STUDIES ON THE COMPARATIVE PHYSIOLOGY OF *CHARA AUSTRALIS*

I. GROWTH PATTERN AND GROSS CYTOLOGY OF THE INTERNODAL CELL

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Summary

The growth of the internodal cell and its parts appears to be allometric. Two growth phases are recognized, an exponential phase in the young cells followed by a linear phase during which the internode expands by up to 200 mm in length. In the exponential phase the cytoplasm to vacuole ratio declines from a very high value to about 0·04–0·06 and then remains constant throughout the linear phase. In the linear phase the structure of the protoplast, at the light-microscope level, is similar for all cells; a unit volume of the protoplast appears similar in cells from 10 to 200 mm in length.

I. INTRODUCTION

The internodal cells of the Characeae have been used extensively over the years for experimental purposes, particularly for permeability and biophysical studies but despite the extensive literature about these cells, comparatively little is known of their general physiology. This lack of information is regrettable because of the tendency amongst plant physiologists to assume that the physiology of the internode resembles that of the parenchyma cells of higher land plants. Indeed the internodal cell is often adopted as a model of these cells. This view may be valid, but there does appear to be a need for detailed comparative studies of the two cell systems. Some aspects of the internodal cells are distinctive, especially the coenocytic condition and their relatively enormous size compared with the small dimensions and uninucleate diploid condition of the cells of the higher plants. Also adaptations to aqueous and terrestrial environments respectively may have resulted in the evolution of two distinct cellular systems.

One aspect of the present project deals with the comparative physiology of the internodal cell and the parenchyma cells of higher land plants. A second aspect deals with problems for which the internodal cell appears well suited: for example, the function of the vacuole, the morphogenesis of organelles, and cell expansion. This first paper of the series is concerned with general observations on the gross cytology and growth patterns of the internodal cell of *Chara australis* var. *nobilis* R. Br., which provided a basis for the subsequent physiological work.

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II. Materials

C. australis var. *nobilis* was selected for the present work because of the large size of the internodal cells and the absence of cortical cells. A brief systematic description of the vegetative plant is given as a basis for comparison with the species of Characeae used for physiological studies in the northern hemisphere.

C. australis is a large, completely ecorticate, dioecious species up to 50 cm high, with 4–12 internodal cells per shoot. Internodes are 1–2 mm in diameter, but are usually less than 0·5 mm in culture. They are generally 4–8 cm long but may attain a length of 20 cm. The cells are bright green when young, and frequently become yellow or whitish and starch-filled or very dark green to black when old. Stipulodes are small (0·3–0·4 mm by 0·08 mm at the base), single or in pairs, and alternate with the branchlets. A whorled arrangement of laterals of limited growth arises from the cells at the node. These branchlets number usually 3–8 per whorl and are 3–5-celled (Fig. 1, *D*). The reproductive structures have been described by Macdonald and Hotchkiss (1956).

III. Gross Cytology of C. australis

The growth pattern of the shoot system is initiated by an apical cell which divides in a plane parallel to the base to give a new apical cell and a segment cell. The segment cell divides giving an upper nodal initial and a lower internodal initial (Fig. 1, *A*; Plate 1, Figs. 1, 2, and 3). The node initial, by a series of longitudinal divisions, gives rise to a group of eight cells consisting of two central and six peripheral cells which form the laterals of limited growth. The internodal cells and the laterals expand but do not divide further. In about 28 days the internodal cell grows from microscopic size to 4–20 cm in length.

Microscopic examination of living apices shows the apical cell, the node, and internode initials to be filled with densely granular cytoplasm containing small inclusions of apparently solid structure and numerous particulate structures, but chloroplasts have not been identified. The nucleus is clearly visible in the apical cell but is not obvious in other cells. In cells immersed in neutral red solution, the dye appears uniformly distributed throughout the cytoplasm, indicating the absence of vacuoles, at least at the light-microscope level.

Protoplasmic differentiation and vacuolation are complete by the time the internode cell is about 1·0 mm in length. After the protoplast has differentiated, chloroplasts, mitochondria, nuclei, vacuole with inclusions, and ground cytoplasm with sol–gel regions are clearly visible under the light microscope in both living and stained material. During the subsequent growth of the cell, the protoplast expands from a few millimetres to full size, but the basic structure does not appear to alter. That is, a unit volume of protoplast has the same microscopic structure in internodal cells of all sizes.

Immediately adjacent to the cell wall is a layer of cytoplasm in which chloroplasts and small dark bodies, possibly mitochondria, are embedded. The boundary between the wall and gel layer is difficult to distinguish and appears stationary under the light microscope. The gel layer merges on the inner side into a region

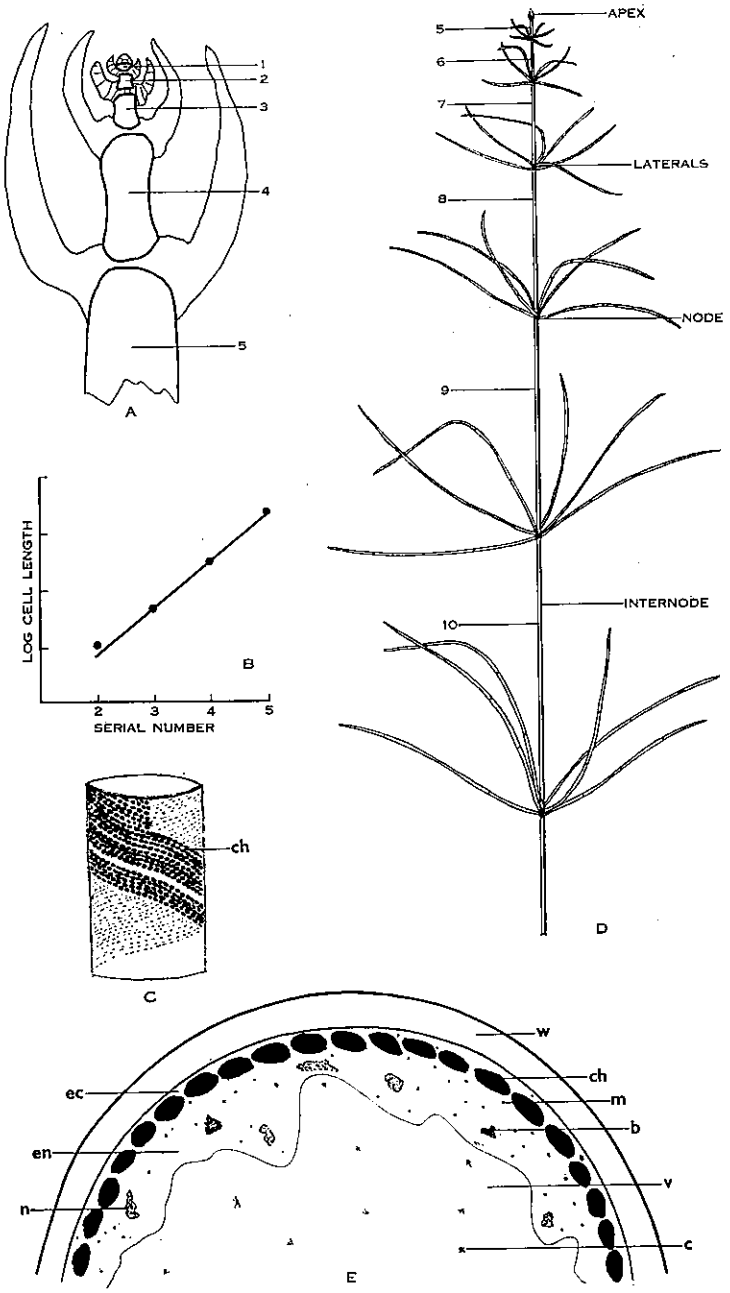

Fig. 1.—*A*, structure of the shoot apex (drawn from longitudinal sections) showing the origin of the internodal cell and the serial numbers (1–5) of the internodal cells in the apex; *B*, logarithm of the length of the internodal cell in a growing apex plotted against cell (or serial) number; *C*, segment of internodal cell showing spiral arrangement of the chloroplasts (*ch*); *D*, growth form of the vegetative system showing the whorled arrangement of the laterals at the nodes; *E*, basic structural features of the internodal cell seen in cross-section: *b*, cytoplasmic bodies; *ch*, chloroplast layer; *ec*, ectoplasm; *en*, endoplasm; *m*, mitochondria; *n*, nuclei; *v*, vacuole with numerous crystals (*c*) and wavy boundary (the tonoplast) bordering the cytoplasm; *w*, cell wall.

of granular cytoplasm which streams rapidly. The path of streaming follows a longitudinal helix round the cell, and upward and downward flow streams are demarcated by a striation of the wall which also has a helical pattern round the cell. Chloroplasts are not found opposite the striation which therefore appears as a thin white line (Fig. 1, *C*). Cytoplasmic streaming is in opposite directions on either side of this region. The chloroplasts are arranged in regular files which also follow the spiral pattern of the streaming cytoplasm and the striation (Plate 1, Fig. 4). Only rarely is a chloroplast seen to be moving in the streaming cytoplasm. The fluid layer of the cytoplasm surrounds the vacuole. During streaming the interface between cytoplasm and vacuole is seen to be in continuous movement as folds and projections of cytoplasm extending into the vacuole appear and disappear. In contrast to the external boundary of the gel layer, the inner boundary of the sol layer is in continuous motion, indicating a dynamic interface where local changes in structure are probably occurring.

The fluid or sol-like layer, the endoplasm, is almost entirely devoid of chloroplasts, although occasionally a few free floating plastids are seen, but bodies about 1 μ in diameter (probably mitochondria) are common. Also present, usually very close to the vacuole and sometimes protruding into it but still enclosed by cytoplasm, are a number of different types of inclusions. Some of irregular shape stained brilliantly with ninhydrin, and so are probably proteinaceous; others may be groups of nuclei and some still remain unidentified. The ground substance of the sol layer in which the various particulate bodies occur is not homogeneous but consists of regions of different optical density ranging in size from less than 1 μ to large clumps 10 μ across (Plate 1, Fig. 4). Frequently the larger clumps have a lumpy, almost semi-solid appearance. Some of these are not definite structures, such as organelles or vacuoles, but are probably transient regions of the cytoplasm which differ temporarily from the surrounding cytoplasm and change continuously during cyclosis.

The vacuole sap usually contains many crystals of different shape which move passively as the vacuole sap is stirred by the streaming cytoplasm. Conductivity measurements indicate that the vacuolar solution contains a high concentration of electrolytes.

From the above description, it is clear that both the cell lineage of the shoot system of *C. australis* and the basic structure of the internode cell are similar to those described for northern hemisphere members of the Characeae (Fritsch 1935; Green 1958), suggesting that the growth pattern of *C. australis* is likely to be similar to that of *Nitella* species as described by Erickson (1959) and Green (1958).

IV. Growth Studies of C. australis

Despite numerous attempts over a number of years using many different culture solutions, growth factors, and vitamins, colonies of *C. australis* were never grown satisfactorily either from spores, nodes, or clones under sterile laboratory conditions.* Occasionally clones derived from nodes were established in the

* In a paper not known at the time by the present authors, Sandan (1956) reports the successful culture *in vitro* of members of the Characeae.

laboratory. These proved useful for photoperiod work and for determining numbers of nuclei in cells of different ages. As plants could not be grown in a predictable way, growth studies were limited to the regrowth of plants under natural conditions in ponds in the Camden district, 30 miles south-west of Sydney. Shoots of the previous growth cycle were cut to base level, allowing regrowth to occur from basal nodes. After an interval of 21–35 days, the regrowth was harvested and the following data obtained for the internode cells—cell number, cell length, cell volume, cell surface area, dry weight, number of chloroplasts, number of nuclei, and concentration of nitrogen-containing compounds (Peebles 1956). In all experiments the shoots used were growing rapidly and mostly had reached only about 50% of the length of mature shoots. At harvest, the length of the shoots and the number of internodes per shoot varied from about 10–15 cm (5 or 6 internodes) to 30–40 cm (9–12 internodes) excluding the internodes of the apices. Regrowth from clones in the laboratory occurred erratically, differences of 3–4 weeks between the start of the growth from different nodes being common. The variation in the shoot length of the field-grown material can be attributed to the time of recommencement of growth from the different nodes rather than to differences in growth rates between shoots.

Growth data for the individual cells and shoot systems were obtained for 30 shoots from field material throughout September–December over two seasons (1955, 1956).

V. Results

(*a*) *Shoot System*

Values for the total length of a shoot system and the logarithms of shoot length plotted against internode or serial number are given in Figure 2. The relative lengths of the laterals in relation to the serial numbers of the internodes to which they are attached are illustrated in Figure 1, *D*.

Some ambiguity exists about the exact value of the serial numbers because a shoot apex contains from four to five internodes (Fig. 1, *A*; Plate 1, Figs. 1, 2, and 3). It was not possible to dissect the apices of all the shoots used in the measurements of growth. It is assumed in the present study that the first internode on which measurements were made corresponded to serial number 5.

(*b*) *Internodal Cells*

The increase in size is enormous. In a few weeks the volume may increase several millionfold, the area several hundred thousandfold, and cell length from microscopic dimensions to 80–160 mm. The length, dry weight, and volume of individual internodes of a single shoot system and their logarithms plotted against serial numbers are given in Figures 2 and 3. Lengths were measured to the nearest millimetre and diameters to the nearest 0·1 mm. Volumes and cell areas were calculated assuming a cylindrical shape for the cells. Dry weights were obtained by drying shoots or individual internodes at 70°C for 24 hr and cooling in a desiccator over calcium chloride for at least 12 hr before weighing. Cell-length data for internode cells for the unexpanded internodes of the apex are given in Figure 1, *B*.

An approximate measure of the volumes of the cytoplasm and vacuole was found by centrifuging cells enclosed in capillary tubes at 250 *g* for 5 min. After the protoplasm had sedimented the mass was measured and the two volumes calculated. For cells longer than about 2 cm the percentage of the cell occupied by cytoplasm and vacuole remained approximately constant at *c.* 4–5% cytoplasm and 95–96%

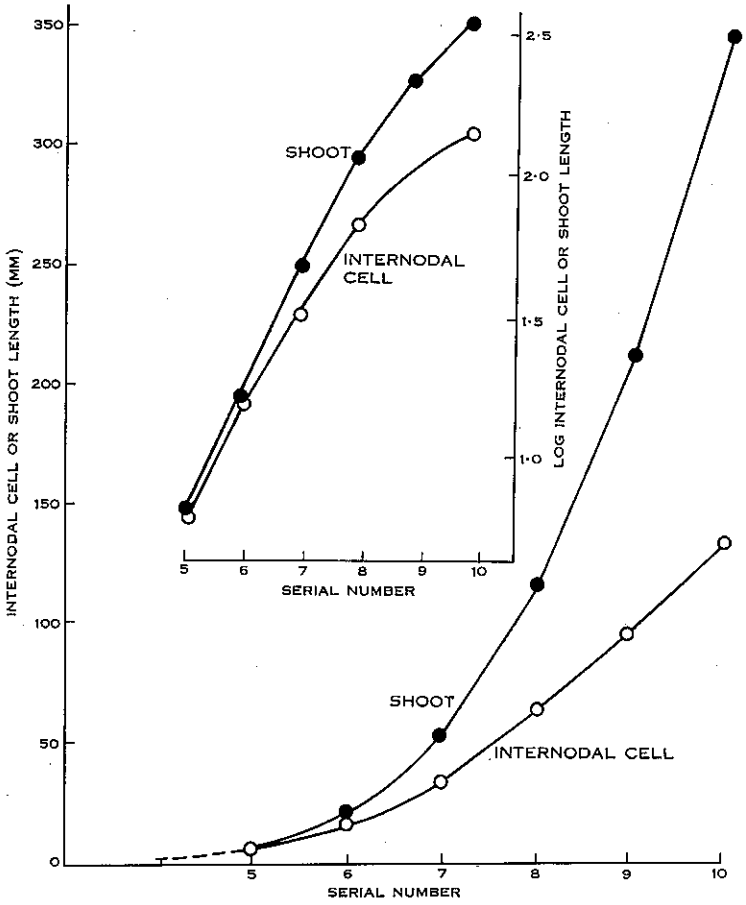

Fig. 2.—Length of the internodal cells and the total length of the shoot plotted against serial numbers. Inset shows the logarithm of the length of the internodal cell and the shoot plotted against the serial number.

vacuole. Very approximate values for the smaller cells were obtained by microscope examination in neutral red. On this basis the volume of the cytoplasm decreases from a possible 100% in the internode initials to about 40% in cells a few millimetres in length (Fig. 4).

Estimates of the number of chloroplasts per cell were made from the total area of the cell surface, assuming a true cylindrical shape, and from the number of chloroplasts per unit area of the cell surface at intervals along the internode. Chloroplast numbers are plotted against cell length in Figure 5.

Estimates of the number of nuclei per cell were made on laboratory-grown shoots and not the field material. It is assumed that these observations illustrate

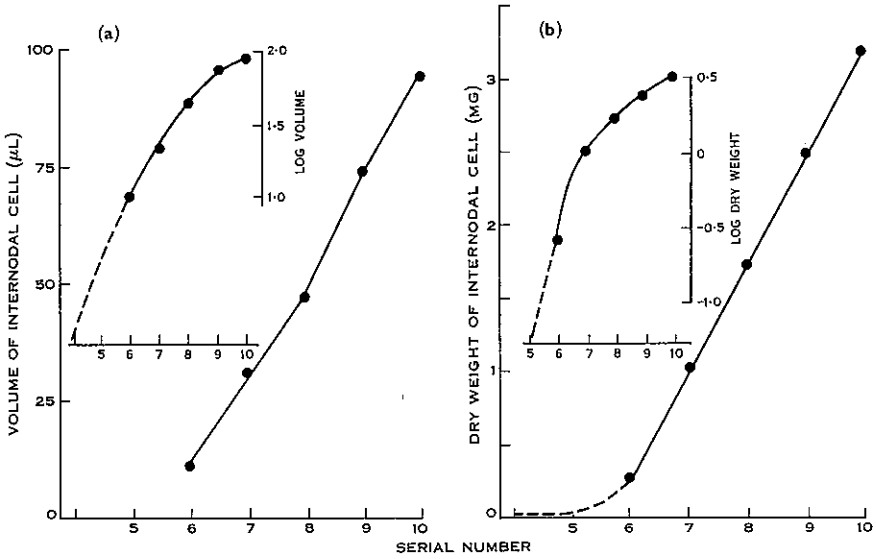

Fig. 3.—Volume (*a*) and dry weight (*b*) of the internodal cell and the logarithms of the volume and dry weight (insets) plotted against serial number.

the general characteristics of the increase in number of nuclei in internode cells. However, because of certain peculiar features about the nuclei, the estimates of

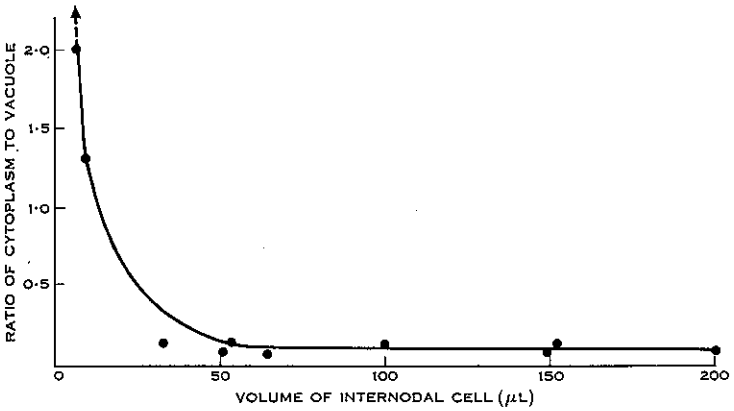

Fig. 4.—Ratio of cytoplasm to vacuole during the growth of the internodal cell as measured by the volume of the cell.

their number per cell are only approximate and limited to low cell numbers. Bodies showing a faint pink–purple Feulgen reaction are common in the internode cells.

These have a coarse, granular, diffuse appearance with vague outlines, are extremely irregular in shape and size (*c.* 3–20 μ), are frequently deeply lobed, and tend to occur

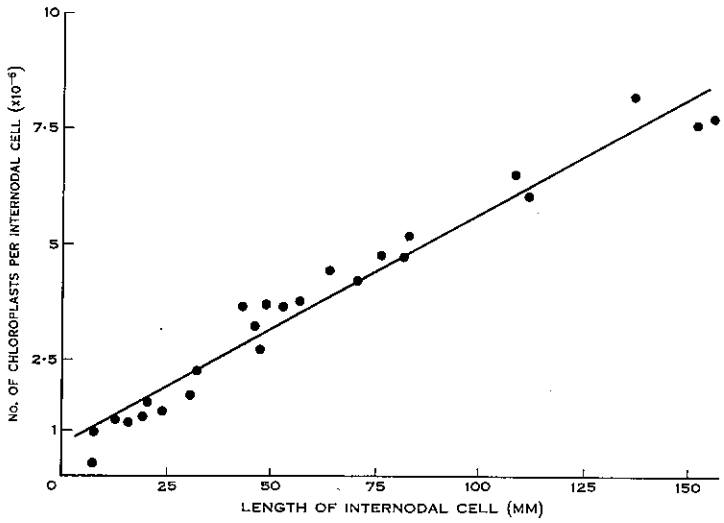

Fig. 5.—Number of chloroplasts per internodal cell during the growth of the cell as measured by the length of different internodal cells.

in smudge-like clumps up to 100 μ in extent. Usually it is impossible to decide whether a clump is really a single much-lobed nucleus or a group of nuclei. In the

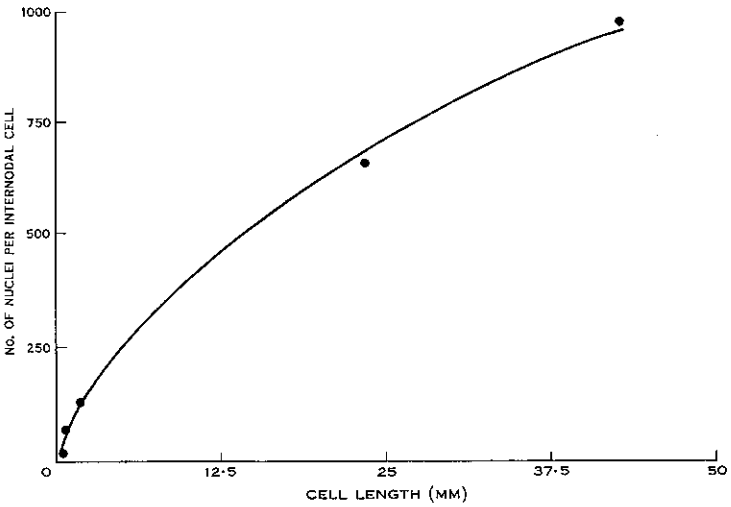

Fig. 6.—Number of nuclei (Feulgen-positive bodies) counted during the growth of the internodal cell, as measured by the length of the cell.

present study any discrete Feulgen-positive body, irrespective of size, was counted as a nucleus. The numbers per cell increased from 1 in the first-formed cell to approximately 1000 in cells *c.* 4 cm in length (Fig. 6).

VI. Discussion

Information in the literature suggests that the sequence of cells in a shoot system of the Characeae may form a growth–time sequence. Askenasky (1880), as described by Erickson (1959), assumed for *Nitella* that internode cells are cut off from the segment cell at equal intervals of time, which he termed a plastochron. That is, new internodes arise at equal intervals of time. Hence the length of internodes in a shoot system plotted against plastochron numbers, i.e. serial numbers, should correspond to the plot of length against time.

Recently Green (1954, 1958), Green and Chapman (1955), and Erickson (1959) showed that Askenasky's assumption about serial numbers as a measure of cell age is valid. From measurements made on shoots growing in culture media and from the changing position of spots on expanding internodes, they concluded that growth of the wall of the internode cell occurs uniformly along the cell axis and that the internode length–time curves are sigmoid in form. Two modes of elongation were recognized, exponential and linear. The former occurred in cell lengths up to a few millimetres and the latter in cell lengths from a few to 10–15 mm. From Green's work it seems highly probable that serial numbers, as assumed by Askenasky, can be used as a measure of cell age in a growing shoot system of the Characeae.

(*a*) *Cell Growth*

In the present study the graphs for cell length against cell number and log cell length against cell number for expanding shoots of *C. australis* (Figs. 1, *B*, and 2), and the graphs for the total length of the shoot against cell number (Fig. 2) closely resemble those obtained by Green for *Nitella axillaris*, suggesting that the pattern of growth of the two genera may be similar. In both *Chara* and *Nitella* elongation is exponential for very young cells, becoming linear for older cells. The exponential phase of *Nitella* (Green 1954) occurs in cells up to a few millimetres in length, whereas in *C. australis* this phase occurs in cells up to a few centimetres in length. This difference probably reflects the large difference between the size of the mature shoot systems in *Nitella* (*c.* 5 cm) and *Chara* (50 cm). It seems that the increase in length of the internode cell and the shoot system of *C. australis* follows the same growth laws as for *N. axillaris*. An expanding shoot system would contain cells at different stages of development, ranging from initials to cells approaching full size, and the sequence of cells down a shoot should correspond to growth stages through which all internodes proceed during cell expansion.

Similarly, the relationships of cell volume, cell dry weight, area of wall, number of chloroplasts, and possibly number of nuclei with serial number have a sigmoid form. Their growth patterns resemble that of cell length, consisting of an exponential phase and a linear phase. Thus the growth of the whole cell, certain fractions, and certain organelles in *C. australis* all follow the same growth pattern: an exponential increase with time during early stages of development, followed by a linear phase during which the cell may expand up to 200 mm in length. Support for this pattern is seen in the decrease in the cytoplasm to vacuole ratio during cell elongation (Fig. 4). The ratio declines from possibly an infinitely high value in the apical cell to a relatively constant value, 4–6%, in cells of higher cell numbers. The period of rapid decrease

in the ratio probably corresponds to the exponential phase, and the constant ratio to the linear phase of growth of the protoplast in which the cytoplasm is maintained at constant thickness as the cell expands many centimetres to full size.

(*b*) *Increase in Number of Nuclei*

The data for nuclei numbers are difficult to interpret, partly because the estimates are only approximate. A striking increase of from 1 to 1000 occurs in cells expanding *c.* 30–40 mm, but the points on the graph log nuclei number *v.* cell length are too scattered for the form of the curve to be determined. Little is known about how nuclei increase to give the coenocytic state in the Characeae, but it is usually ascribed to a process of amitosis (Fritsch 1935). This view is favoured by the fact that definite chromosomes and division figures have not been observed in the elongating internodes of *C. australis*. Sharp (1934) has stressed that many examples of amitosis, in both plant and animal cells, are really aberrant forms of normal mitosis. According to this view the atypical shapes of the nuclei in *C. australis* are those of degenerate organelles which presumably are no longer capable of division, and therefore should lead to a decreasing number being concerned with the production of new nuclei. This possibility seems unlikely in *C. australis* since it would not give the observed exponential increase in numbers in the early stages of growth. Irrespective of the process, whether amitosis or mitosis, all nuclei must be involved in the production of new nuclei in the exponential phase. If the growth pattern of the nuclei is similar to that of the rest of the cell, then the rate of increase in numbers must gradually become linear. Further work is required before the mechanism of nuclei increase in the internode is resolved.

(*c*) *Chloroplast Growth*

The growth of the chloroplasts during expansion of the internode is interesting because they increase both in total numbers and individually in volume with cell age. Chloroplasts increase some fiftyfold in volume from small structures at the limits of resolution of the light microscope to large, elliptical, disc-shaped bodies, while the increase in the numbers has the same growth characteristics as the cell, i.e. an exponential and a linear phase.

(*d*) *Allometric Growth*

During the development of the internode the relation between cell volume, cell length, cell area, and chloroplast number and other quantities appears to be allometric (Huxley 1932). The double logarithmic plots of cell volume and cell length tends to fall on a straight line (Fig. 7). Assuming the correct curves are straight lines, the growth of the protoplast and its parts can be expressed by the heterogeny formula

$$x = bz^k,$$

where x is a part of the protoplast, z the protoplast, b a constant, and k the allometric coefficient expressing the ratio between the relative growth rates of the cell.

Unfortunately, it was not possible to determine by direct observation, for example from marks on the surface of the protoplast, the distribution of growth throughout the protoplast during development. But the allometric data and the exponential characteristics suggest that growth probably occurs uniformly rather than locally, for example at the ends of the protoplast. Consequently the growth of the surface of the protoplast is likely to be distributed over the entire surface. Green (1954) has shown conclusively that the growth of the cell wall in *Nitella* is evenly distributed throughout the entire wall. It seems possible, therefore, that both wall and protoplast have similar growth characteristics, and that the growth

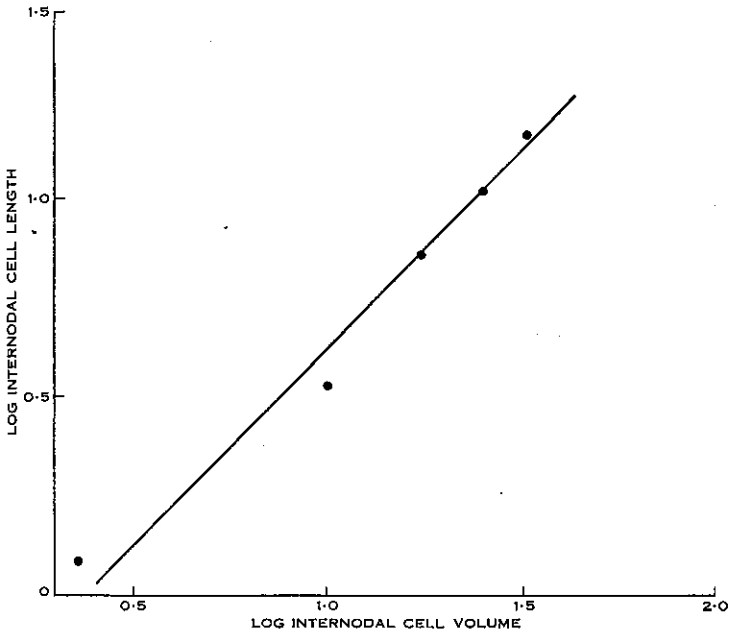

Fig. 7.—Logarithm of the length of the internodal cell plotted against the logarithm of the volume of the internodal cell.

of the wall reflects the expansion growth of the protoplast. Actually Green's speculation that wall growth may be determined by the amount of protoplasm in the cell, and that in the linear phase the amount of protoplasm and wall synthesizing enzymes are in fixed quantities, is consistent with the trend seen here in the cytoplasm to vacuole ratio. In the linear phase the cytoplasm is maintained as a layer of constant thickness as expansion occurs.

(*e*) *Conclusion*

It appears that the sequence of internodes in a shoot system can be used to describe the growth of the internode. During cell expansion the whole cell and its parts increase exponentially in the early stages with the relative growth rates of the

separate parts remaining constant; later, the relative rates fall gradually to zero as the cell expands to maximum length during the linear phase, but their ratios with reference to the whole cell remain constant. In other words, as expansion proceeds the proportions between the cell and parts should not alter. This conclusion is similar to that reached from the light-microscope observations, namely that the cytological structure of the cell does not appear to change during development. From the present data, a unit volume of the cell appears similar, apart from differences in the size of the chloroplasts, in cells from 10 to 200 mm in length.

Both the cytological and growth studies indicate that the sequence of internodes in a shoot system of *C. australis* should be a suitable system for studying the physiology of the internodal cell, and also provide a satisfactory basis for comparative studies of internodal cells and cells of higher plants. Later papers based on these conclusions will deal with particular aspects of the physiology and development of the internodal cell.

VII. Acknowledgments

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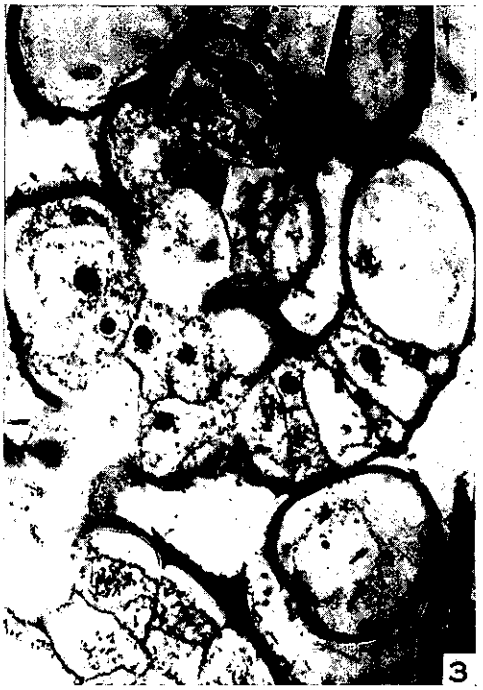
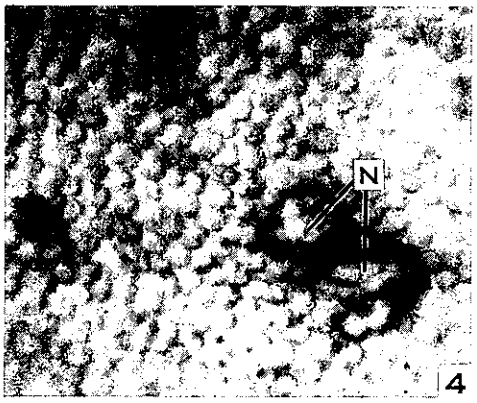
COMPARATIVE PHYSIOLOGY OF CHARA AUSTRALIS. I





Explanation of Plate 1

Figs. 1–3.—Longitudinal sections of the terminal apex showing serial numbers of the internodal cells (Figs. 1 and 2) and longitudinal section of the axillary bud (Fig. 3) of the shoot of *C. australis* after fixation in dilute chromic–acetic acid solution, dehydration in t-butanol, embedding in wax, and staining with safranin, crystal violet, fast green, and orange G (Figs. 1 and 2) and safranin and Harris' haematoxylin (Fig. 3). × 185, 370, and 360, respectively.

Fig. 4.—Photomicrograph of a mature, living, streaming internodal cell of *C. australis*. Note stationary layer of chloroplasts beneath which are large irregularly shaped particles (*N*) which may be nuclei. These latter were moving with the mobile cytoplasmic layer when the micrograph was made. High-speed Agfa record film (ASA = 1200) was used. ×1800.