplasmic drop. We determined the concentration of organic dry matter (D_o) using the following relationship:

$$D_o = OPD(t)^{-1}(a)^{-1} \quad (7)$$

where D_o is the concentration of organic dry matter (in percent). t is the diameter of the spherical drop (in m). a is the specific refractive increment (0.0018·100 ml·g^{-1}).

The concentration of organic dry matter (D_o) in the endoplasm is 4.3±0.2 (57) g (100 ml)$^{-1}$ and 4.0±0.2 (104) g (100 ml)$^{-1}$ for *Nitellopsis* and *Chara*, respectively. If we assume that the majority of the organic dry matter is protein, and that the specific volume of the protein is 0.75, then we can calculate the percent of the volume of the endoplasmic drop that is occupied by the salt solution (W_s) using the following formula:

$$W_s = 100 - 0.75(D_o) \quad (8)$$

where W_s is the percentage (v/v) of the salt solution in the endoplasmic drop (in percent). D_o is the concentration of organic dry matter (in g (100 ml)$^{-1}$ = percent). 0.75 is the specific volume of the organic matter (protein).

The endoplasmic drops of *Nitellopsis* and *Chara* contain 96.8%±0.1 (57) and 97.0%±0.1 (104) salt solution, respectively. Since the densities of the salt solutions are 1,006.22 kg m^{-3} and 1,004.23 kg m^{-3}, respectively, we can calculate the contribution of the salt solution to the densities of the endoplasmic drops by multiplying the volume fraction (on a scale from 0–1) of the salt solution by its den-

Table 3 The density of the endoplasm of *Nitellopsis* and *Chara*

Method	Species	
	Nitellopsis obtusa kg m^{-3}	*Chara corallina* kg m^{-3}
Interference microscopy	1,016.6±0.5 (57)	1,014.1±0.4 (104)
Immersion refractometry	1,016.8	1,013.7
Average of the two methods	1,016.7	1,013.9
SD	0.1	0.3
SE	0.1	0.2

In order to obtain D_{en} in units of kg m^{-3}, the value in g (100 ml)$^{-1}$ is multiplied by 10.

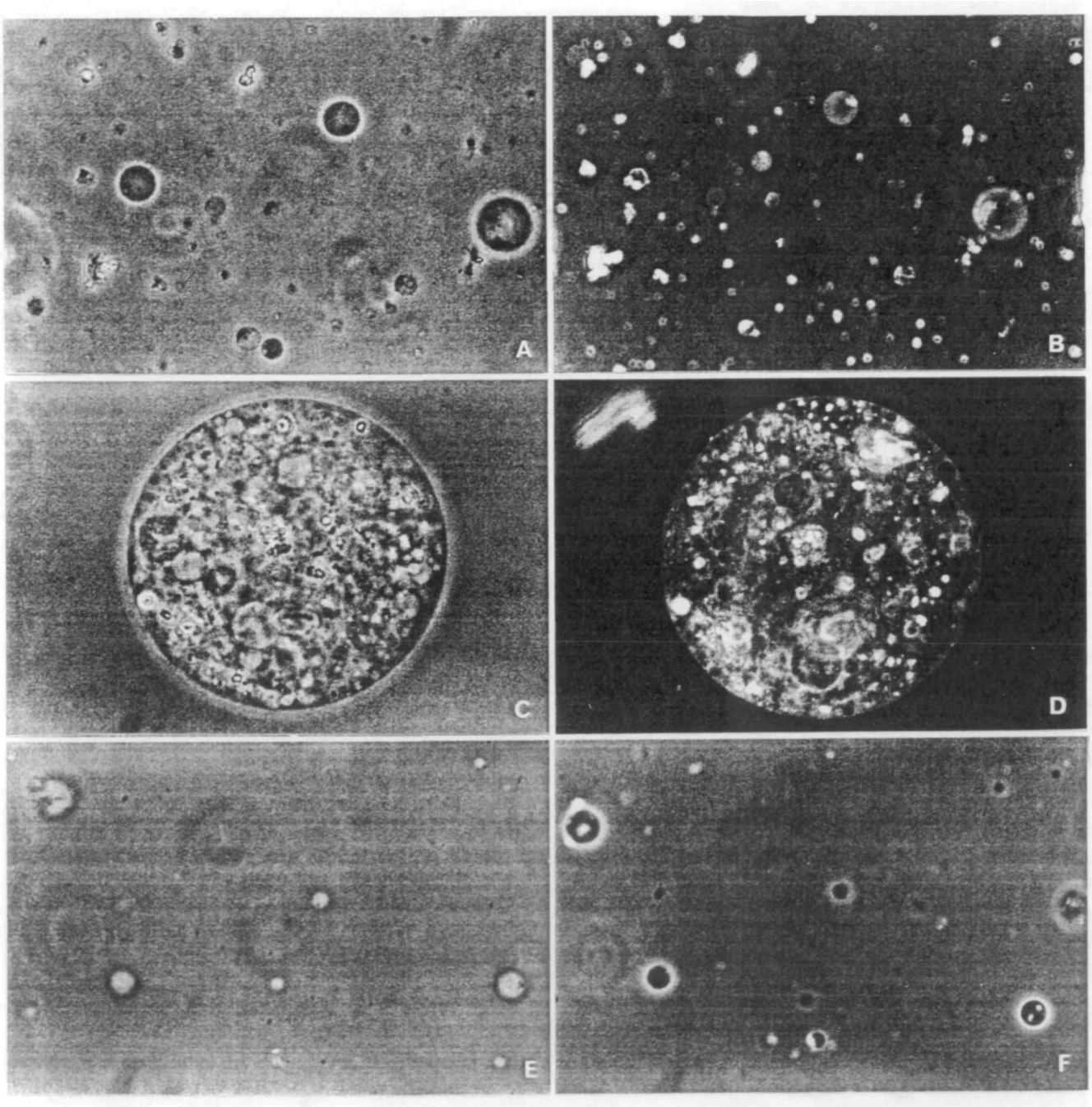

Fig. 3 Micrographs of living endoplasmic drops containing motile organelles taken with a phase contrast microscope. A, C and E were taken with a positive low phase contrast objective. B, D and F were taken with a negative high phase contrast objective. (A and B) drops in ACS. (C and D) drop in ACS plus 4.3% BSA. (E and F) drops in ACS + 7% BSA. The magnifications of all the photomicrographs are 400×. Note: as one looks down the micrographs taken with the positive low phase contrast objective (A to C to E), the halos get darker as the concentration of BSA increases. As one looks down the micrographs taken with the negative high phase contrast object (B to D to F), the halos get lighter as the concentration of BSA increases.

sity. The contribution of the salt solution to the total density of the endoplasmic drops (D_s) is thus 97.4 g (100 ml)$^{-1}$ for both *Nitellopsis* and *Chara*. We can now calculate the density of the endoplasmic drops (D_{en}) using the following formula:

$$D_{en}=D_s+D_o \quad (9)$$

We find that the densities of the endoplasmic drops of *Nitellopsis* and *Chara* are 1,016.6±0.5 kg m^{-3} and 1,014.1±0.4 kg m^{-3}, respectively, which are similar to the values determined by Kamiya and Kuroda (1957b) for *Nitella* (1,014–1,015 kg m^{-3}) (Table 3). However, we feel that our measurements may be an underestimate of the density since the observed value of the density depends on the diameter of the endoplasmic drops and we assume that less endoplasm is lost from the smaller drops than the larger drops during their formation. The regression equations are:

$$D_{en}=1{,}019.89-0.10298 \text{ (diameter in } \mu\text{m)} \quad (10)$$

for *Nitellopsis*, and

$$D_{en}=1{,}016.65-0.09681 \text{ (diameter in } \mu\text{m)} \quad (11)$$

for *Chara*.

While the regressions are significant, ($p<0.001$) the correlation coefficients (r^2) are so low; 0.371 and 0.114 for *Nitellopsis* and *Chara*, respectively, that we cannot use the Y intercept to predict the density of an infintesimally small volume of endoplasm. So at this point, we can only discard the hypothesis that there is no correlation between the diameter and density of an endoplasmic drop (Figure 2).

In order to test independently whether the value determined by quantitative interference microscopy is correct, we decided to perform quantitative immersion refractometry with a phase contrast microscope (Figure 3). We varied the density of the medium by adding Bovine Serum Albumin, which is impermeant, to ACS and determined the percentage of cells that had either a light or dark halo around them. Using this method, the endoplasmic densities of *Nitellopsis* and *Chara* were determined to be 1,016.8 and 1,013.7 kg m^{-3}, respectively (Figure 4).

Density of the cell wall—Lastly, we determined the density of the cell walls using the equilibrium method. We submerged the isolated cell wall fractions in various concentrations of Optiray until we found the solution where the wall would neither sink nor float. We found that the cell walls of *Nitellopsis* and *Chara* have densities of 1,371 kg m^{-3} and 1,355.3 kg m^{-3}, respectively. The average thickness of the cell walls of both *Nitellopsis* and *Chara* used for the density measurements were 4.39 μm.

We used a number of techniques to estimate the densities of the cell sap, endoplasm and cell wall of *Nitellopsis obtusa* and *Chara corallina*. Summary diagrams of the densities of the major compartments of the cells are shown in Figure 5. While the values obtained for each compartment are similar, the densities of the cell sap, endoplasm and cell wall of *Nitellopsis* are always greater than those of

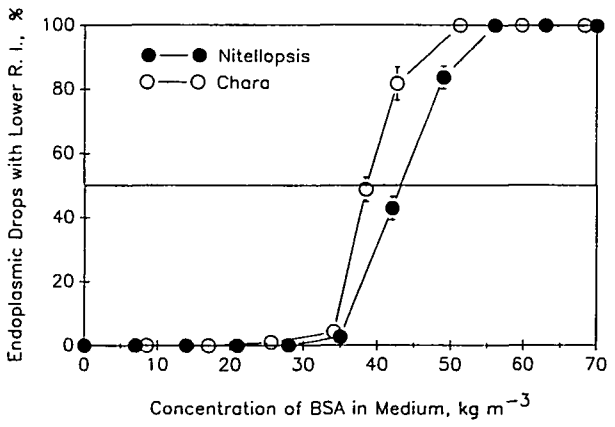

Fig. 4 Determination of the concentration of organic dry mass in endoplasmic drops using immersion refractometry with a phase-contrast microscope. The ordinate is the percentage of cells that have a lower refractive index than the medium and appear bright when viewed with a positive low phase contrast objective and dark when viewed with a negative high phase contrast objective. (●) *Nitellopsis obtusa*. (○) *Chara corallina*.

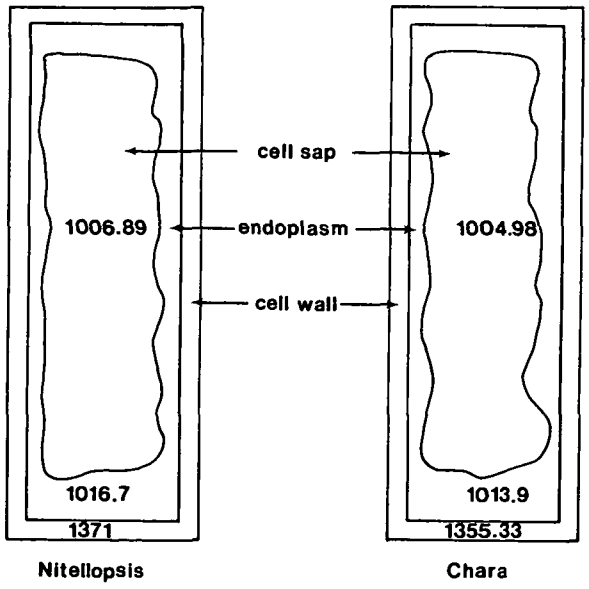

Fig. 5 Summary diagram of densities of cell components of *Nitellopsis obtusa* and *Chara corallina*. Densities are given in kg m^{-3}.

Chara even though they are growing under identical conditions, indicating that differences do exist between these genera. The difference in densities between the endoplasm and cell sap for *Nitella* is 4 kg m^{-3} while it is 9.8 and 8.9 kg m^{-3} for *Nitellopsis* and *Chara*, respectively, indicating that the motive force per unit area calculated for *Nitellopsis* by Wayne et al. (1990) using the densities estimated for *Nitella* is underestimated by a factor of 2.45. The corrected motive force per unit area is between 0.29 and 0.34 N m^{-2}.

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