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Pectin Synthesis during the Wall Regeneration of Plasmolysed Tobacco Leaf Cells

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1. Discs of tobacco leaf lamina were floated on media containing D-[U-^{14}C]glucose. Glucose uptake was mainly through the cut edges, and diffusion through the veins and intercellular spaces was slow. 2. Radioactivity was detected in all polysaccharide fractions extracted from the discs, including those associated with the wall. 3. Plasmolysis in sorbitol or KCl decreased the incorporation of radioactive material into all fractions. 4. Incorporation of arabinose into pectins was increased or unaffected by plasmolysis, but the incorporations of other sugars were decreased. Removal of the lower epidermis did not affect this result. 5. Separate mechanisms for arabinan and polygalacturonorhamnan syntheses must exist, and these must differ in their responses to the physicochemical, structural and organizational changes that accompany plasmolysis.

The regeneration of cell walls by isolated plant protoplasts is abnormal in its early stages, owing to the diffusion of pectic material into the media. Eventually the network of cellulose fibrils becomes sufficiently dense to trap the matrix polysaccharides, and so a strong wall can develop (Prat & Roland, 1971; Burgess & Fleming, 1974; Hanke & Northcote, 1974). Gabriel (1970) has shown that algal protoplasts in liquid culture form a fibrillar network on their outer surfaces, but do not produce a normal wall. If pectin is added to the culture medium, a normal wall is formed.

Protoplast preparation invariably involves mild plasmolysis of the cells, and a convenient way to prevent loss of pectins from the protoplast surface is by leaving the old wall in place. Benbadis (1972) has shown the regeneration of a wall within the old wall of plasmolysed tobacco leaf mesophyll cells, under conditions in which isolated protoplasts do not form a wall. The nature of the pectins synthesized by soyabean callus protoplasts changed during the 40h after their isolation (Hanke & Northcote, 1974), and it would be interesting to know the cause of such changes and their possible effects on the nature of the wall being formed.

During plant cell growth the physical properties of the cell wall play a dominant role in influencing the nature of the growth, and the pectic polysaccharides are especially important during primary growth (Northcote, 1969). The physical characteristics of pectins can be related to such factors as the degree of esterification of the polygalacturonorhamnan backbone, to the proportion of rhamnose present in the backbone, and to the presence of neutral blocks of arabinan or arabinogalactan (Rees, 1967, 1969; Rees & Wight, 1969; Northcote, 1972).

There is strong evidence for the separate syntheses of arabinan and of polygalacturonorhamnan before their being joined together by a transglycosylase (Stoddart & Northcote, 1967). The arabinose content of the pectins of several tissues has been shown to change during differentiation (Thornber & Northcote, 1961; Northcote, 1969; Wright & Northcote, 1973), and Rubery & Northcote (1970) have demonstrated that auxin causes an increase in the incorporation of arabinose into the pectin of sycamore callus cells, probably by increasing the activity of the synthetase which transfers arabinose from UDP-arabinose to the pectic arabinogalactan.

In the present paper we report that plasmolysis of tobacco leaf mesophyll causes a decrease in overall pectin synthesis, but stimulates the incorporation of arabinose. The same, or similar, plasmolysing conditions have been used widely for protoplast preparation from many tissues, including tobacco leaf mesophyll (Cocking, 1961, 1972; Nagata & Takebe, 1970; Benbadis, 1971; Watts *et al.*, 1974) and consequently many effects of plasmolysis have been described. The effects of plasmolysis on polysaccharide synthesis are discussed in relation to the structural changes reported by other workers.

Methods and Materials

When necessary, all media and equipment were sterilized by autoclaving at 120°C for 30min. Chemicals were all of analytical grade, and glass-distilled water was used throughout.

Sample preparation

The lower, fully expanded leaves of *Nicotiana tabacum* (cultivar Xanthi) plants, between 100 and 130 days old, were used for these experiments. These were sterilized by washing in 96% (v/v) ethanol for 30s, followed by a brief rinse in sterile water before soaking for 3min in undiluted Milton sterilizing fluid (Richardson–Merrell Ltd., London W.1, U.K.), which was then washed away by repeated rinses in a total of about 500ml of sterile water. In some experiments the lower epidermis was stripped away by using fine forceps. Discs were cut from the lamina, avoiding all main veins, by using cork borers.

About 6cm^2 total area of discs were then floated, lower epidermis down, on 4 or 5ml of the medium of Nagata & Takebe (1971), modified so that no carbon source was present. Where plasmolysis was required, D-sorbitol was added, to give a 0.7M solution. The discs were then kept in the dark at 25°C, except for brief periods in daylight during sampling of the media. When discs were kept like this for 3 days, *myo*-inositol and 1% (w/v) sucrose were present in the media, but were replaced by carbon-free media about 3h before radioactive glucose was added.

Radioactive labelling

D-[U-^{14}C]Glucose (The Radiochemical Centre, Amersham, Bucks., U.K.), specific radioactivity 268mCi/mmol, was made up in the medium of Nagata & Takebe (1971) without any other carbon compound which could be metabolized by the tobacco cells (i.e. *myo*-inositol and sucrose were not included). Between 10 and 40μCi was added to each dish, in no more than 100μl of solution. After mixing, duplicate 10μl samples of medium were removed from each dish, and each was put on a strip (4cm×1cm) of Whatman no. 1 chromatography paper for subsequent determination of the radioactive glucose initially in the medium.

After 5h the discs were washed over a period of about 1h with several portions (6ml) of full medium, with or without 0.7M-sorbitol, containing 1% (w/v) unlabelled glucose in place of the radioactive glucose. In some cases the discs were left in this medium for up to 6h. After a quick rinse in water, each sample of discs was dropped into 10ml of boiling 96% ethanol, and was refluxed for 10min. This was repeated with another 10ml of 96% ethanol, and the discs were then desiccated over P_2O_5 under vacuum.

Polysaccharide extractions

Pectin and starch. The desiccated discs were treated with 10ml of 2% (w/v) sodium hexametaphosphate, adjusted to pH3.7 with 2M-HCl, at 99°C for 2h (Stoddart *et al.*, 1967). After filtration through Whatman no. 1 filter paper, the solution was adjusted to pH7 with 2M-NaOH, and NaCl was added to give a final concentration of about 0.05M; salivary amylase was added, and samples were kept at 37°C, with a few drops of toluene, for 4h to allow the digestion of starch (Olaitan & Northcote, 1962). Salts, maltose and any free sugars were removed by dialysis of the solutions against 4×2 litres of distilled water, over a period of at least 12h, with stirring. The non-diffusible material (pectin, plus some RNA) was evaporated to dryness at 40°C under reduced pressure.

Hemicellulose. After extraction of pectin and starch, hemicelluloses were removed from the residues by treatment, under N_2 at 18°C, with 5% (w/v) KOH for 2h, followed by 24% (w/v) KOH for a further 2h. The solutions were pooled and dialysed against water, after neutralization by acetic acid, and the non-diffusible material (hemicellulose) was evaporated to dryness at 40°C under reduced pressure. The solid remaining after alkaline extraction was α-cellulose.

Analysis of pectin

The pectins were hydrolysed by treatment with approx. 4% (w/w) H_2SO_4 at 120°C, 103kPa (15lb/in^2) for 1h. The hydrolysate was neutralized by the addition of $BaCO_3$, from which it could be decanted after centrifugation in a bench centrifuge. Remaining traces of Ba^{2+} were removed by pouring the solution through a short column of Amberlite IR-120 (H^+ form) cation-exchange resin (BDH Ltd., Poole, Dorset, U.K.). The solution was concentrated to low volume by rotary evaporation, and was then applied to Whatman no. 1 chromatography paper for electrophoresis in buffer at pH2 (80ml of acetic acid plus 20ml of formic acid, made up to 1 litre with water) under white spirit, with a potential gradient of 111V/cm, for 30min, with a mixture of non-radioactive sugars and amino acids as markers (Northcote & Pickett-Heaps, 1966).

After elution of the sugars from the region of the origin of the pH2 electrophoretogram, they were separated by descending chromatography on Whatman no. 1 paper, for 24h, in ethyl acetate–pyridine–water (8:2:1, by vol.) as the solvent. Galactose and glucose were resolved by re-chromatography in the same system for 72h. Ribose and rhamnose were resolved by re-chromatography on Whatman no. 1 paper, in butan-1-ol–ethanol–water (5:1:4, by vol.), the phase which was immiscible with water being used as solvent. Galacturonic acid and glucuronic acid were separated by electrophoresis on Whatman no. 1 paper, in pH3.5 buffer (40ml of pyridine, 400ml of acetic acid, 7560ml of water) under white spirit, by using a potential gradient of 89V/cm for 45min.

Counting of radioactivity

Chromatograms and electrophoretograms were dried and then cut into strips (4cm×1cm) which were assayed for radioactivity by scintillation counting in glass vials (5cm×1.3cm) containing 0.6ml of scintillant [8.75g of 2,5-diphenyloxazole plus 0.125g of

1,4-bis-(5-phenyloxazol-2-yl)benzene in 2.5 litres of sulphur-free toluene], by using a Nuclear–Chicago Unilux III counter (counting efficiency approx. 40% for paper strips). Solutions were assayed by taking 10 or 20μl samples and applying them to strips of chromatography paper, 4cm×1cm, which, when dry, were treated as above. All results have been corrected for background radioactivity, and sugar proportions are in terms of numbers of molecules, corrections having been made for the presence of only five carbon atoms in arabinose, ribose and xylose.

Radioautography

Leaf discs, which had been treated with boiling 96% ethanol to remove free sugars, were desiccated under vacuum, and were then left in contact with the emulsion of a Kodak AR 10 plate, in the dark at 4°C, for 13 days. A non-radioactive disc was included as a control. The plate was developed in Kodak D-163 developer and fixed in Kodafix solution.

Microscopy

Bright-field light-microscopy with an Ultraphot II microscope (Carl Zeiss) was used to see cytoplasmic streaming in pieces of leaf lamina, and to look for contaminating micro-organisms when sterility was required.

Callus culture

Pieces of sterile leaf were placed on the full medium of Nagata & Takebe (1971) with or without 0.7M-sorbitol, solidified by 1% (w/v) special Agar Noble (Difco Laboratories, Detroit, Mich., U.S.A.), in sterile plastic pots (Sterilin Ltd., Richmond, Surrey, U.K.), and were kept in the dark at 25°C.

Results

Sites of uptake and incorporation of radioactive material

Four dishes, each containing 5ml of medium without carbon source, were prepared, and leaf discs of different diameters were floated on these (Fig. 1). After 5h in the dark, at 25°C, 100μl of a solution of radioactive glucose in water, containing about 10μCi/100μl, was added to each dish, and duplicate 20μl

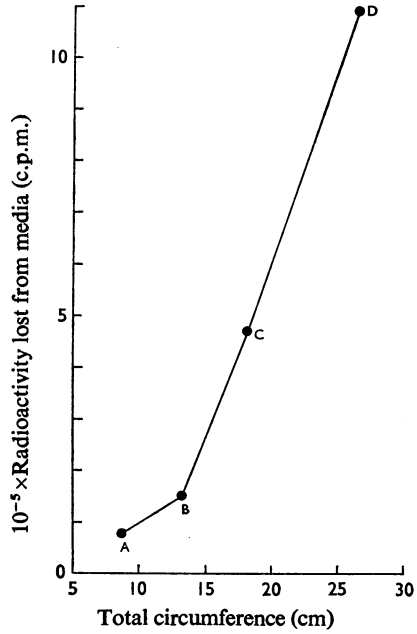


Fig. 1. *Relationship between radioactivity lost from media during* 6.8*h and the total circumference of the leaf discs*

Each sample of leaf discs was floated on 5ml of medium containing about 10μCi of D-[U-^{14}C]glucose for 6.8h, after 5h on non-radioactive medium.

Sample	Disc diam.	No. of discs	Total area	Total circumference
A	2.75cm	1	5.9cm^2	8.7cm
B	2.1cm	2	6.6cm^2	13.2cm
C	1.45cm	4	6.6cm^2	18.2cm
D	0.85cm	10	5.7cm^2	26.7cm

Table 1. *Uptake of* D-[*U*-^{14}C]*glucose by leaf discs from 5 ml of media during 5 h incubation after 3 days in non-radioactive media, and distribution of radioactivity in the fractions extracted from the discs*

Discs (approx. 1 cm diam.) were floated, in the dark, on medium (5 ml) with or without sorbitol before incubation with D-[U-^{14}C]glucose. Fractions were prepared from the discs as described in the text. The radioactivity in samples (20 μl) of the soluble material was counted and starch was determined from the loss in radioactivity after amylase treatment and dialysis. Cellulose was hydrolysed before counting.

Sample		$10^{-6}\times$Radioactivity in media (c.p.m)			Radioactivity in fractions (% of uptake)					
		Initial	Uptake	Uptake (%)	Ethanol extract	Starch	Pectin	Hemi-cellulose	α-Cellulose	Remainder (mainly respired)
Unplasmolysed	1	38.2	3.6	9.4	4.0	0.4	4.4	7.5	3.2	80.5
	2	38.2	4.5	11.8	3.7	0.7	6.1	8.4	4.7	76.4
Plasmolysed	1	38.1	2.3	6.0	5.6	0.5	1.9	4.4	2.6	85.0
(0.7 M-sorbitol)	2	38.1	2.2	5.8	3.6	0.4	1.6	4.6	1.2	88.6
	3	38.1	2.3	6.0	5.4	0.4	2.8	5.0	2.4	84.0

samples of media were removed for measurement of the initial concentrations of radioactive glucose. The sampling was repeated after a further 6.8 h in the dark, and the discs were then washed with water before being boiled in 96% ethanol before radioautography.

The net uptake of radioactive material from the media was seen to depend on the total circumference of the discs, and was unrelated to their total area (Fig. 1). Radioautography of the discs (Plate 1) showed that incorporation was highest at the cut edges of the discs, and also around damaged areas and along veins, with spots of activity possibly corresponding to the positions of stomatal pores.

Nature of incorporation

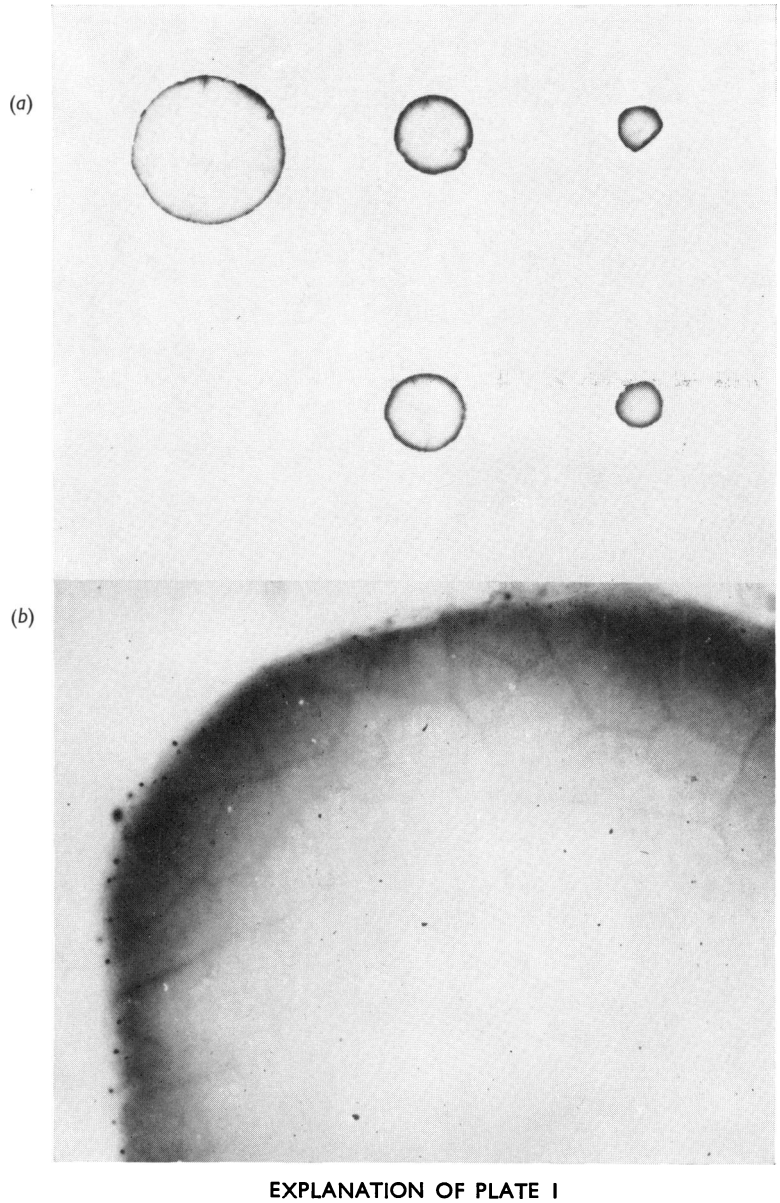
Ten dishes of full medium were prepared, half of which contained 0.7 M-sorbitol, and leaf discs (approx. 1 cm diam.) were floated on each, under sterile conditions throughout. After 3 days in the dark at 25°C, all the samples appeared to be free of microbial contamination, and microscopic examination of one plasmolysed and one unplasmolysed disc showed that both were very healthy, judged by the widespread, vigorous, cytoplasmic streaming, and by the general appearance of the cells. The full media of three plasmolysed and two unplasmolysed samples were replaced by 5 ml portions of media without carbon source (but still with 0.7 M-sorbitol for the plasmolysed discs) containing radioactive glucose (about 33 μCi/5 ml). After sampling of the media, the dishes were put in the dark at 25°C.

At the end of 5 h in the radioactive media, duplicate samples were taken from the media, which were then replaced by full media with or without 0.7 M-sorbitol, including 1% (w/v) non-radioactive sucrose. After soaking for 30 min in each of three batches of these media, the discs were transferred to boiling 96% ethanol, and the polysaccharides were extracted. Table 1 shows that radioactive material was present in every fraction prepared, and that plasmolysis decreased the incorporation into every fraction, although not all fractions were affected to the same extent.

In an experiment in which the 5 h incubation with radioactive glucose was followed by washing with non-radioactive media, the pectin fractions of two plasmolysed and two unplasmolysed samples were hydrolysed, and were shown to be free of radioactive amino acids (electrophoresis at pH 2). Chromatography of the material which moved only slightly on pH 2 electrophoresis, coinciding with sugar markers, allowed the proportions of radioactive sugars in the pectins to be measured. The results (Table 2) show that the pattern of incorporation is altered by plasmolysis, causing the proportion of radioactive arabinose to increase at the expense of uronic acids and other sugars. The absolute incorporation of every sugar into the pectins is decreased by plasmolysis after 3 days, but arabinose is not as severely affected as the uronic acids and other neutral sugars.

After the 5 h flotation on radioactive media, these discs had been placed on several changes of non-radioactive full media, with or without sorbitol, containing 1% sucrose and 1% glucose (both w/v), and they were left on 8 ml of these for 6 h. Only a small amount of radioactive material appeared in these media, in keeping with the washing-out of intercellular radioactive glucose. The pectin was still found to be radioactive, so the incorporation cannot be 'chased' out of the pectin under these conditions.

When another experiment was performed with only 2 h, instead of 3 days, between the disc preparation and the start of the 5 h incubation with radioactive glucose, the radioactive sugar compositions of the pectins were not the same as those from the corresponding samples in the 3-day experiment (Table 3). Nevertheless, plasmolysis still had the effect of decreasing the incorporations of all the sugars except



EXPLANATION OF PLATE 1

Radioautographs of leaf discs after incubation with medium containing D-[*U*-^{14}*C*]*glucose*

Discs were floated on non-radioactive medium for 5h, followed by 6.8h on radioactive medium. After extraction of free sugars with boiling 96% ethanol, the discs were desiccated. (*a*) Radioautograph after 13 days in contact with five radioactive discs. A non-radioactive disc was placed at the left end of the lower row. Magnification ×0.6. (*b*) Enlarged view of part of the autoradiograph (from the upper right disc). Radioactivity was most intense at the edge of the disc, but was not limited to damaged areas. The traces of small veins can be seen, and also several spots, possibly caused by radioactivity in stomatal pores. Magnification ×11.

arabinose, which actually appears to be incorporated more rapidly in freshly plasmolysed than in unplasmolysed tissue. Two samples were plasmolysed by using 0.35M-KCl in place of 0.7M-sorbitol. The results (Table 3) show that the effects on pectin synthesis of KCl as plasmolysing agent are very similar to those of sorbitol.

Discs were prepared from leaf lamina from which the lower epidermis had been stripped, and these were incubated with radioactive glucose for 5h, after flotation for 1h on non-radioactive media. The radioactive sugar compositions of the pectins from plasmolysed and unplasmolysed samples were measured (Table 4). Again the incorporation of all sugars, except arabinose, into the pectins is decreased by plasmolysis.

In all these experiments the samples of leaf discs were examined for cytoplasmic streaming at the ends of the incubations, to check that the cells were still healthy. A further check on the viability of the cells under these experimental conditions was made by placing small pieces of sterile leaf lamina on media solidified by agar, with the lower epidermis stripped away from some of the leaf pieces. Those pieces on medium without sorbitol rapidly gave rise to callus along their cut edges, or all over in the case of stripped pieces. Those on medium containing sorbitol did not

Table 2. *Radioactive-sugar composition of pectins extracted from leaf discs incubated with* D-[*U*-^{14}C]*glucose for 5h after 3 days in non-radioactive media*

Discs (approx. 1.8cm diam.) were floated on media (4ml) without any carbon source, with or without sorbitol, and were kept in dim light at 25°C before incubation in the dark with about 40μCi of D-[U-^{14}C]glucose per disc, after which they were washed with media containing non-radioactive sucrose and glucose (both 1%, w/v) for 6h. Pectin was extracted and analysed as described in the text.

	Radioactivity (%)				
	Unplasmolysed		Plasmolysed (0.7M-sorbitol)		
Sugar	Sample 1	Sample 2	Sample 1	Sample 2	Ratio of incorporations (plasmolysed/unplasmolysed)
Uronic acids (mainly galacturonic)	41.6	39.7	23.6	21.7	0.1
Galactose	16.6	15.4	21.8	23.3	0.3
Glucose	3.0	1.7	2.6	3.5	0.2
Mannose	0.8	1.8	1.8	2.0	0.3
Arabinose	11.0	13.4	28.3	27.9	0.5
Xylose	0.9	1.2	—	—	—
Rhamnose	3.6	2.8	5.4	4.0	0.3
Ribose (from RNA)	19.3	19.5	15.2	13.2	0.1
10^{-3}×Radioactivity in pectin (c.p.m.)	233	234	52	39	0.2

Table 3. *Radioactive-sugar compositions of pectins extracted from leaf discs incubated with* D-[*U*-^{14}C]*glucose for 5h after 2h in non-radioactive media*

Discs (approx. 0.9cm diam.) were floated on media (5ml) without any carbon source, with or without sorbitol or KCl, and were kept in the dark at 25°C before and during incubation with about 17μCi of D-[U-^{14}C]glucose per ten discs, after which they were washed with media containing non-radioactive glucose (1%, w/v) for 2h. Pectin was extracted from each sample of ten discs and was analysed as described in the text.

	Radioactivity (%)							
	Unplasmolysed		Plasmolysed with 0.7M-sorbitol		Ratio of incorporations (plasmolysed/ unplasmolysed)	Plasmolysed with 0.35M-KCl		Ratio of incorporations (plasmolysed/ unplasmolysed)
Sugar	Sample 1	Sample 2	Sample 1	Sample 2		Sample 1	Sample 2	
Uronic acids	38.5	22.3	17.4	21.5	0.4	28.9	22.5	0.3
Galactose+glucose	33.2	55.5	41.8	45.0	0.6	32.0	47.6	0.3
Arabinose	4.9	6.7	19.6	23.1	2.3	15.2	16.5	1.0
Ribose+rhamnose	12.4	12.3	9.0	7.4	0.4	10.3	9.4	0.3
10^{-3}×Radioactivity in pectin (c.p.m.)	38	40	25	25	0.6	16	12	0.4

Table 4. *Radioactive-sugar compositions of pectins extracted from leaf discs stripped of lower epidermis, incubated with* D-[*U*-^{14}C]*glucose for 5 h after 1 h in non-radioactive media*

The lower epidermis was stripped from a leaf lamina, and discs (approx. 2 cm diam.) of the remaining lamina were floated on media without any carbon source, with or without sorbitol, and were kept in daylight at 18°C before incubation in the dark with about 10 μCi of D-[U-^{14}C]glucose per disc, after which they were washed with media containing non-radioactive glucose (1%, w/v) for 1 h. Pectin was extracted and analysed as described in the text.

	Radioactivity (%)				
	Unplasmolysed		Plasmolysed (0.7M-sorbitol)		
Sugar	Sample 1	Sample 2	Sample 1	Sample 2	Ratio of incorporations (plasmolysed/unplasmolysed)
Uronic acids (mainly galacturonic)	28.3	34.2	32.8	31.8	0.3
Galactose	43.2	33.9	30.9	34.2	0.2
Glucose	18.1	14.4	9.2	13.4	0.2
Arabinose	2.5	3.7	13.3	12.8	1.2
Ribose+rhamnose	5.4	5.6	5.0	2.6	0.2
10^{-3} × Radioactivity in pectin (c.p.m.)	34	28	13	3	0.3

appear to grow; however, after 34 days they were transferred to medium without sorbitol and, after 2 weeks, all had produced a considerable amount of callus.

Discussion

The results shown in Fig. 1 and the radioautographic studies clearly indicate that the radioactive glucose entered the leaf discs through their cut edges and, to some extent, through stomatal pores. It then slowly diffused through the intercellular spaces in the parenchyma and along veins. Incorporation of radioactive material was not restricted to the immediate vicinity of cut cells, as the silver-grain density of the radioautographs was significant several millimetres in from the edge of each disc. Moreover, since the extent of incorporation into the polysaccharides was not drastically decreased by flotation on non-radioactive media for 3 days before incubation with radioactive glucose, the incorporation was not predominantly a wound response.

Longer incubations with radioactive glucose gave correspondingly higher incorporation of radioactively labelled material into polymers, which facilitated their analysis; however, short incubation periods were preferable, since the pattern of incorporation changed over a period of 3 days. If several small discs were used, rather than one large disc, perfectly adequate incorporations were obtained without the use of long incubations or high concentrations of radioactive glucose.

Plasmolysis had the effect of generally decreasing the incorporation of sugars into all polysaccharide fractions. This was not merely the result of some slight metabolism of sorbitol or of some impurities in the sorbitol, but was caused by placing the tissue in hyperosmotic solutions: 0.35M-KCl produced results similar to those of 0.7M-sorbitol.

Plasmolysis affected the incorporation of arabinose into pectic material in a different way from its effect on other sugars. In freshly plasmolysed tissues the incorporation of arabinose was stimulated, whereas those of other sugars were decreased. The leaf discs contained epidermal cells, including stomatal cells, as well as mesophyll cells. So the changes in the radioactive sugar composition of the pectins on plasmolysis could have occurred if two types of cells produced pectins which differed from each other in their arabinose content, and if plasmolysis caused an increase in pectin synthesis in those which produced an arabinose-rich pectin and a decrease in the pectin synthesis of the others. There was radioautographic evidence for incorporation at stomatal pores, although it is impossible to say if this was in the guard cells or in the mesophyll cells near to the pores. However, the plasmolysis still increased arabinose incorporation and decreased uronic acid incorporation in discs from which the lower epidermis and stomata had been removed. The upper epidermis was not only a very small proportion of the remaining tissue, in terms of cell numbers and weight, but was also farthest removed from the media. It would consequently have to be synthesizing pectin at a quite disproportionate rate to have any influence on the pectin composition of the whole tissue. Although the mesophyll cells were present as spongy and pallisade types, they have very similar functions in the leaf, and are of a common origin, so it is unlikely that they would produce greatly different pectins or respond in opposite ways to plasmolysis.

In many different tissues the composition of the

wall pectins changes during the development and differentiation of the constituent cells (Thornber & Northcote, 1961; Stoddart & Northcote, 1967; Bowles & Northcote, 1972; Wright & Northcote, 1974). These changes have been related to the changes in the physical properties of the wall which occur as the functions of the cell alter during its development (Rees, 1969; Northcote, 1972). The properties of the pectin are considerably modified by the presence of large blocks of neutral sugars (arabinans, galactans, arabinogalactans) joined to the polygalacturonorhamnan backbone, and the cells must therefore be able to exert separate controls over the syntheses of these two constituent parts of their pectin. Stoddart & Northcote (1967) have investigated such a system in sycamore callus. The present results are in keeping with the idea of separate synthetic mechanisms for arabinan and polygalacturonorhamnan syntheses, and it appears that these mechanisms respond differently to the stimulus of plasmolysis.

The process of plasmolysis involves several interdependent events, both chemical and structural. Hyperosmotic solutions cause the loss of water from the cells, and so the activities of enzymes may be altered by changes in the ionic strength of the cytoplasm; a decrease in water potential may occur if rapidly permeating osmotica are used. Greenway & Leahy (1970) have shown that in the roots of *Zea mays* seedlings slowly permeating osmotica, such as mannitol, are far more effective in decreasing respiration, glucose uptake and the synthesis of methanol-insoluble compounds, than are rapidly permeating osmotica, such as ethylene glycol. They conclude that these effects are the result of water loss or structural changes, or both, rather than of low water potentials, and these results may be applicable to our system, where only slowly permeating osmotica have been used. Tobacco mesophyll cells contain large vacuoles, and so it is probable that the homeostatic mechanisms of the cytoplasm will maintain a constant ionic strength in the cytoplasm, with all water loss being from the vacuole.

One very obvious structural consequence of plasmolysis is the decrease in total surface area of the plasmalemma. The general decrease in incorporation of radioactive material on plasmolysis could be related to a decrease in the rate of glucose uptake, which might be expected if the sites of glucose transport through the plasmalemma remain at a constant density over the membrane. However, this cannot explain the increase in arabinose incorporation which occurs on plasmolysis. Loss of contact between much of the cell wall and the plasmalemma may cause significant changes in the environments of enzymes bound to the outer surface of the plasmalemma, and this could result in disruption of cellulose synthesis, which is thought to take place on the outside of the plasmalemma of most plant cells (Frei & Preston, 1961; Northcote, 1969). Pectin synthesis, on the other hand, occurs inside the cell in the membrane system of the dictyosomes (Northcote, 1969, 1974), so changes in the structure of the plasmalemma cannot be directly responsible for changes in the nature of pectin synthesis. Prat (1972) has shown that plasmolysis causes the disappearance of dictyosomes from onion root cells, and this change would account for the decreased pectin synthesis that we have seen in tobacco cells.

Withers & Cocking (1972) have demonstrated the presence of plasmodesmata linking the mesophyll cells of tobacco leaves, and some of these links are broken by plasmolysis. Wright & Northcote (1973) have discussed the importance of cell–cell interactions in an organized tissue, where the nature of the wall of a cell must be related to its position in the tissue; they suggest that the regulation required may involve the exchange of material between adjacent cells, and that the plasmodesmata play an important part in this exchange. It is therefore possible that plasmolysis of the leaf tissue is effectively producing a conglomeration of separate cells from the previously integrated tissue, and that this loss of organization removes a constraint from the arabinan synthesis which more than offsets the general decrease in synthesis.

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