The Presence of Ribonucleic Acid in the Cell Walls of Higher Plants

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A method for isolating extensively purified cell walls from higher plants is described; the preparations contain no detectable chloroplast or nuclear material and the protein content (2–5% of the dry wt. of walls) indicates that there is little contamination with cytoplasm. Incubation of purified cell walls with 0.3N-potassium hydroxide for 17 hr. at 37° liberates ribonucleotides, which can be purified by adsorption on charcoal and by ion-exchange chromatography. Ribonucleotides are also liberated by incubating the walls with ribonuclease, but not with deoxyribonuclease. The RNA content varies from 0.5 to 6mg./g. dry wt. of walls, depending on the nature and age of the tissue, and at 3mg./g. dry wt. of walls accounts for about 7% of the total RNA of the tissue. Less than 0.2% of the RNA of the walls is due to the presence of bacteria in the preparation. The base composition of the cell-wall RNA is identical with that of ribosomal RNA.

Endoplasmic reticulum, Golgi bodies and microtubules have all been implicated in the synthesis and organized deposition of the material of the plant cell wall on the basis of their association, in electron micrographs, with developing cell walls (Albersheim, 1965). Direct chemical evidence of a role for these organelles in the synthesis of wall materials is almost entirely lacking, except for the demonstration (Northcote & Pickett-Heaps, 1966) that pectic substances are formed in the Golgi apparatus and are transported to the cell wall in the Golgi vesicles. If the rough endoplasmic reticulum is part of the mechanism for laying down cell walls then it is possible that some RNA may be present in isolated cell walls. Ribosomes have not been identified in electron micrographs of cell walls, but Kivilaan, Beaman & Bandurski (1959) did detect trace amounts of RNA (1.5mg./g. dry wt.) in a preparation of the cell walls of *Zea mays* coleoptiles. They did not investigate the possibilities that this RNA could be due to the presence of bacteria or of residual cytoplasm. We have isolated highly purified preparations of cell walls from a number of plants and have found that RNA is invariably present; it is firmly bound into the wall and less than 0.2% of it is attributable to the presence of bacteria. A preliminary report of some of this work has been published (Phethean & Hallaway, 1965).

MATERIALS AND METHODS

Materials. Peas (*Pisum sativum* var. Kelvedon Wonder) and beans (*Vicia faba* var. The Sutton) were grown as

described by Bucke, Leech, Hallaway & Morton (1966); barley (*Hordeum vulgare* var. Impala and var. English Proctor) was grown on vermiculite in the dark or light at 15–18°. All other plant materials were obtained from the University Botanic Gardens.

Chemicals were obtained from the following sources: ribonuclease, deoxyribonuclease and yeast RNA from British Drug Houses Ltd. (Poole, Dorset); nucleoside 2'(3')-phosphates from C. F. Boehringer and Soehne G.m.b.H. (Mannheim, Germany) and Koch-Light Laboratories Ltd. (Colnbrook, Bucks.); sodium dodecyl sulphate from Kodak Ltd. (Kirkby, Lancs.).

Preparation of the cell walls. Cell walls were prepared by two methods.

Method A (standard procedure). The tissue was suspended in 67mM-potassium phosphate buffer, pH 6.0 (10ml./g. of tissue), and was macerated with an Ultra-Turrax homogenizer (Janke und Kunkel K.G.) at 4–5° for 6 min. in twelve 30sec. periods. Between each maceration the suspension was filtered on a sintered-glass Buchner funnel (no. 1 porosity) and the residue was resuspended in fresh buffer. After maceration the fine suspension was collected on a sintered-glass Buchner funnel (no. 1 porosity) and washed thoroughly with 67mM-phosphate buffer, pH 6.0 (1l. of buffer/10g. of tissue), then four times with boiling 80% (v/v) ethanol, three times with ice-cold 5% (w/v) trichloroacetic acid, three times with ethanol-diethyl ether (3:1, v/v) or until the filtrate was neutral, three times with 80% ethanol, once with 100% ethanol and finally three times with diethyl ether. About 120ml. of each solvent was used in each wash. The walls were sucked dry on the funnel and stored in a desiccator at 4°. The usual yield of dried walls was about 1g./50g. of tissue. For comparison the lipids and acid-soluble nucleotides were removed from some preparations by the method of Holdgate & Goodwin (1965): after being washed on the funnel with buffer, the walls were homogenized in 85% (w/v) methanol,

collected by filtration and then washed three times with 10% (w/v) trichloroacetic acid at 2°, twice with 90% (w/v) ethanol saturated with sodium acetate at 2°, once with 95% (w/v) ethanol at 2°, twice with ethanol-chloroform (3:1, v/v), twice with ethanol-diethyl ether (1:1, v/v) and once with diethyl ether.

Method B. A few preparations were made by the method of Kivilaan *et al.* (1959), in which the tissue is homogenized in glycerol and the wall fraction is separated by filtration on a bed of fine glass beads.

Release and purification of nucleotides from dried cell walls. The walls were incubated in 0.3N-KOH for 17 hr. at 37° (Schmidt & Tannhauser, 1945; Millikan & Pickett, 1963); this treatment hydrolyses RNA, but not DNA, to the constituent nucleotides. The alkaline extract was adjusted to pH 2 with 60% (w/v) HClO₄ and the precipitate of KClO₄ removed by filtration. Any DNA removed from the preparations by alkaline hydrolysis would be precipitated with the KClO₄. Acid-washed activated charcoal (prepared by washing British Drug Houses Ltd. charcoal with 3N-HCl and then with water until the washings were neutral and drying it overnight at 110°) was added to the supernatant (1 g. of charcoal/0.5 g. of dried walls) and the charcoal was collected by filtration on a bed of Celite (503) on a sintered-glass Buchner funnel (no. 3 porosity). The colourless filtrate contained some material absorbing at 260 m μ . The nucleotides were eluted from the charcoal by allowing small volumes of ethanol-aq. NH₃ (sp.gr. 0.88)-water (79:2:19, by vol.) to percolate through it. The yellow filtrate was poured immediately on to a column (1 cm. $\times$ 6 cm.) of Dowex 1 (X8; Cl⁻ form; 200-400 mesh), on to which the nucleotides were bound. The column was washed with water until the effluent was neutral, then with 0.10M-NH₄Cl until the effluent was again neutral and finally with water. The nucleotides were eluted from the column with HCl at pH 1 and were either determined immediately or reabsorbed on charcoal and eluted from it with ethanolic NH₃ as above. The ethanol and NH₃ were distilled off in a rotary evaporator and the concentrated aqueous solution of the ribonucleotides was stored at -15°.

Highly polymerized yeast RNA was completely hydrolysed to nucleotides in 17 hr. at 37° in 0.3N-KOH, and increasing the duration of hydrolysis to 40 hr. caused no loss of ribonucleotides. Increasing the duration of incubation of the cell walls with 0.3N-KOH to 40 hr. gave no increase in the yield of ribonucleotides. The recovery of standard mixtures of ribonucleotides from charcoal was over 95% and from Dowex 1 (X8; Cl⁻ form) was over 99%. Holdgate & Goodwin (1965), using a different charcoal (acid-washed Norit OL), obtained recoveries of nucleotides of only about 75%. Our procedure for release and purification of nucleotides is similar to the industrial process described by Munden, Crook & Donald (1963), though it was developed independently.

Extraction of total RNA. Barley coleoptiles were subjected to the procedure of Holdgate & Goodwin (1965) for extracting total tissue RNA.

Extraction of ribosomal RNA. RNA was extracted from barley coleoptiles by the method of Glitz & Dekker (1963). The RNA was precipitated from aqueous solution with 2 vol. of ethanol and the precipitate was further extracted according to the procedure of Hastings & Kirby (1966), to give ribosomal RNA free from DNA, carbohydrate and low-molecular-weight RNA.

Analytical methods. Calibration curves for the determination of ribonucleotides by measuring phosphate content, ribose content and E_{260} were prepared by using at first yeast RNA (not highly polymerized) and later an equimolar mixture of 2'(3')-AMP, 2'(3')-GMP, 2'(3')-CMP and 2'(3')-UMP as standard, and the analytical results are expressed in RNA equivalents. Ribonucleotide samples were analysed by at least two of these techniques, and usually by all three.

Determination of phosphate. Phosphate was determined by either the method of King (1932) or that of Allen (1940). Samples of nucleotide solutions were either hydrolysed in a final concentration of 40% (w/v) HClO₄ at 100° for 1 hr., which released phosphate from the purine nucleotides, or were ashed in 1N-HClO₄ for 4 hr. to liberate all the phosphate as inorganic phosphate.

Determination of ribose. The orcinol reaction (Dische, 1955), which measures the ribose from purine nucleotides, was used.

Measurement of E_{260} . In samples freed from protein E_{260} was linearly related to ribonucleotide concentration. The E_{260}/E_{230} ratio was used as a rough guide to the purity of the nucleotide preparations, values above 2 indicating that the level of contaminating u.v.-absorbing material is low (Millikan & Pickett, 1964). All spectra were recorded in a Unicam SP.800 Spectrophotometer.

Determination of DNA. The precipitate obtained when the alkaline hydrolysate was acidified with HClO₄ was treated according to the method of Osborne (1962), in which DNA in the well-washed precipitate is hydrolysed with 0.5N-HClO₄, excess of perchlorate is removed as KClO₄ and the deoxyribonucleotides are determined by the diphenylamine reaction (Burton, 1956) or by measuring E_{260} .

Determination of chlorophyll. Chlorophyll was determined by the method of Arnon (1949).

Assay of protein. The total nitrogen of cell-wall preparations was measured by the micro-Kjeldahl method with Markham's apparatus. The N content, adjusted for the contribution by RNA, was multiplied by 6.25 to give the protein content.

Thin-layer chromatography. Thin-layer chromatography of the ribonucleotides was carried out on cellulose (Whatman CC41) thin-layer plates prepared with a Shandon applicator. The developing solvents used were propan-2-ol-conc. HCl-water (85:22:18, by vol.) (Wyatt, 1951) and redistilled pyridine-water (2:1, v/v). Authentic nucleotides were run as markers and the air-dried plates were sprayed with fluorescein (0.01%, w/v, in ethanol) to facilitate detection of the nucleotide spots in u.v. light.

Measurement of base ratios. This was done by cation-exchange chromatography (Katz & Comb, 1963).

RESULTS

Purification of the ribonucleotides released from cell walls by 0.3N-potassium hydroxide. Fig. 1 shows the spectra of samples at three stages of the purification procedure. The alkaline extract had no clear extinction maximum at 260 m μ , but the eluate from Dowex had a sharp maximum, with the E_{260}/E_{230} ratio 2.9 showing that the nucleotide had been purified extensively (Millikan & Pickett, 1964).

However, some ribose- and phosphate-containing compounds other than RNA were still present, since the amount of RNA as determined by phosphate content and ribose content (5.8mg. and 5.3mg. respectively) was higher than that estimated by u.v. absorption (4.7mg.). Table 1 gives the analyses of samples from pea leaf cell walls at the same stages. Clearly more ribose and phosphate are liberated by 0.3N-potassium hydroxide than are accounted for by the E_{260} value, and the E_{260} value is decreased by 80% during purification. The equivalence of the ribonucleotide content of the eluate from Dowex, as measured by three independent assays, indicates that the content of interfering compounds has been greatly decreased during purification. The presence of 2'(3')-AMP, 2'(3')-GMP, 2'(3')-CMP and 2'(3')-UMP in the eluate from

Dowex was established by thin-layer chromatography with authentic ribonucleotides as markers.

Effect of ribonuclease on cell-wall RNA. When purified cell walls were incubated with pancreatic ribonuclease at pH 5.0 ribonucleotides were liberated, accounting for about one-third of the total cell-wall RNA (Table 2). Treatment with deoxyribonuclease liberated only a trace of u.v.-absorbing material under the same conditions. This confirmed that the ribonucleotides released from cell walls by 0.3N-potassium hydroxide came from RNA.

Bacterial contamination of the cell-wall preparations. Since subcellular fractions from plants are often heavily contaminated with bacteria (see Hallaway, 1968), the possibility that the RNA in the cell-wall preparations was due to the presence of bacteria was examined. Samples of preparations after the washing with buffer but before the extraction with hot ethanol were plated on nutrient agar. About 10^6 – 10^7 bacteria/g. dry wt. of walls were present (Table 3), but, even after multiplying these

Fig. 1. Absorption spectra of cell-wall extract at different stages of the purification. A 1g. sample of dried cell walls from 8-day-old barley coleoptiles was incubated with 0.3N-KOH. Curve 1, u.v. absorption of the alkaline extract (vol. 100ml.) diluted 1:5; curve 2, u.v. absorption of eluate from charcoal (vol. 80ml.) diluted 1:2 (E_{260}/E_{230} ratio 1.5); curve 3, u.v. absorption of eluate from Dowex (vol. 100ml.) (E_{260}/E_{230} ratio 2.9).

Table 2. *Effect of incubation with ribonuclease on the RNA content of cell walls*

Two samples (0.5g.) of cell walls from 7-day-old barley coleoptiles were incubated in 20ml. of 0.1M-sodium acetate-acetic acid buffer, pH 5.0, for 15hr. at 31° with or without 70μg. of ribonuclease. The cell walls were collected by filtration and washed and the ribonucleotide contents of the control and experimental combined filtrate and washings were determined directly. The cell walls were incubated with 0.3N-KOH and the released ribonucleotides were purified and determined.

Treatment	Total RNA (mg.)
RNA in 0.5g. of cell walls incubated in 0.1M-acetate buffer	2.42
RNA remaining in 0.5g. of cell walls incubated in 0.1M-acetate buffer+ribonuclease	1.60
RNA released by incubation in 0.1M-acetate buffer	0
RNA released by incubation in 0.1M-acetate buffer+ribonuclease	0.79

Table 1. *Purification of ribonucleotides from cell walls of pea leaves*

A 1g. sample of dried cell walls was incubated with 0.3N-KOH and the ribonucleotides were isolated as described in the Materials and Methods section. The E_{260} , ribose content and total phosphate content were assayed at different stages.

Stage of purification	RNA content (mg./dry wt. of walls)		
	Calc. from E_{260}	Calc. from phosphate content	Calc. from ribose content
Extract in 0.3N-KOH	17.6	17.9	48.2
Eluate from charcoal	4.9	6.7	17.0
pH 1 HCl eluate from Dowex (E_{260}/E_{230} 2.0)	3.4	3.7	4.0

Table 3. *RNA of bacterial origin in cell-wall preparations*

Cell walls were isolated from 8-day-old barley coleoptiles and 5-day-old bean embryos. Diluted samples of the preparations after the washing with buffer but before extraction with ethanol were plated out on Oxoid nutrient agar plates (3%, w/v) and incubated at 30° for 24 hr. The total number of colonies was multiplied by 10 to allow for bacteria that would not grow on this medium. The RNA content of the bacteria was calculated assuming the dry weight of 10^6 bacteria to be 2.5×10^{-7} g. and the RNA content of bacteria to be 20% of the dry wt. (McQuillen, 1965).

Source of cell wall	Dry wt. of cell wall (mg.)	RNA content of cell wall (mg.)	$10^{-7} \times$ total colonies	Total RNA (μ g.)	RNA of bacterial origin (% of total)
Barley coleoptile (English Proctor)	330.0	2.05	1	0.5	0.025
Barley coleoptile (Impala)	461.5	2.3	1	0.5	0.022
Bean embryo (The Sutton)	413.0	2.69	6	3.0	0.11

Table 4. *Effect of washing cell walls with deoxycholate, or saline, and of adding RNA on the RNA content of cell walls*

Samples of cell walls, after the washing in buffer but before extraction with hot ethanol, were homogenized in 67 mM-phosphate buffer, pH 6.0, containing 5% (w/v) sodium deoxycholate, or were washed with 1 M-NaCl and 0.5 M-NaHCO₃, or with 0.15% NaCl or 0.2 M-borate buffer, pH 8.5. Ribosomal RNA or yeast RNA was added to other samples, which were then washed by filtration. All samples were then taken through the remaining stages of the standard procedures for purifying the walls and for releasing and determining the ribonucleotides.

Treatment	RNA content (mg./g. dry wt. of walls)	
	Control	Treated
Homogenization in deoxycholate	2.75	2.61
Washing with 1 M-NaCl	2.64	2.62
	3.90	3.44
Washing with 0.15% NaCl	2.75	2.72
Washing with 0.2 M-borate buffer	2.87	2.28
Yeast RNA (1 mg.) added	2.75	2.82
Barley ribosomal RNA (20 mg.) added	2.89	2.87

values by 10 to allow for bacteria that failed to grow on the medium provided, the amount of RNA of bacterial origin in the cell wall was less than 0.2%.

Purity of the cell-wall preparations. The most probable source of the RNA in cell-wall preparations is in ribosomes attached to elements of endoplasmic reticulum passing through plasmodesmata. Other possible sources are cytoplasmic ribosomes or fragments of nuclei, chloroplasts or mitochondria in cytoplasm adhering to the walls. The repeated washing and filtration to which the walls are subjected during isolation by either method A or B are likely to wash out most of the subcellular particles, which will have been fragmented by the prolonged maceration in method A. Samples (0.25 g.) of dried walls contained no DNA, and examination of the preparation in the light-microscope revealed no intact cells. All chlorophyll was extracted by the organic solvents. Preparations made by either method A or B contained 2–5% of protein on a dry-

weight basis, depending on the tissue. This is at the lower end of the range (2.5–10% of protein on a dry-weight basis) of the protein contents found in cell walls isolated by a variety of techniques from a number of tissues; Lampion (1965) and Thompson & Preston (1967) consider most of this protein is protein bound into the cell wall and is not attributable to cytoplasmic contamination. [However, King & Bayley (1965) found traces of cytoplasm in electron micrographs of cell walls prepared by method B that contained 2.5–5% of protein.]

Neither maceration of the tissue in the presence of 0.5–5% (w/v) sodium deoxycholate to disrupt endoplasmic reticulum nor addition of yeast RNA or barley ribosomal RNA to preparations of barley coleoptiles during maceration altered the amount of RNA recovered in the cell-wall fraction (Table 4). This indicates it is unlikely that the RNA in the dried cell walls is derived from rough endoplasmic reticulum or from cytoplasmic RNA adsorbed on

Table 5. *Effect of the procedure for extracting lipids and acid-soluble nucleotides on the RNA content of barley coleoptile cell walls*

Barley coleoptiles of different ages were homogenized and washed with buffer as described in method A; samples of washed cell walls were extracted either by the procedure of Holdgate & Goodwin (1965) or by the standard procedure. Ribonucleotides were released from the walls, purified and then determined by measuring their E_{260} values.

Age of tissue (days)	RNA content (mg./g. dry wt. of walls)		E_{260}/E_{224} of ribonucleotides	
	Walls extracted by standard procedure	Walls extracted by Holdgate & Goodwin method	Walls extracted by standard procedure	Walls extracted by Holdgate & Goodwin method
5	5.23	5.21	2.39	2.27
6	5.15	5.17	2.11	2.09
7	4.71	4.73	2.00	2.03
8	4.22	4.20	1.75	1.74

Table 6. *Effect of extraction with phenol on the RNA content of cell walls*

(a) A preparation of cell walls from barley coleoptiles was divided into three portions. One portion was shaken with phenol saturated with water for 20 hr. at room temperature then the walls were filtered off and washed with ethanol, dried and weighed. The second portion was shaken for 3 hr. at room temperature with phenol saturated with water and containing 0.5% (w/v) sodium dodecyl sulphate (SDS). The walls were then filtered off, washed and dried and reweighed. The third portion was untreated. All three samples were then incubated with 0.3N-KOH, and the released ribonucleotides were purified and determined. (b) Three samples (i, ii and iii) of barley coleoptiles of different ages were homogenized (in two 30sec. bursts) in an Ultra-Turrax homogenizer and each sample was divided into two. One portion was taken through the standard procedures for washing and release and purification of nucleotides. The other portion was subjected to the standard extraction of lipids without further washing. Each sample of partially washed walls was then divided into two. One portion was not treated. The other portion was shaken with phenol saturated with water and containing 0.5% (w/v) sodium dodecyl sulphate, then was washed with ethanol and dried. Both the phenol-extracted and the untreated, partially washed walls were then incubated with 0.3N-KOH and the released nucleotides purified and determined as usual. Samples (i), (ii) and (iii) were shaken with phenol for 3 hr. at room temperature, for 48 hr. at 35° and for 7 days at 30° respectively.

Treatment	RNA content (mg./g. dry wt. of walls)		
	(i)	(ii)	(iii)
(a) Walls prepared by standard procedure			
No phenol extraction		4.41	
Extracted with phenol		4.69	
Extracted with phenol+SDS		4.71	
(b) Walls partially washed			
No phenol extraction	14.1	8.68	7.73
Extracted with phenol+SDS	12.1	7.08	7.93
RNA extracted by phenol+SDS	2.0	1.60	—
Walls prepared by standard procedure	5.57	4.69	4.52

them during homogenization. When the walls were freed from lipids and acid-soluble nucleotides by the method of Holdgate & Goodwin (1965) both the amount of RNA recovered in the walls and the $E_{\max.}/E_{\min.}$ ratio were identical with those in walls prepared by the standard procedure (Table 5). The Holdgate & Goodwin (1965) technique includes two washes with ethanol-chloroform (3:1, v/v), which would effectively disrupt lipoprotein membranes.

Hornby, Lilly & Crook (1966) succeeded in removing protein that was physically attached to

CM-cellulose by washing the cellulose successively with 1M-sodium chloride, 0.5M-sodium hydrogen carbonate and 1M-sodium chloride. Inclusion of this treatment after the washing with buffer decreased the RNA content by about 12% in one case, by less than 1% in another (Table 4). A washing of the walls with either 0.15% sodium chloride or 0.2M-sodium borate buffer, pH 8.5, had negligible effect on the RNA content, though these methods have been used to remove adsorbed protein (Glasziou, 1959; Arnold, 1966).

As phenol has been used frequently to extract RNA from ribosomes (Kirby, 1964) the effect of treating the walls with phenol with or without 0.5% (w/v) sodium dodecyl sulphate was determined to investigate the possibility that the RNA was due to the presence of adsorbed ribosomes. No RNA was removed by phenol, with or without sodium dodecyl sulphate, from cell walls that had been prepared in the standard way. If, however, walls were subjected to only two washes with buffer before extraction with lipid solvents in the usual way, then the RNA content of the dried walls was higher than in the thoroughly washed controls, but phenol extraction removed only part of the additional RNA, leaving cell walls that still contained more RNA than the controls (Table 6). Finally, homogenization of the tissue in buffers of different pH, which might be expected to alter the amount of any cytoplasm absorbed on the wall, did not alter the amount of RNA recovered in the dried cell walls (Table 7). It was concluded from all these experiments that most of the RNA detected in the cell-wall preparations was associated with the cell walls, and could not be attributed to contamination of the preparations by other RNA-containing subcellular fragments, but the possibility that a small amount is due to contamination cannot be completely excluded.

Table 7. *Effect of pH of homogenizing buffer on the RNA content of cell walls*

Cell walls were prepared from two different samples of shoots of pea seedlings by the standard procedure, with either 0.1M-tris-HCl or 0.1M-phosphate buffer as the homogenizing and washing medium.

Buffer pH	RNA content (mg./g. dry wt. of walls)	
	0.1M-Tris-HCl buffer	0.1M-KH ₂ PO ₄ - K ₂ HPO ₄ buffer
4	1.9	2.15
6	2.0	2.23
8	1.87	2.2

Base ratios of cell-wall RNA. The base composition of the RNA from the exhaustively cleaned cell walls of barley coleoptiles is identical with that of ribosomal RNA, and significantly different from that of total cell RNA (Table 8).

RNA contents in cell walls from a variety of tissues. RNA was present in the cell walls of all the tissues examined. The content on a dry-weight basis tended to be higher in younger tissues than in mature tissues. The content ranged from 0.4mg./g. dry wt. in tobacco pith to 6.2mg./g. dry wt. in 5-day-old barley coleoptiles (Table 9). The cell-wall RNA of barley coleoptiles at 3mg./g. dry wt. of walls accounted for 7.2% of the total RNA of the tissue.

Table 9. *RNA content of cell walls from a variety of plant tissues*

Cell walls were isolated by method A and the ribonucleotides released from them and determined as described in the Materials and Methods section.

Tissue	RNA content (mg./g. dry wt. of walls)
<i>Pisum sativum</i>	
Young leaf	2.2
Stem	1.2
Root	0.8
<i>Nicotiana tabacum</i>	
Young leaves	2.3-2.5
Shoot tip	3.5-3.6
<i>Tradescantia</i> sp.	
Shoot tip	2.95
<i>Saponaria officinalis</i>	
Shoot tip	4.45
<i>Mentha aquatica</i>	
Shoot tip	3.02
<i>Hordeum vulgare</i> coleoptile	
5 days old	6.2
9 days old	3.25
20 days old	0.79

Table 8. *Molar proportion of bases in total ribosomal and cell-wall RNA from barley coleoptiles*

Methods for isolating total and ribosomal RNA, for releasing the nucleotides and for determining the base ratios are described in the Materials and Methods section. The standard deviation for the method was determined on six replicate samples of ribosomal RNA.

Sample	Molar proportions (%)			
	Adenine	Cytosine	Guanine	Uracil
Total RNA	23.44	21.18	36.11	18.44
Ribosomal RNA	22.07	23.19	33.59	21.18
Cell-wall RNA	21.70	23.36	33.50	21.42
s.d. ...	0.63	1.14	0.43	0.62

DISCUSSION

The origin of the ribosomal RNA present in higher-plant cell walls is unknown. Rough endoplasmic reticulum is associated with both primary and secondary cell walls in electron micrographs (Albersheim, 1965), and it is possible that some endoplasmic reticulum with attached ribosomes is trapped in the cell wall as it is formed. In an electron micrograph published by Porter & Machado (1960) there is apparently an element of the reticulum within the cell walls lying parallel to the plasmalemma, an observation that supports this possibility.

Bouck & Galston (1967) have found that treatment of pea stems with 2,3,5-tri-iodobenzoic acid results in deposition of disorientated wall materials; within these masses vesicles of plasma membranes are trapped, and the vesicles may contain ribosomes. It is not known, however, how far the process of wall deposition in 2,3,5-tri-iodobenzoic acid-treated tissues resembles the normal process.

The experiments described here indicate that most, if not all, of the RNA of the preparations is held within the walls, and cannot be released from them except by hydrolysis either with alkali or with ribonuclease. The mechanism by which the RNA is held cannot be deduced from these observations, since both RNA bound by covalent linkages to wall protein or carbohydrate and RNA held physically within the cellulose network would be released by hydrolysis to mono- or oligo-nucleotides. The necessity of hydrolysing the RNA has also prevented study of its molecular size.

Investigations on the release of undegraded RNA by treating the walls with pectinase and cellulase, or with borate (Arnold, 1966), should aid in answering these questions.

The function, if any, of the RNA of cell walls has also to be investigated. Albersheim (1965) noted the possibility that some of the proteins of the cell wall might be synthesized *in situ*. However, protein synthesis requires an elaborate system of enzymes and nucleic acids that is unlikely to be present within the wall. Lamport (1965) concluded that the hydroxyproline-rich cell-wall protein is synthesized in the cytoplasm and is transferred to the wall, where it is firmly bound.

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