

COMPOSITION OF DEVELOPING PRIMARY WALL IN ONION ROOT TIP CELLS. I. QUANTITATIVE ANALYSES^{1, 2}

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An understanding of the factors influencing and directing cell development must rest on knowledge of the changes occurring during the course of cell development on both a morphological and biochemical level. Previous work on onion root tips has outlined some of these changes that occur early in the development of the cell (7, 8, 9, 11). The wall is one of the most conspicuous parts of the cell and undergoes great changes during the life of the cell. Very little is known, however, of the chemical composition of the wall during the early stages of formation. The analyses reported here attempt to enlarge the picture of the composition of the developing primary cell wall.

The root tip is an excellent material in which to study cell development, as the various stages are arranged in approximately linear order. Thus, the analysis of consecutive sections yields information on changes accompanying cell development. The cell develops rapidly, particularly during the early stages, and to follow the development, sufficiently thin sections must be used. The first 20 consecutive 100 μ sections of the onion root tip are analyzed in the present work.

The usual methods of cell wall analysis based on gravimetric measurements or on paper chromatography could not be used on this level. Lignin is not present in this region of the root and as all the other wall components can be analyzed in terms of carbohydrates (hexoses and pentoses) or simple carbohydrate derivatives (hexuronic acids), colorimetric procedures were used. Microchemical techniques were developed to handle the material and measure the sugars. A standard extraction procedure of the wall components is used and the various extracts analyzed for the amount of hexuronic acids, hexoses, and pentoses present. The results are expressed as per cell and per unit surface area per cell.

MATERIALS AND METHODS

Sets of *Allium cepa* var. white globe were placed on vials of tap water and kept in the dark at 25° C. Roots between 1 and 2 cm in length developed in 48 hours. The first 5 to 10 mm of carefully selected uniform roots were excised and quickly frozen in iso-

pentane cooled with liquid nitrogen. The root tips were then placed in methyl alcohol at -30° C. The methyl alcohol was changed once during the first 24 hour interval and three times during the second 24 hours, finally being replaced with toluene at the same temperature. After 2 hours the roots were brought to room temperature and the toluene changed. They were then paraffin infiltrated and imbedded. The roots were carefully aligned in groups of three during the imbedding.

The roots were sectioned transversely on a regular Spencer microtome at either 10 or 25 μ . As the analyses required 100 μ segments of the root, ten 10 μ sections or four 25 μ sections were collected and placed in a 1 ml centrifuge tube. Actually, when 10 μ sections were used only nine were placed in the tube, while the tenth was mounted on a microscope slide and stained. These sections acted as controls on the alignment of the roots and the condition of the tissue. The 10 μ sections were used in preliminary analyses while the 25 μ sections were used in the later replications. As the analyses required nine 100 μ segments, nine roots were cut and the identical sections placed in the same tube. This was facilitated during imbedding by aligning roots in groups of three in the paraffin.

Once the tissue was placed in the centrifuge tubes it was never removed. Changing the various liquids in the tubes without loss of tissue was a very critical step. Pipettes drawn from glass tubing were drawn a second time so that the opening was narrower in diameter than the smallest section. A holder rigidly supported the pipette which was connected to a flask and ultimately to a vacuum line. A stopcock in the top of the flask controlled the pressure in the flask and hence the rate of removal of the liquid. The centrifuge tube containing the tissue and solution was raised to the pipette by means of a small movable platform. A magnifying lens mounted in front of the tube, and a mirror mounted at one side aided in observing the tissue and in aligning the tube with the pipette. Care was taken to keep the tip of the pipette just below the surface of the liquid and to remove the liquid slowly. Where the liquid removed was to be saved, a rubber bulb with a small hole in the top replaced the vacuum line. By closing the hole with a finger, the bulb could be used to draw off the liquid. Opening the hole equalized the pressure in the bulb and the liquid remained in the pipette and was not carried up into the bulb. The same stands for pipette and tube were used as in the case of the vacuum line. The tubes in either case were usually centrifuged in a small micro

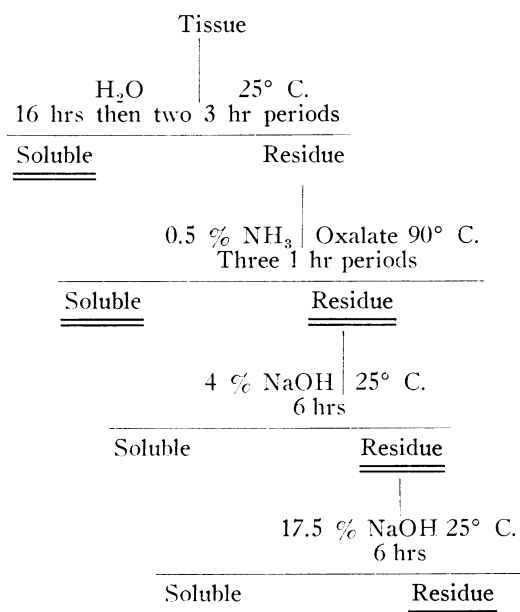
¹ Received July 16, 1959.

² Supported in part by a research grant from the National Science Foundation (G-4286).

centrifuge to force the tissue to the bottom before removal of the liquid.

The tubes were covered with aluminum foil wherever possible to reduce the amount of dust and lint falling into the tubes. Aluminum blocks 6in \times 6in \times 2in in which 100 holes $\frac{3}{8}$ in in diameter and $\frac{7}{8}$ in in depth had been drilled, were used for heating or cooling the tubes. These gave uniform heat conduction on a hot plate and could be cooled in a deep freeze.

The tissue was deparaffinized with toluene, rinsed with 100 % ethyl alcohol, and air dried. Analyses for total carbohydrate, hexoses, pentoses, and hexuronic acids were made directly on the tissue at this point, otherwise the sections were extracted according to the following schedule:



The extracts containing the soluble fraction were in each case combined and brought to dryness in an oven at 50° C. In the case of the 4 % NaOH extract, an equal volume of 4 % acetic acid was added to neutralize the base and then the solution evaporated. At all the underlined points in the above schedule, the extracts or residues were measured for amount of hexoses, pentoses, and hexuronic acids present. Of all the cell wall components fractionated in this scheme, only the 4 % NaOH and the 17.5 % NaOH soluble fraction had to be determined by difference in the residues before and after extraction, rather than directly on the extract. This was necessary when it was found that after treatment with NaOH neither the hexoses nor pentoses would react in the color reactions necessary for their determination.

This extraction schedule was worked out with great care. Longer and more numerous extraction periods were tested in most cases but the schedule given above proved to give the most satisfactory separation of wall components. Wherever possible alternate procedures were used to verify particular

fractions. The ammonium oxalate extraction was replaced by extraction with 1*N* HCl and similar results were obtained. Balance sheets for the various carbohydrates were also run combining various extraction procedures.

The extraction procedure used here is similar to but not identical with that of Boroughs and Bonner (4) and differs from that of Jermyn and Isherwood (10) principally in not first extracting with 70 % alcohol and obtaining a cold water and ammonium oxalate fraction instead of a single hot water fraction. It also differs from that of Bishop, Bayley, and Setterfield (2) where a 10 % NaOH and a 17.5 % NaOH extraction are combined so that only one NaOH fraction was measured. Burström (5), who generally followed Jermyn and Isherwood also combined the alkali soluble fractions. A search of the literature revealed no two identical extraction schedules. This indicates that every material must be handled as a separate case.

The procedures listed below were used for the carbohydrate analyses. They are based on macro or semi-micro procedures (1,6), but in all cases have been further reduced in volume and modified.

I. TOTAL PENTOSE AND HEXOSE—ORCINAL REACTION. To the sample was added 50 μ l H₂O, 50 μ l freshly prepared 2 % orcinol in 20 % H₂SO₄ and 500 μ l 60 % H₂SO₄. The mixture was heated for 30 minutes at 80° C in an aluminum block and then cooled in a second, cold aluminum block. A "flea" of glass coated ferrum reductum was added and the solution stirred by moving the flea with a magnet. The absorption was measured at 425 m μ in a Beckman spectrophotometer model DU. The range of this procedure is 0.5 to 10 μ g \pm 0.1 μ g. Glucose, fructose, galactose, mannose, ribose, and arabinose give equal absorption per μ g, while xylose gives twice this absorption. Glucuronic and galacturonic acids give values of less than a tenth of the hexoses and pentoses while ascorbic acid gives no color at all. The orcinol reaction was used to measure the total combined hexoses and pentoses in the untreated tissue.

II. HEXURONIC ACIDS—CARBAZOLE REACTION. To the samples was added 50 μ l H₂O and 300 μ l concentrated H₂SO₄. The tubes were heated for 20 minutes at 100° C in an aluminum block and then cooled in a cold aluminum block for 5 minutes. Then 10 μ l of a freshly prepared solution of 0.1 % carbazole in 95 % ethyl alcohol was added. A flea was used to stir the solution and the absorption measured at 535 m μ in a Beckman spectrophotometer. The procedure has a range of 0.5 to 5 μ g \pm 0.1 μ g. Glucuronic and galacturonic acids give identical values. Pentoses and ascorbic acid do not react and there is negligible interference from hexoses. The carbazole reaction was used to measure hexuronic acid in the untreated tissue and in the various extracts.

III. PENTOSSES—CYSTEINE REACTION. The tubes containing the samples were cooled in a cold aluminum block and 50 μ l cold H₂O and 200 μ l cold concen-

trated H_2SO_4 were added. After shaking, the tubes were removed from the block, fleas added, and the tubes allowed to come to room temperature. The solutions were kept at room temperature for 2 hours with regular stirring. Then 5 μl of freshly prepared 3% aqueous cysteine was added and the mixture stirred. After 15 minutes, the absorption at 390 $m\mu$ and at 425 $m\mu$ was read in a Beckman spectrophotometer. The difference of absorption at 390 $m\mu$ to 425 $m\mu$ is proportional to the amount of pentose present. The range is 0.5 to 5 $\mu\text{g} \pm 0.1 \mu\text{g}$. There was no reaction to hexoses, hexuronic acids, or ascorbic acid. The greatest difficulty with this procedure is that xylose gives 4.5 times the color per μg as does arabinose or ribose. In all cases the pentose data are expressed as arabinose. This cysteine reaction was used to measure pentoses in the untreated tissue, the extracts, and the tissue residues.

IV. HEXOSES—CYSTEINE REACTION. To the sample was added 50 μl H_2O . The tubes were then placed in an ice water bath and 500 μl of a six part H_2SO_4 :one part H_2O mixture was added. After a few minutes the tubes were removed from the ice water, allowed to warm to room temperature, and then heated in boiling water for 3 minutes. They were then cooled in a water bath at room temperature and 100 μl 3% aqueous cysteine solution added. The solutions were stirred with a flea and the absorption at 415 $m\mu$ and 380 $m\mu$ read in a Beckman spectrophotometer. The difference in absorption at 415 $m\mu$ and 380 $m\mu$ equals the amount of hexose present. The range of the procedure is 0.5 to 5.0 $\mu\text{g} \pm 0.1 \mu\text{g}$. Glucose, fructose, galactose, and mannose give almost identical absorption values. There is no interference from pentoses, hexuronic acids, or ascorbic acid. This cysteine reaction was used to measure hexoses in the untreated tissue, the extracts, and the tissue residue.

V. TOTAL HEXOSES AND PENTOSES—INDOLE REACTION. To the sample was added 50 μl H_2O , 500 μl 75% H_2SO_4 and 20 μl 1% alcoholic (95% ethyl alcohol) indole solution. This mixture was heated for 10 minutes at 100° C and cooled. The absorption was measured at 470 $m\mu$ in a Beckman spectrophotometer. The range of the procedure is 0.5 to 5.0 $\mu\text{g} \pm 0.1 \mu\text{g}$ for hexoses and pentoses. Glucose, fructose, mannose, galactose, arabinose, and ribose have equal absorption while xylose is 1½ times higher. Galacturonic and glucuronic acid react intensively while ascorbic acid gives less intense, but still appreciable, color. Consequently, the indole test was used only on the 4% and 17.5% NaOH tissue residue where neither the hexuronic acids nor ascorbic acid is present.

In all of the carbohydrate analyses two kinds of standards were used. In one the standards were treated the same as the tissue or extract and carried through the entire procedure. This standard acted as a control of the effect various chemicals and heating periods might have on the carbohydrates. The

second standard was run only through the color reaction. In most cases there was little difference between the two standards.

The carbohydrate procedures given above were worked out in great detail. All hydrolysis times and temperatures were tested and complete absorption curves run in every case. Each procedure was tested for the possibility of interference by higher concentrations of sugars not normally reactive in that procedure. Even at ranges of more than five times the amount of the carbohydrate normally present in the tissue, no interference in any of the tests was found.

Attempts were made to remove the protoplasm from the cells before analysis of the wall by treatment with alcoholic KOH. These attempts were abandoned after determinations showed large losses of hexuronic acids, hexoses, and pentoses. As there is little hexuronic acid in the cell other than that associated with the cell wall and the total amount of pentoses found in all the nucleic acids in the cell give an insignificant color under the conditions used here, it was concluded that not only the protoplasm but much of the cell wall material was being removed. Onion root tips do not contain starch and it would appear safe to assume that the significant amount of the hexuronic acids, hexoses, and pentoses, is either associated with the cell wall or is soluble.

The roots were analyzed morphologically in the same way as reported earlier (9). A group of bulbs different from that used in previous work was used in this study. The roots from the new group of bulbs had slightly different cell numbers and cell dimensions. For these reasons the cell number, mean cell cross sectional area, mean cell length, and mean cell surface area are presented here. The number of mitosis also were counted.

RESULTS

MORPHOLOGY. The first 2 mm of the root tip are represented diagrammatically in figure 1. The graph immediately below the diagram presents the cell number per 100 μ section. The upper curve is the total cell number while the lower represents the number of cells per section minus the number of rootcap cells. Figure 1 presents the average cross sectional area, length, and surface area of the cells in each 100 μ section. The percent of nuclei in division is also presented in figure 1. A detailed morphological analysis of the onion root has been published (9) and complete discussion of the morphology of the root will not be undertaken here.

The general pattern of cell development can easily be seen from the graphs of figure 1. The cell, basal to the apical initials, first enters a period of marked radial enlargement. This period lasts from 400 to 1,400 μ from the tip. Elongation begins some 800 to 1,000 μ from the tip and extends beyond the 2 ml of the tip analyzed here. From 800 to 1,400 μ there is a period in which the cell is both enlarging radially and elongating. The amount of cell elongation, however, is small until after approximately 1,400 μ from

the tip. The period where elongation and radial enlargement overlap will be termed the first period or phase of elongation, while elongation after 1,400 μ will be referred to as the second phase. This pattern is in general agreement with that of Scott et al (11).

The distribution of cell divisions must also be considered in relation to the various stages of cell development. The apical initials are very low in number of cells in division. This feature of the root has been discussed elsewhere (9). During the period of radial enlargement, the number of cells in division increases and the maximum number of divisions occurs at the beginning of elongation. During the first phase of elongation, cell divisions are as numerous as during the first phase of radial enlargement. As the second phase of elongation is approached, cell divisions decrease and by 1,600 μ have ceased. This pattern of divisions is similar to that reported earlier by Jensen and Kavaljian (9). In the roots used by them, however, the rootcap was more extensive and the first 2 mm of the root analyzed, essentially did not contain the second period of elongation. Moreover, they used fluctuating temperatures while a constant, slightly elevated (25° C) temperature was used in the present work.

The cells of the rootcap in front of and surrounding the apical initials undergo rapid development resulting in large, heavy walled cells. The presence of the rootcap cells surrounding the apical initials must not be forgotten when considering the data from the early sections which contain large numbers of rootcap cells as well as the apical initials and other non-

rootcap cells. The amount of carbohydrate present in the apical initials can be calculated if it is assumed that the amount of carbohydrate per rootcap cell immediately in front of the apical initials, is the same as in the rootcap cells surrounding the apical initials. Since the amount of carbohydrate in the rootcap cells in front of the apical initials is known from section II, and the number of rootcap cells present in section III is known from the morphological analysis, then simple multiplication of these two figures will result in the amount of carbohydrate in section III that will be accounted for by rootcap cells. If this figure is then subtracted from the total amount of carbohydrate in section III, the remainder will be the carbohydrate present in the apical initials. Dividing this figure by the number of apical initials, results in an estimate of the amount of carbohydrate present per cell of the apical initials. This figure is really only an approximation, but it is nonetheless interesting and useful.

CARBOHYDRATE DETERMINATIONS. The results of the carbohydrate determinations on the whole tissue and after the various extraction procedures, are presented graphically per cell and per μ^2 surface area per cell, in figures 2 and 3. All data have been expressed per μM or per $\mu\mu M$ so that the data on the various sugars can be compared directly.

Before describing the results a word must be said regarding terminology. Two general systems of cell wall terminology are apparently in existence. One is based on solubility criteria so that all hot water or ammonium oxalate soluble materials become pectin

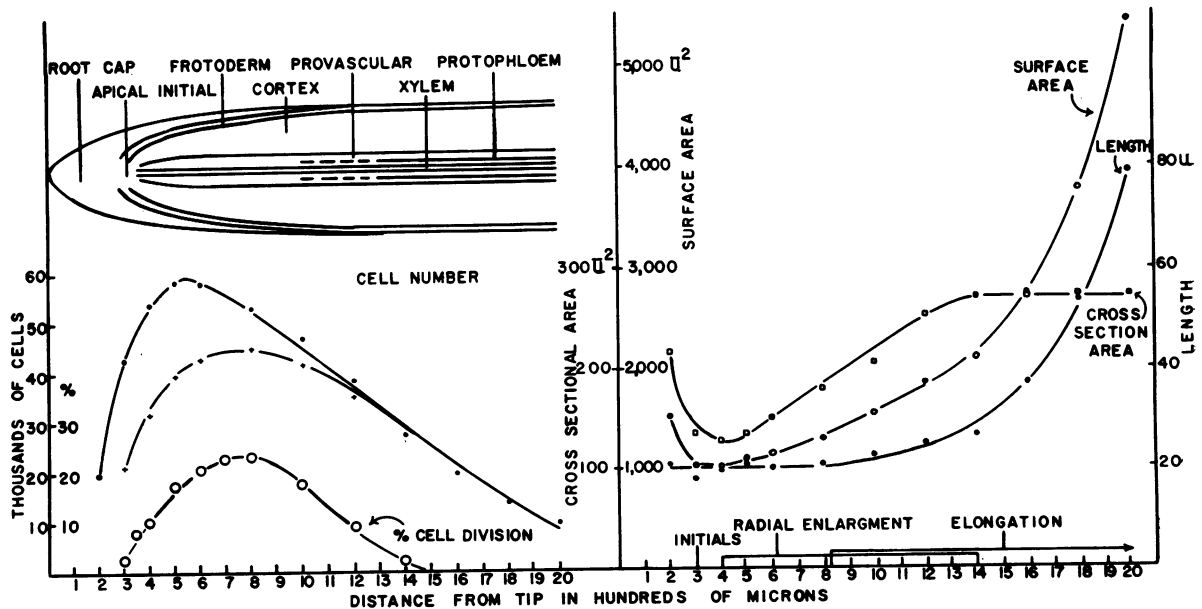


FIG. 1 (left graph). Number of cells per 100 μ section. (• = total number of cells per section, + = non-rootcap cells per section) and percent cells in division. The diagram of the root at the top is on the same scale as the graph.

(Right graph) Average cell cross section area, length, and surface area expressed as a function of distance from tip of section analyzed.

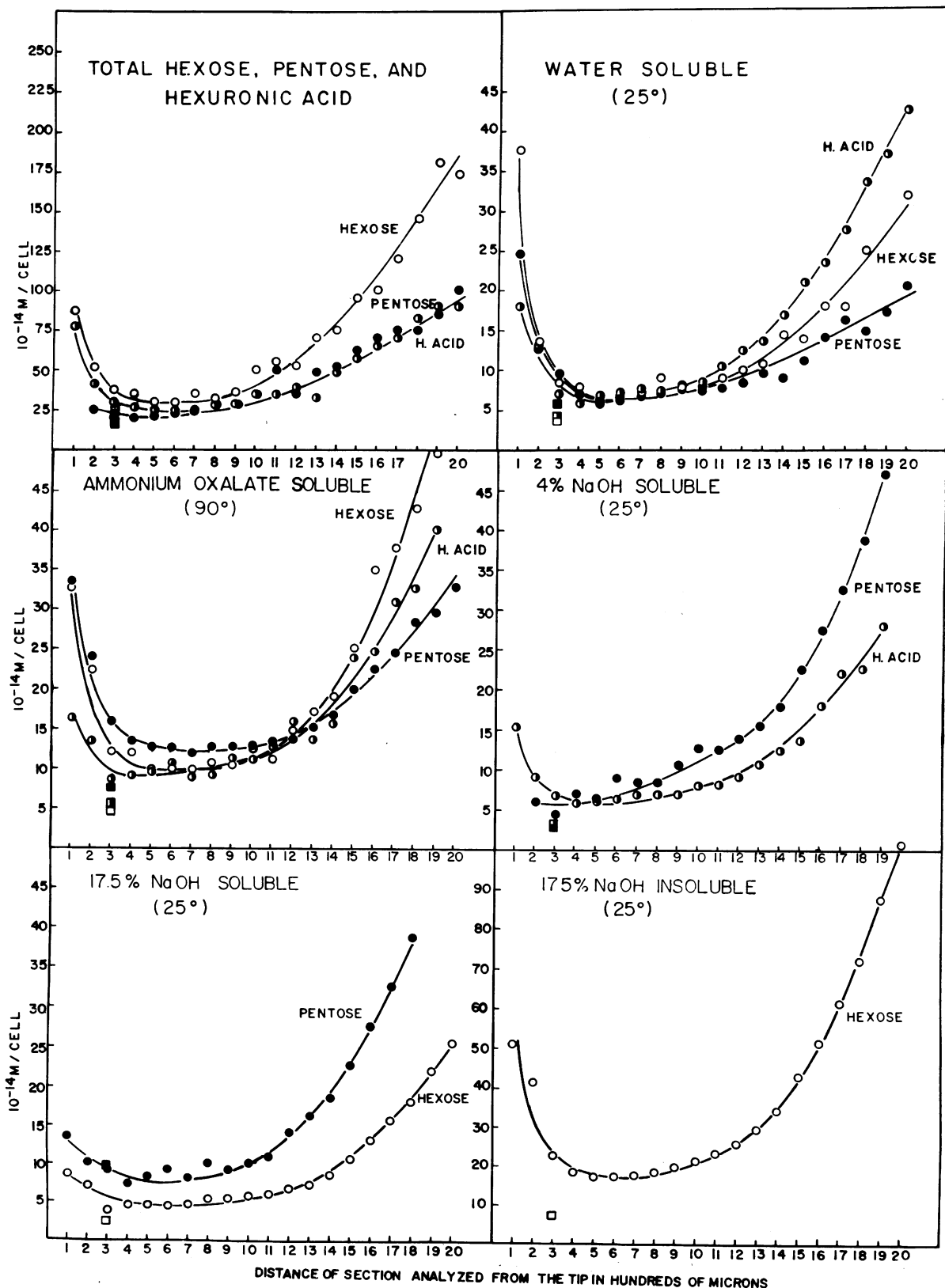


FIG. 2. Results of carbohydrate analyses expressed per cell. Open circle is hexose; closed circle is pentose; half-open circle is hexuronic acid. The squares indicate calculated values for the apical initials (see text for explanation).

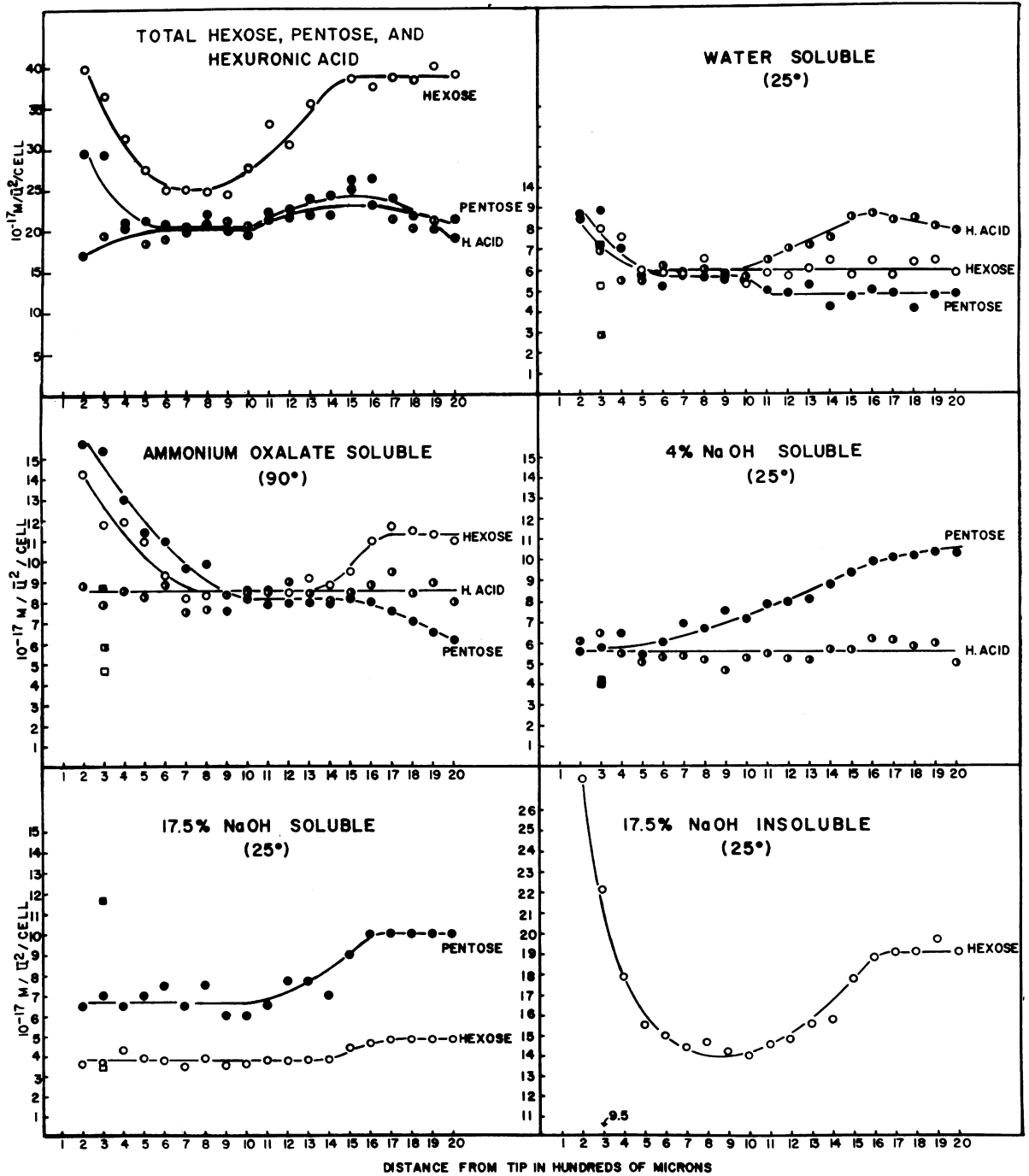


FIG. 3. Results of carbohydrate analyses expressed per surface area per cell. Open circle is hexose; closed circle is pentose; half-open circle is hexuronic acid. Squares indicate calculated values for apical initials.

and alkali soluble materials become hemicelluloses (10). The other is based on chemical composition criteria so that only pure polygalacturonic acid compounds are pectic substances, while hemicelluloses contain hexuronic acid and pentose, and noncellulosic polysaccharides composed of hexoses and pentoses may be found either in the hot water-ammonium oxalate soluble or alkali soluble fraction (3). This picture is further complicated by dividing the pectic substances (in a chemical sense as polygalacturonic acid) into pectin and protopectin on solubility characteristics. Only cellulose and lignin are clearly uncontroversial terms.

The following definitions will be used: pectin—water soluble hexuronic acids; protopectin—water insoluble, ammonium oxalate soluble hexuronic acids; pectic substances—the sum of the water and ammonium oxalate soluble hexuronic acids; soluble non-cellulosic polysaccharides—water insoluble, ammonium oxalate soluble hexoses and pentoses; hemi-cellulose—ammonium oxalate insoluble, 4% NaOH soluble hexuronic acids and pentoses; insoluble non-cellulosic polysaccharides—4% NaOH insoluble, 17.5% NaOH soluble hexoses and pentoses; cellulose—17.5% NaOH insoluble hexose.

Quantitative comparison of the present data with previous work is almost impossible. This is because the present data is expressed per cell and almost no data on a similar basis exist in the literature. Moreover, it is not possible to obtain the dry weight of the sections making it impossible to express the present data in terms of percent dry weight as is common in the literature.

To understand the implications of the data expressed per unit surface area per cell it is necessary to realize that there need be no connection between the surface area of the cell and the area of the wall. When the data are expressed per unit area they mean that the amount of wall material per cell is being compared with the surface area of the cell. If the amount of wall material and the area of the cell both increase uniformly from one section to another the amount of wall material per unit area will remain constant; if the wall materials increase more than the surface area, the amount of wall material per unit area will increase. There is no assumption that the wall is continuous or entire. From figure 4, it is clear, that except for the cell in rapid elongation, the wall is not a simple, entire sheet surrounding the cell. When the unit area data are viewed in this way and the factors involved in changes in surface area are examined, a picture of cell wall development can be outlined.

The important points of the determinations can be summarized thus:

A. TOTAL HEXURONIC ACID, HEXOSE, AND PENTOSE. The three major groups of carbohydrates present in the root tip show, in the analysis of the total tissue, a unity and relationship that belies the diversity of their distribution within the cell wall. The cells of the root cap are high in both pentoses and hexoses while relatively low in hexuronic acids.

All three groups are relatively low in the cells undergoing radial enlargement and increase proportionally to the increase in surface area of the cell. Correlated directly with the beginning of elongation there is an increase in amount of all three groups of carbohydrates. This increase in amount is greater than the increase in surface area of the cell. As the cells enter the second phase of elongation, the amount of hexuronic acids, pentoses, and hexoses continues to increase, but only the hexoses continue to increase at a rate greater than the increase in surface area.

These determinations of total hexuronic acid, hexose, and pentose were also used as a check on the measurement of the various fractions. When the hexuronic acid, hexose, and pentose content of the various fractions are added together they should equal the total content of these carbohydrates measured directly. The direct and indirect total amounts were found to differ by less than 3% for the hexoses and less than 8% for the hexuronic acids. The pentose difference was almost 30%, however, with the indirect total being higher than the direct. The pentoses are the most difficult to measure of the three carbohydrates, requiring a procedure that does not allow the tissue to be heated. This fact makes it highly probable that the direct total amount is lower than the indirect total because of difficulties in obtaining complete hydrolysis of the wall.

B. WATER SOLUBLE FRACTION. All three groups of carbohydrates were found in the water soluble fraction in relatively large amounts. As the tissue was in contact only with toluene or absolute methyl or ethyl alcohol from the time of initial freezing until the water extraction, we believe that no measurable amount of carbohydrate was lost.

The hexuronic acids may compose part or all of the water soluble pectins. The cells of the root cap

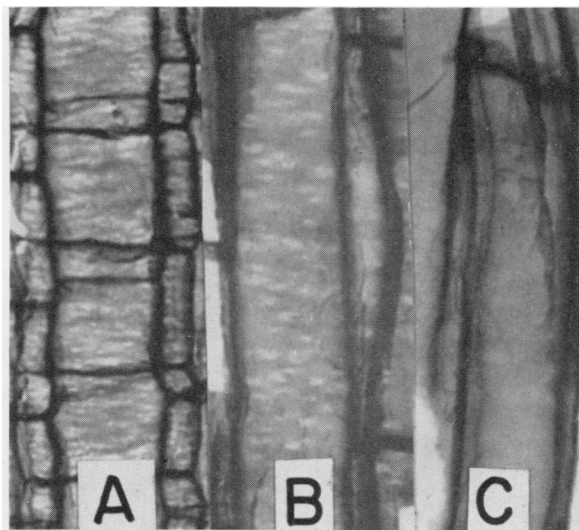
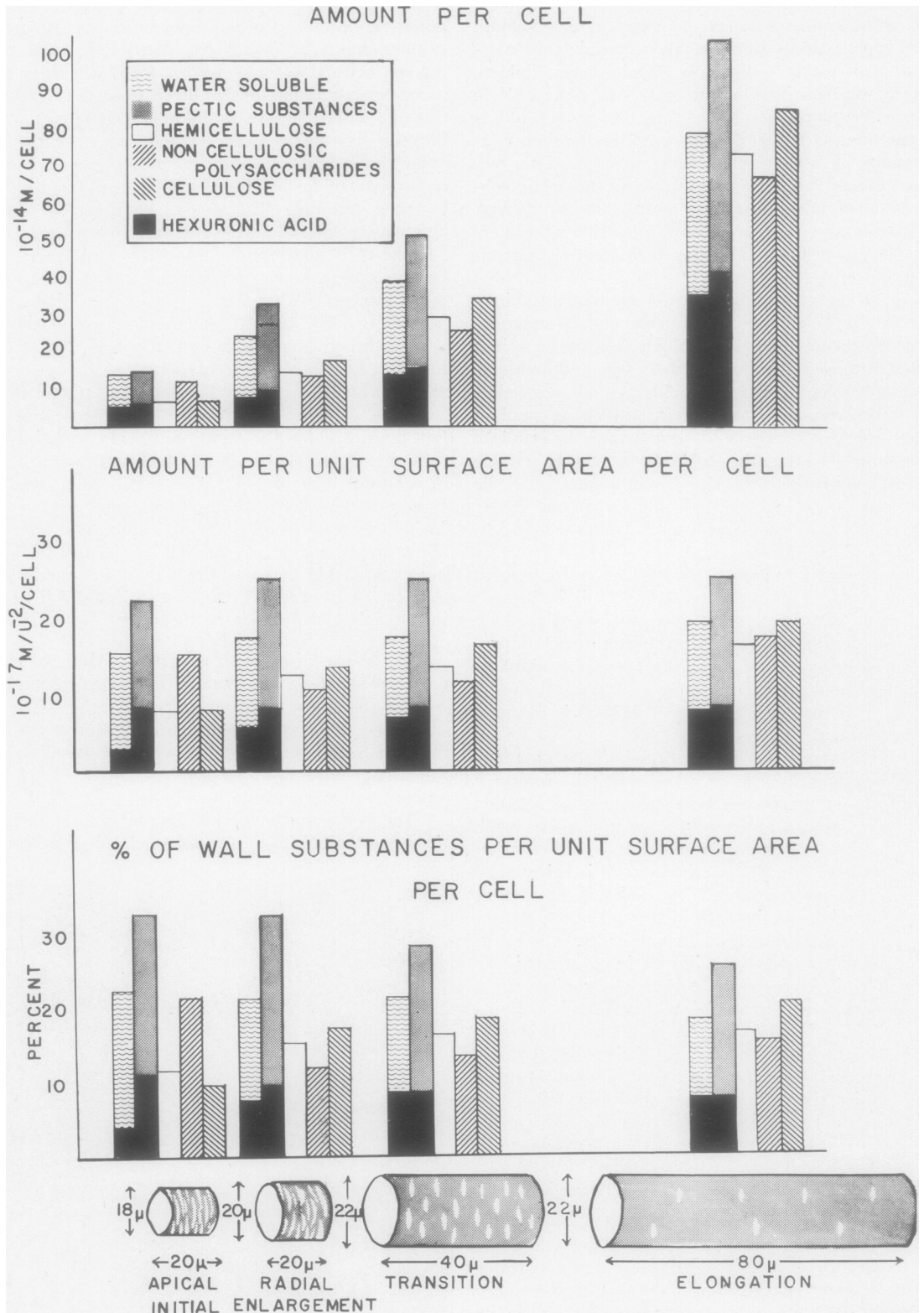


FIG. 4. Microscopic structure of cell wall. A. Wall of cells in radial enlargement. B. Wall of cells in transition zone. C. Wall of rapidly elongating cells.



STAGES OF CELL DEVELOPMENT

are relatively high in hexuronic acid. The cells in radial enlargement contain a relatively low amount which appears to be directly proportional to the surface area of the cell. As soon as elongation begins, however, the amount of hexuronic acid increases at a rate greater than the increase in surface area. As the cell reaches the second phase of elongation, the rate of increase of the hexuronic acid falls below the rate of increase of surface area and the amount of hexuronic acid per unit area progressively declines.

Hexose content of the root cap cells is also high, particularly in the extreme cells. In the cells in radial enlargement the amount of hexose is lower than in the rootcap. As cell elongation begins, hexose content per cell increases. This increase, however, is directly proportional to the increase in cell surface area so that the amount per unit area remains constant.

The pentose content per cell is similar to the hexose pattern except that at the beginning of elongation there is a small but consistent drop in pentose amount per unit area.

C. 0.5% AMMONIUM OXALATE SOLUBLE. Again, all three groups of carbohydrates were found and in relatively large amounts.

The hexuronic acids, which may be considered the protopectins and the pectates, are present in amounts directly proportional to the cell surface area in all stages of development represented in the first 2 mm of the root including the rootcap. The protopectins make up 6% of the total carbohydrate content of the first section and increase to 10% by the eighth section and then remain relatively constant. The sum of the pectin and protopectin follows a similar pattern, starting at 13%, increasing to 18% and then remaining constant.

Hexoses, probably in the form of a non-cellulosic polysaccharide, are present in large amounts in cells that have undergone or are undergoing cell elongation. The cells of the rootcap appear very high in hexoses and undoubtedly influence the amount per unit area between 500 to 800 μ from the tip. During radial enlargement the amount of hexose is relatively low and directly proportional to cell surface area. This proportionality is maintained during early elongation but as the cells enter the second phase of elongation there is a sharp rise and a new proportionality at a higher level is established.

Pentoses, probably in the form of a non-cellulosic polysaccharide, are present in large amounts in the cells of the rootcap. Cells in radial enlargement and early elongation contain relatively less pentoses but the amount is directly proportional to the increase in surface area. As the rate of elongation increases,

the increase in pentose content does not remain proportional but decreases so that the amount of pentose per unit area of the wall decreases.

D. 4% NaOH SOLUBLE FRACTION. This fraction contained only hexuronic acid and pentoses and thus fits one commonly accepted definition of the polyuronide hemicelluloses (3).

The hexuronic acid content per cell is low in the cell in radial enlargement and increases as the cells enter elongation. In all phases of cell development the amount per cell is directly proportional to surface area.

Pentose content per cell is directly proportional to surface area only during the very early stages of cell development. During this period the ratio of pentose to hexuronic acid is 1:1. While the cells still are undergoing radial enlargement, the amount of pentose per cell begins to increase at a rate greater than the increase in cell surface area. This condition persists until the second phase of elongation when the amount of pentose decreases slightly in relation to increase in surface area of the cell.

This pattern of hexuronic acid and pentose change suggests that the hemicellulose present consists of chains of hexuronic acid-pentose units in a ratio of 1:1. The extra pentose molecules may form cross linkages between the hexuronic acid-pentose chains.

E. 17.5% NaOH SOLUBLE. This fraction contained only hexose with an occasional trace of pentose and represents almost pure cellulose.

The hexose content of the rootcap is high while the cells in radial enlargement are relatively low. As cell elongation begins, the hexose content of the wall begins to increase at a rate greater than the increase in surface area. When cell elongation enters the second phase, however, the rate of increase of hexose per cell becomes constant in relation to the increase in surface area.

DISCUSSION

The composition of the primary cell wall in the root tip presents a surprisingly complicated picture. The problem is to relate the changes in the chemical composition of the wall with the morphological development of the cell. The region of the root analyzed contains cells of the rootcap, the apical initials, and cells in the following stages of morphological development: radial enlargement, a transition stage where radial enlargement and elongation are both going on, and elongation. The data for the apical initials and the various stages of cell development are summarized in figure 5.

FIG. 5. Composition of cell wall expressed as a function of stages of development of cell. The noncellulosic polysaccharides referred to in the legend are the insoluble noncellulosic polysaccharides of the text. The hexuronic acid content of the water soluble and pectic substances fraction are shown because of importance of the hexuronic acids in definitions of pectin and protopectin. The black bars in both cases represent the amount of hexuronic acid in each fraction.

The cells of the rootcap actually are present in all stages of development but the mature, fully differentiated cells predominate. These cells are large and possess very thick walls. From the chemical analyses it is clear that the walls contain large amounts of cellulose and soluble non-cellulosic polysaccharides. The cells also contain fairly large amounts of water soluble hexoses, pentoses, and hexuronic acids. Protopectin, hemicellulose, and the insoluble non-cellulosic polysaccharides are present in much lower amounts and are directly proportional to cell surface area.

The apical initials represent a relatively small group of cells that can only be treated indirectly on the basis of calculations previously described. The striking feature of the apical initials is their low incidence of division. They appear to be metabolically inactive insofar as they have been examined. Morphologically, the apical initials are small cells with thin, delicate appearing cell walls that in no sense form a continuous sheet of material. On a per cell basis, the apical initials are low in all cell wall components, except the insoluble pentose non-cellulosic polysaccharides, when compared with the other cells in the root (fig 5). On a per unit surface area basis, however, all the pentose containing fractions, except the pentose in hemicellulose, are either equal to or greater than the same fractions in the cells in radial enlargement. Pectin and protopectin are low on either basis but the wall is proportionally richer in protopectin than is the wall in radial enlargement. Cellulose is also low but the proportion of cellulose to pectic substances is the same in the apical initials and the cells in radial enlargement. It is clear that the cells of the apical initials do not lack cellulose, nor are very high in pectic substances or composed only of pectic substances and cellulose.

The cells in radial enlargement are increasing primarily in diameter with little increase in average length. Cell divisions are numerous during this stage and increase in number. The wall is morphologically a loose open structure with large gaps (the primary pit fields) running at right angles to the long axis of the cell (fig 4a). The wall is low in all carbohydrates although all components are present in roughly equal amounts. If all the water and ammonium oxalate soluble carbohydrates are taken together and treated as pectic substances they amount to some three times the amount of cellulose present. If, however, only the hexuronic acids of these fractions are considered as pectic substances they are then equal to the cellulose present. During this period of radial enlargement all the wall components increase uniformly in relation to one another and in direct proportion to the increase in cell surface area. The cells in radial enlargement are often considered as the meristem and treated as being simple meristematic cells. In this connection it should be emphasized again that the wall does not lack cellulose nor is it composed primarily of pectic substances.

Radial enlargement continues until approximately 1,400 μ from the tip, while elongation begins some 800 to 900 μ from the tip. Thus, there is a region

where the cell is still increasing in diameter while at the same time beginning to increase in length. Cell division in the root reaches a peak at the beginning of this stage and decreases as the stage progresses and elongation becomes more pronounced. During this stage the morphology of the wall changes to a more continuous structure with the larger gaps being filled in, although still visible, and the primary pit fields becoming smaller (fig 4b). This change in the morphology of the wall is reflected in the amount and composition of the various wall components. All the carbohydrate components of the wall increase during this period but three (pectin, soluble pentose non-cellulosic polysaccharide, and cellulose) increase more than the surface area of the cell.

As radial enlargement ends and several hundred microns later cell divisions cease, the cell enters the stage of rapid elongation. The morphology of the wall changes again, becoming continuous with all but the final traces of the primary pit fields nearly invisible (fig 4c). The change to straight elongation has some very interesting effects on the carbohydrates of the cell wall. All components continue to increase per cell but it is in relation to surface area that surprising changes occur. From 1,600 to 2,000 μ from the tip a steady state seems to exist between the increases in cell wall components and the increase in cell surface area (all cell wall components increase directly proportional to surface area). This steady state has been arrived at differently, however, for the different components. In the case of protopectin and hemicellulosic hexuronic acid no change was involved as they remained directly proportional to surface area through all the stages of development. For cellulose and pectin which were from the beginning of elongation increasing faster than the surface area, the final state is a result of synthesis now becoming directly proportional to growth in area. For soluble hexose noncellulosic polysaccharides and the insoluble non-cellulosic polysaccharides, the end of radial enlargement resulted in a period of increased synthesis that terminated in the establishment of a new proportionality to surface area but at a higher level. In all cases the end result is a condition where each component is being synthesized in direct proportion to the increase in surface area of the cell. The composition of the wall is different, however, than it was before. Per unit area, the wall is higher in cellulose, pectin, hemicellulose, soluble hexose noncellulosic polysaccharide, and the insoluble noncellulosic polysaccharides while remaining constant with regard to protopectin and water soluble hexose. Only water soluble pentose decreased in amount.

What essentially appears to be happening during this stage of development is that the gaps in the cell wall associated with radial enlargement are being filled in with cellulose, pectin, hemicellulose, soluble hexose noncellulosic polysaccharide, and the insoluble noncellulosic polysaccharides. This accounts for the increase of these components per unit area immediately after the cessation of radial enlargement and for the

fact that they then become directly proportional to surface area at a higher level while the wall appears no thicker, if not thinner, morphologically. When this stage is compared with the preceding one where both radial enlargement and elongation are occurring simultaneously, it seems possible that the amount of elongation in the earlier stage may be limited by the fact that the wall is still too fragmentary for rapid elongation to occur. Only after the wall is an approximately continuous unit, can elongation occur to any great extent. This point of conversion would appear to be a logical place to study the effect of growth substances on roots.

The data presented here are difficult to compare with other cell wall analyses due to differences in the techniques and the means of expression employed. Insofar as comparison is possible the data reported here are in general agreement with other analyses of primary walls using quantitative biochemical techniques (3, 4, 5, 10). The notable exception is the work of Bishop et al (2), who reported small amounts of pectic substances in the primary wall of *Avena coleoptiles*. The striking difference in the materials may, however, very well account for the differences in the results. We agree with Bishop et al in the conclusion that the hemicelluloses and noncellulosic polysaccharides may be more important in cell wall development than generally recognized.

SUMMARY

The first 20 consecutive 100 μ sections of the onion root tip were analyzed for water soluble, 0.5% ammonium oxalate soluble, 4% NaOH soluble, 17.5% NaOH soluble, and 17.5% NaOH insoluble hexose, pentose, and hexuronic acids. The data were expressed per cell and per μ^2 surface area per cell.

Besides the rootcap and apical initials, this region of the root contains the following stages of cell development: radial enlargement, a transition stage where both radial enlargement and elongation occur, and elongation. On the basis of the present analysis the cell wall in these stages can be characterized. The rootcap cell is high in cellulose and soluble noncellulosic polysaccharides, fairly high in water soluble hexoses and pentoses and pectin, and low in protopectin, hemicellulose, and insoluble noncellulosic polysaccharides. The apical initials were low in all cell wall components although all were present, the pectic substances and cellulose being present in equal amounts. In radial enlargement the wall was still low in all components but the amounts of the major

components were approximately equal. During the transition stage all components increased per cell while on a per unit area basis the only component that showed an increase that could be correlated with the beginning of elongation was pectin. When radial enlargement ceased there was a marked increase in the amount of cellulose, pectin, soluble hexose noncellulosic polysaccharide, and insoluble noncellulosic polysaccharides per unit area. This increase can be correlated to morphological changes in the wall. During the stage of elongation all components increased in direct proportion to the increase in surface area of the cell.

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