XXV. OBSERVATIONS ON THE NATURE, DISTRIBUTION AND DEVELOPMENT OF CERTAIN CELL-WALL CONSTITUENTS OF PLANTS.

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THE view has frequently been advanced that the pectic substances of plants, whatever their precise chemical nature, are not to be regarded as fixed and permanent constituents of the cell-wall, but rather as labile substances that may undergo further changes, possibly of very profound nature, leading to their almost complete disappearance from the older tissues. By such transformations of pectin, two classes of substances have been supposed to arise, (i) lignin, (ii) the hemicelluloses. It has in fact been suggested [O'Dwyer, 1926; 1928; 1932] that all three types of compound are interrelated, the passage from pectin to lignin taking place through certain hemicelluloses.

With regard to the former, Fuchs [1927] has advanced a theory of lignification in which he emphasises the fact that pectin and cellulose disappear as lignification sets in—a point previously made by von Fellenberg [1918]. Fuchs sees further a parallel to this development in the individual plant in the apparent development of a more and more modified cell-wall as one passes from the Algae to the higher plants; thus the algal cell-wall (he states) consists of pure cellulose, while in the mosses, incrusting substances (pectins) are present, lignins only appearing at a higher evolutionary level. As conceived by Fuchs, lignification involves the oxidation of the primitive cell-wall to "acid saccharides," and especially to pectin, with subsequent transformation of these, by unknown stages, into lignin. Ehrlich also [1930] considers that the acid polysaccharides form the chief cell-wall material, and that the formation of lignin represents a process involving enzymic dehydration and reduction of these bodies and affecting particularly the pectins. He claims that from flax stems (*i.e.* a partly lignified tissue) it is possible to isolate an "altered pectin," giving rise on hydrolysis to less galacturonic acid than normal pectin and containing also xylose residues in the molecule; with this is associated a brown substance having many of the properties of lignin, giving the characteristic colour tests of the latter, having almost the same empirical composition and containing a similar proportion of methoxyl [Ehrlich and Schubert, 1926; 1928]. This brown substance he regards as a substance derived from the pectin and intermediate between it and lignin.

It has been claimed also that microchemical staining tests show that there is a replacement of pectin by lignin in older tissues [Mehta, 1925], while Ritter [1925] states that 0.5 % ammonium oxalate, a recognised reagent for extraction of pectins, does not remove any pectic substance from basswood, red alder or pinewood (age of wood not stated), and he infers therefore the absence of pectin from woody tissues.

Finally the common possession of a high methoxyl content, both by soluble pectin and insoluble lignin (at least, as isolated), has been regarded as evidence

of their interrelation. Ehrlich [1930] also considers that the hydroxyl and acetyl groups of the lignin molecule represent those present in the parent polysaccharide.

No complete scheme representing the course of the supposed transformation of pectin into lignin seems to have been formulated—indeed, in view of the lack of understanding of the structure of lignin, none is possible as yet—but such changes if taking place must necessarily be of the most profound nature.

A relationship between the pectins and the bodies known as structural hemicelluloses is much more readily understood. The acid saccharides or polyuronides in general comprise three groups of structural units, *viz.* the anhydrides of hexoses, hexuronic acids and pentoses, combined in widely varying proportions. Chemically, the hexoses are considered to represent the parent substances forming the cell-wall material as originally laid down. By oxidation of their free primary alcoholic groups, after condensation of the sugars themselves to hexosans, there would be formed uronic anhydride units, and by subsequent decarboxylation of these, pentosan units—the whole process taking place actually within the cell-wall. Stereochemical considerations show that, by such a process, arabans would arise from galactans, while glucosans would pass through glucuronic anhydrides to xylans. It is evident that from a parent hexosan, even if based on a single sugar, a very wide variety of intermediate products could arise as the result of such transformations; it is thus not surprising that very different substances, all legitimately classed as hemicelluloses or acid saccharides, have been obtained. Among these substances the pectins represent a type of unusually highly oxidised hexosan, with but little of the unchanged parent hexose and little of the ultimate pentose constituent. Pectins thus are mainly distinguished from the hemicelluloses in that they contain some 70 % of uronic anhydride, whereas in the hemicelluloses the figure rarely exceeds 25 %.

Support has been found for the suggestion that the pectins are the precursors of the hemicelluloses in the frequently-repeated observation that the former predominate in young, fleshy or unlignified tissues, while in older lignified tissues, of lower moisture content, the position is reversed. From the more purely chemical side, the *in vitro* transformation of pectins into hemicellulose-like bodies has been reported by Candlin and Schryver [1928] and Linggood [1930] who used a method involving decarboxylation by heating under pressure; and by Norman and Norris [1930] who employed a process of mild oxidation.

It is evident therefore that, taken as a whole, the evidence for the existence of a relationship between the three groups of substances is considerable. All these suggestions, however, need some further examination. In the first place there seems to be no record of any investigation dealing with the distribution of pectin, hemicelluloses and lignin, and possibly other allied carbohydrates, in a given plant or tissue during its life-period, the statements concerning the relative amounts of these substances present in young or old material being based rather upon isolated observations on different types of material. Thus speculation has been founded upon data concerning the relative proportions of the different groups of constituents, rather than concerning the absolute amounts of the substances present in a plant from time to time. It is by no means impossible that observations which have been interpreted as indicating the transformation of one substance into another really indicate a changing course of metabolism in the plant, giving rise to increasing amounts of a new type of product, while formation of the original type ceases—that already present remaining intact, however. Preece [1930], for instance, records the presence of 0.4 % of pectin in boxwood, while O'Dwyer [1925] actually isolated a small amount of pectin from beechwood 80 years old. Although these relative proportions are low, the total weight

of pectin in the whole plant must have been considerable. If pectin were formed only in the early stages of growth, while subsequent thickening of the cell-wall involved the laying down of large amounts of non-pectin material, the low concentration of the pectin would be accounted for, without the necessity for postulating any extensive subsequent alteration of pectin.

Concerning the chemical work mentioned above, the products obtained by the various investigators certainly had a much lower uronic anhydride content than the original pectins, but still considerably higher than met with in any of the hemicelluloses extracted from plant materials, with the sole exception of O'Dwyer's beech hemicellulose B [1926] which contained some 60 % of uronic anhydride, a figure not approached in any other hemicellulose recorded. Again the yields of the products were generally very low (Candlin and Schryver [1928] report a yield of some 15 %), and in some cases may have consisted in part at least of hemicelluloses present as impurity in the original pectin. The nature of the sugar residues in these products is not recorded.

As regards the evidence based upon microchemical staining tests this must be accepted with caution. The extremely complex nature of the cell-wall, the constituents of which may be present in the form of an intricate mosaic, and not differentiated into sharply divided layers, and the admitted lack of specificity of many of the stains used render the evidence from this direction of doubtful value, at least when taken alone.

Thus it will be seen that the evidence for the transformation of pectin into hemicellulose and/or lignin *in vivo* is by no means conclusive, although perhaps, in the mass, impressive. It seemed therefore appropriate to attempt experiments which would yield more direct evidence of the occurrence or possibility of the transformations suggested. Since the ready lability of the pectins is assumed in all the theories outlined above, attention was in the first place directed to these substances, and experiments were made with the object of ascertaining whether such assumptions are justified. At the same time, the investigations furnished much useful information in other directions. For instance, a search through the literature revealed the paucity of direct information concerning the cell-wall constituents of the leaf, and certain particulars concerning these bodies came to light during the course of the work. Again, observations were made concerning the availability of some of the supposed cell-wall materials as reserve carbohydrate for purposes of metabolism.

Concerning the methods of investigation which seemed suitable, the most obvious consisted in following out the distribution of the various substances under consideration in a given plant during its full period of development. To carry out thoroughly such an investigation on a typical woody plant would obviously be a matter of considerable difficulty. On the other hand, it is possible to select a part of a plant which in the course of a season's growth will show an alteration in character somewhat resembling that met with in the development of woody tissues as a whole. Data of this type may be found in the rather isolated observations published by Schulze and Pfenniger [1910] regarding the hemicellulose content of the pods of garden peas and beans. In the case of beans particularly, the young pods are of fleshy nature, with a high water content, a state regarded as quite typical of tissues in which pectins predominate; while with maturity the water content decreases enormously, and the tissues become hardened and fibrous, resembling externally at least tissues in which much hemicellulose may be found. Staining tests also show that some lignin-like material has developed in the final stages of ripening. Schulze and Pfenniger found that the proportions of hemicellulose in the pods increased considerably with maturity.

No figures for pectin content are given by these authors—in fact, the methods for estimation of these substances had not then been established. Further, Schulze and Pfenniger estimated their hemicellulose by a method of difference, and therefore possibly included certain other substances under this heading. In the present work, the cell-wall constituents of runner bean pods and the pods of sweet peas were investigated.

Although as regards steadily diminishing water content these tissues resemble to some extent those parts of the plant undergoing lignification, yet they cannot be considered as typical lignified materials, and at the most the changes taking place during their ripening could only be expected to simulate the very early stages of lignification. It was therefore desirable to carry out a similar investigation dealing with the growth of a true woody tissue. Suitable material may be found in the hard-wooded shrubs. For instance, roses normally throw up, in the spring, strong growths from the base of the plant, whose early growth is extremely rapid, and which are very definitely of a fleshy nature. During the summer and autumn these shoots become “ripened,” i.e. lose their water slowly and undergo true lignification. Opportunity arose to conduct a (single) controlled experiment upon such growths, as described below.

Apart from such direct studies as those outlined above, artificial conditions may be set up which will tend to promote transformation of cell-wall materials where such is possible. To this end examination was made of detached leaves, (i) after a period of “starvation” in darkness, (ii) after allowing the leaves to undergo a process of slow drying in sunlight. The first-mentioned process was carried out by allowing the detached leaves to remain, with their petioles in water, in darkness for varying periods of time (7 days and upwards) until, in extreme cases, visible wilting had taken place. Under these circumstances, photosynthesis ceases, respiration continues, and there is an ample water supply. Thus it is to be expected that, after exhaustion (by respiration) of the readily available sugars, there might be some oxidation of reserve polysaccharides, either directly or after hydrolysis to simple sugars. On these lines, if decarboxylation of uronides is a process of mild oxidation, as suggested by Norman and Norris [1930], then prolonged starvation might possibly lead to such decarboxylation, the pectins with their high uronic anhydride content reflecting this process. Some early experiments on these lines were recorded by Deleano [1912] and by Ahrns [1924]. The former subjected leaves of *Vitis* to starvation for periods up to 21 days, but observed breakdown only of simple sugars and of starch; no figures for pectin were recorded. Ahrns, using much less drastic methods, obtained somewhat corresponding results.

The second process, that of slow drying, may be regarded as somewhat analogous to the conversion of grass into hay. In this case, both photosynthesis and respiration might be expected to continue during the earliest stages of the process, but would rapidly be terminated by depletion of water-supply. Thus respiration would probably be too short-lived to occasion any mobilisation of possible reserve carbohydrate; the diminished water supply would, in fact, be the most important factor altering the course of metabolism. As Spoehr and McDougal [1918] and McDougal *et al.* [1919] have shown, a depleted water supply induces a conversion of saccharides into anhydrides and leads to an accumulation of pentosans. The thickening of tissues (*e.g.* during lignification) is accompanied by a progressive decrease in their water content, a factor which may well influence the building up of the cell-wall or the modification of its constituents. If the pectins become converted with increasing age into hemicelluloses, and possibly lignin, then it might be expected that the same process

would be encouraged by the gradual withdrawal of water from the tissue by slow drying.

Apart from the above-discussed suggestion, regarding interconversion of cell-wall constituents, the statement has frequently been made that pectins are formed in tissues during periods of rapid growth, hemicelluloses arising with less rapid development. It seemed therefore appropriate to examine the distribution of these cell-wall constituents in plants of the same variety grown under conditions that would produce different rates of growth, *i.e.* plants grown normally and "forced." A brief experiment of this type is recorded.

The various lines of attack outlined would in the sum be expected to yield information of the kind sought and at the same time to furnish useful data in the other connections mentioned. The isolation of the different types of hemicellulose from leaves has not been recorded hitherto. Some of these substances were of necessity handled in the present research, and information regarding their chemical nature is recorded.

Apart from the difficulty of providing suitable material (*e.g.* for any prolonged series of investigations on the development of lignin in a typical woody plant), there are considerable difficulties of analysis to contend with. While for instance the calcium pectate method for determination of pectin may be regarded as satisfactory for fruits *etc.*, where extraction is easy, the physical nature of wood might make thorough extraction much more difficult, although pectins existed in the tissue. Again the methods of estimation of the hemicelluloses so far available are far from satisfactory. The term, as usually applied, and as used in the present work, indicates those constituents not extracted by water, but removed by extraction with 4 % hydroxide solution. (Their solubility after extraction frequently alters). Thus certain substances, whose chemical nature would warrant their inclusion in the group, would be excluded; among such would be the water-soluble galactans of wood [Schorger and Smith, 1916], of fruits [Widdowson, 1931] and the unextracted xylan found in close association with cellulose and called by Hawley and Norman [1932] "cellulosan."

EXPERIMENTAL.

1. General analytical methods.

Since the investigation involved a very large number of routine analyses, it will save repetition if the methods usually employed are summarised in the first place, reference to any special modifications being made in dealing with the individual experiments.

(a) *Preparation of material.* In the case of leaves, these were dried in a large steam-heated oven through which a current of air was drawn; drying was carried out as soon after picking the leaves, and as rapidly, as possible. The material was usually allowed to remain in the oven for 4–5 hours and was then transferred to stoppered glass jars, cooled, weighed and ground finely. Before using, the material was passed through a sieve, and the portion of 30–60 mesh size (per inch) taken for experiment; this was dried overnight in an oven before being weighed out for extraction.

In some cases (noted below) drying was carried out by dropping the material into six times its weight of boiling 95 % alcohol; after boiling for a sufficient time, the material was transferred to the steam-oven and the process completed as described.

(b) *Estimation of pectin.* Suitable samples (1–5 g.) of the dried material were extracted with 0.5 % ammonium oxalate solution in a water-bath at 95° for

6 hours; the residues were treated twice more in the same manner. In the extract, pectin was estimated as calcium pectate, following a preliminary precipitation with acidified alcohol, according to the method of Emmett and Carré [1926]. An amount of extract was taken sufficient to provide 0.02–0.04 g. of calcium pectate for weighing.

(c) *Estimation of hemicelluloses.* Hemicelluloses were estimated by a method similar to that of Preece [1930], and already described in detail by the present author [1934]. According to this method, the material, after removal of pectin, was treated once with dilute alcoholic sodium hydroxide (1 % NaOH in 50 % alcohol), and then exhaustively extracted with 4 % sodium hydroxide. The hemicellulose precipitated by acidification and addition of excess of alcohol was, after suitable washing, weighed directly on a Gooch crucible. Differentiation into “hemicellulose A” (pptd. on acidification only) and “hemicellulose B plus C” (requiring excess of alcohol for pptn.) was made where desirable. No separation of the fractions B and C was attempted. In all cases corrections for ash and for protein present were made, as suggested by Preece. Amounts of material were chosen to give 0.15–0.30 g. of hemicellulose for weighing.

(d) *Other estimations.* Lignin was estimated by the method of digestion with 72 % sulphuric acid, according to the details given by Dorée [1933]. Cellulose was estimated by the Cross and Bevan chlorination technique. Estimations of furfuraldehyde were made by the usual distillation method, the furfuraldehyde being weighed as phloroglucide, while uronic anhydride was determined where necessary by the method of Nanji *et al.* [1925].

In the cases of the pectin and hemicellulose estimations, duplicate estimations were in all cases performed upon aliquot parts of the extracts and, except in a few cases where lack of material prevented, duplicate extractions were carried out. The other estimations were normally carried out in duplicate.

2. *The distribution of cell-wall materials in pods of beans and sweet peas during development.*

The material used was obtained from the open garden during the summers of 1933 and 1934, no special cultivation being made. In the case of the bean pods, all were picked at the same time and graded according to size in five or six batches, this being regarded as sufficient guide to their stage of development. In the case of the sweet pea pods, dated labels were attached to the separate flowers as the petals faded, so that the actual age of the pods themselves, from the time of forming, was recorded.

Drying of the material was carried out by the alcohol method, after removal of the seeds, only the shells being used for experiment. The figures given in Table I for percentage content of pectin *etc.* are calculated on the dry weight of material after alcohol treatment; values are also given for actual weight of the constituents per 100 pods.

The results in all cases showed a steady development of both pectin and hemicellulose throughout the whole period of the experiment. The very young pods contained relatively less pectin than the older, in which both pectins and hemicelluloses remained at a fairly constant level; both types of substance seemed therefore to be produced throughout the whole period of development. It was of interest to note the very different ratios of pectin to hemicellulose in the two types of tissue; the pods of the sweet pea are relatively of a much drier nature than those of the bean, especially in their earlier stages, and the preponderance of hemicellulose therein is in accordance with common experience.

Table I. *Distribution of pectins etc. in pods of Phaseolus and Lathyrus.*

Series I. <i>Phaseolus</i> . Tall variety, grown at Chelsea, 1933.						
Picked 15. viii. 1933.						
Batch No.	...	...	...	...	...	...
Average length of pods when picked (cm.)	1	2	3	4	5	6
Wt. of alcohol-dry residues per 100 pods (g.)	2.5	5.0	7.5	10.0	15.0	21.0
Pectin, as calcium pectate, % of dry weight	3.82	7.55	17.9	27.7	69.0	99.2
Hemicellulose, % of dry wt.	9.8	9.5	10.3	14.1	—	15.0
Pectin, g. per 100 pods	(17.2*)		18.9	16.3	19.0	18.3
Hemicellulose, g. per 100 pods	0.37	0.72	1.68	3.52	—	14.9
	(0.98*)		3.35	4.50	13.1	18.2
Series II. <i>Phaseolus</i> . Dwarf variety, "Little Gem"; grown at Outwood, Surrey, 1934.						
Picked 19. x. 1934.						
Batch No.	...	...	...	...	...	...
Average length of pods (cm.)	1	2	3	4	5	
Wt. of alcohol-dry residues per 100 pods (g.)	4	7	9	11	13	
Pectin, as calcium pectate, % of dry wt.	4.55	7.84	29.8	35.6	71.0	
Hemicellulose, % of dry wt.	11.1	11.4	14.4	15.4	13.1	
Pectin, g. per 100 pods	14.0	13.8	13.1	16.4	17.3	
Hemicellulose, g. per 100 pods	0.50	0.89	4.29	5.49	9.3	
	0.64	1.07	3.90	5.84	12.3	
Series III. <i>Lathyrus</i> . Grown at Outwood, Surrey, 1933.						
Batch No.	...	...	...	...	...	...
Age when picked, (days)	1	2	3	4		
Wt. per 100 pods (g.)	3	8	16	23		
Pectin, as Ca pectate, % of dry wt.	2.2	7.4	12.1	23.2		
Hemicellulose, % dry wt.	2.63	3.75	4.26	4.31		
Pectin, g. per 100 pods	29.2†	26.0	27.7	22.25		
Hemicellulose, g. per 100 pods	0.06	0.28	0.52	0.99		
	0.64†	1.94	3.35	5.11		

* Average of batches 1 and 2; insufficient material for separate determinations.

† One extraction only.

The values for hemicellulose content of the bean pods are in fairly good agreement with those of Schulze and Pfenniger [1910].

This investigation was extended to include examination of fully grown bean pods in both the green and the brown (fully ripened) conditions, in order to see whether the pectin content was altered in the final stages of development. Micro-chemical examination of brown pods showed that they contained some amount of thickened tissue, giving the reactions of lignin; in this state the pods were almost completely "air-dry." Green and brown pods of the same size were chosen, assuming that equivalent development had taken place in each case.

In the 1934 series, the "brown" pods were of two types, (i) allowed to remain on the plants while ripening, and (ii) detached when fully grown, but still green, and allowed to become brown by remaining in sunlight under glass (*cf.* the process of slow drying described below, p. 207). After removal of the seeds, drying, sampling *etc.* were carried out as usual (see Table II).

From these figures it seemed evident that the pectins remained unaffected even at the end of tissue development; in the 1933 series there was even a small increase of pectin at the end of the period—possibly owing to the "green" pods having been gathered rather before they had fully developed. Complete ripening resulted in a small loss of total dry matter in each case and in a definite loss of hemicellulose. It may be pointed out that in the case of the sweet peas, batch no. 4, the pods were turning brown, and a relative decrease in hemicellulose was to be observed there also. According to Schulze and Pfenniger, part of the hemicellulose is withdrawn from the pods during the later stages of ripening and translocated to the seeds themselves. It is interesting to compare certain of the

Table II. *Distribution of pectins etc. in mature bean pods.*

Material	...	...	...	...	Outwood, 1933 (Dwarf type, short pods)		Chelsea, 1934 (Tall type, long pods)		
					— Green	On plant Brown	— Green	On plant Brown	Detached Brown
Ripened	...	...	...	...	—	On plant	—	On plant	Detached
Dry wt. of material insol. 80 % alc., per 100 pods (g.)					62.5	56.6	109.0	132.0*	92.0
Pectin, as Ca pectate, % of dry material					12.6	15.0	17.8	14.5*	21.2
Hemicellulose, % of dry material					14.7	13.2	21.4	12.5*	17.9
Lignin, % of dry material					13.2	14.9	8.5	9.4*	—
Pectin, g. per 100 pods					7.99	8.49	19.4	19.1	19.5
Hemicellulose, g. per 100 pods					9.17	7.60	21.1	16.5	18.0
Lignin, g. per 100 pods					8.25	8.43	9.26	12.4	—

* Material not dried by alcohol. (See also Table V, p. 206.)

figures for corresponding batches grown in the two seasons, 1933 and 1934, *viz.* Series II, batch 5, of Table I, with "Outwood, 1933, green" (Table II); and Series I, batch 6, Table I, with "Chelsea, 1934, green" (Table II). In each case, total growth is greater in the 1934 batch, probably owing to the greater rainfall during the growing season; and at the same time, the relative proportions of both pectins and hemicellulose are higher. This point is further commented upon below (p. 214).

Among the other figures given, attention may be called to the small increase in lignin in the 1933 series and the very definite increase in the pods ripened on the plant in 1934.

3. *Changes in the cell-wall constituents of wood of rose during lignification.*

A vigorous hybrid tea rose ("Leni Neuss") was pruned severely in April 1934; it was noted that within a few weeks two new shoots had appeared from a dormant bud on one stem, and were making rapid growth. Their rates of growth were similar, and a terminal bud appeared on each at the end of June. On July 4th the length of each was 53 cm., with 13 internodes; it seemed therefore that the two shoots might be regarded as identical at this stage. From each shoot the apical bud and side leaves were removed. One shoot was cut off, 5 cm. from its base (*i.e.* at the lowest node) on that date. The second shoot was allowed to remain on the plant, any fresh growths from axillary buds being removed immediately, until October 24th. Shoot no. I when removed was mainly of fleshy growth, although the lower part had already become considerably hardened. During the warm dry summer of 1934 the second shoot had become thoroughly "ripened," and was hard and woody throughout its length. A small increase in length (measured from the first node) had occurred, and the water content of the shoot was much less (Table III).

Each shoot on removal was sliced thinly and dried by the alcohol method, and the necessary analyses were performed, the results of which are shown in Table III. In the calculations of the percentage composition, allowance was made for the quantity of material removed in the alcoholic extracts. From shoot no. I only single extractions were possible; the cellulose determination was carried out on the residues from the extraction of hemicellulose.

From these results it appeared that formation of pectin continued throughout the whole period of development, the percentage showing however a decrease in the older shoot. In contrast, there was a much greater development of hemi-

celluloses, and still more so of lignin, the total amount of which in the shoot increased six times. The significance of these results is further considered below (pp. 212, 216).

Table III. *Distribution of cell-wall constituents in woody shoots of rose.*

	Shoot I	Shoot II
Age when cut (weeks)	12	28
Length (cm.) when cut	48	55
Fresh wt. when cut (g.)	27.0	27.5
Total dry wt. (g.)	4.06	10.51
Pectin, % dry wt.	5.03	4.77
Hemicellulose, % dry wt.	13.46	18.93
Cellulose (C and B), % dry wt.	24.0	26.3
Lignin, % dry wt.	7.94	18.36
Total pectin in shoot (g.)	0.204	0.49
Total hemicellulose in shoot (g.)	0.55	0.99
Total cellulose in shoot (g.)	0.97	2.76
Total lignin in shoot (g.)	0.33	1.93

4. *Changes in cell-wall constituents of leaves during "starvation".*¹

Three types of material were used in these experiments: (a) leaves of runner-bean, (b) leaves of vine, (c) laminae of maize. Picking was in all cases carried out in the early morning, when but little starch was present. The batches of leaves were divided into "control" and "experimental" lots, care being taken to obtain fair average samples in every case. The control leaves were dried immediately in the oven, after removal of their petioles; the experimental leaves were placed with their petioles in jars of water and kept in darkness throughout the experimental period. The water in the jars was changed daily, and on each occasion it was found desirable to remove a small portion of the tip of the petiole. At the end of the period, the petioles were removed, and the laminae dried under the same conditions as the controls. In the case of the maize plants, a somewhat different procedure was necessary; whole plants of as nearly the same size as possible, were chosen, and were usually found to be of almost the same fresh weight (within 5 g. on a weight of 75–100 g.). The lower ends of the stalks were sufficiently trimmed to equalise the weights of control and experimental batches (4 whole plants in each batch), and the complete plants were treated, *i.e.* starved, dried *etc.* It was noted that both vine and maize were far more resistant to the starvation process than was the bean; in one experiment maize plants remained for 45 days without any appreciable wilting. Further, in the case of maize, the loss of dry matter during starvation was comparatively little. Table IV summarises the results of these experiments.

It was evident from these figures that the pectins remained practically unaffected by the process of starvation. In only one case was any appreciable loss of pectin noticed; in one experiment on the bean leaves. The loss of 3.6 % of the total pectin, here recorded, was probably outside the limits of experimental error; and it is perhaps noteworthy that in this case, the only one in which loss of pectin could be suspected, there was an extreme loss of total material. (In the case of maize, 1933, the total pectin was so low that the differences in amount between the two batches are of doubtful significance.) The leaves used in this test were younger than in the other cases, and contained more water-soluble matter, a fact which may have accounted for the unusually great loss of material.

¹ Many of the analyses recorded in this section were carried out by Dr V. H. Chambers, who was responsible also for the preparation of the material (bean, 1932 batches, and vine).

Table IV. *Effect of starvation on cell-wall constituents of leaves.*All values (pectin *etc.*) are given as % on dry weight of original tissue.

Material	% loss of solids	Pectin (as Ca pectate)	% change of pectin	Hemicellulose "A"	% change	Hemicelluloses "B"- "C"	% change	Hemicellulose total	% loss
Bean, 1932, I									
Control	—	6.55	—	1.09	—	2.14	—	3.23*	—
Starved 7 days	31	6.31	-3.6	0.97	-11.0	1.32	-38	2.29*	-29
Bean, 1932, II									
Control	—	7.74	—	1.06	—	1.59	—	2.65*	—
Starved 7 days	17.5	7.92	+2.3	1.09	+2.8	1.23	-23	2.32*	-12
Bean, 1933									
Control	—	8.96	—	1.77	—	5.22	—	6.99*	—
Starved 10 days	21	9.03	+0.8	1.78	+0.6	3.22	-38	5.00*	-28
Vine, 1932									
Control	—	6.90	—	1.84	—	2.50	—	4.34*	—
Starved 21 days	27	6.75	-2.2	1.92	+4.3	0.84	-66	2.76*	-36
Maize, 1933									
Control	—	0.90	—	—	—	—	—	25.5	—
Starved 46 days	6	1.04	(+15)	—	—	—	—	24.5	- 4
Maize, 1934									
Control	—	1.39	—	10.9*	—	14.7	—	25.6	—
Starved 19 days	10	1.43	+2.8	11.4*	+4.6	11.9	-19	23.3	- 9

* Values calculated, by sum or difference; other values by direct estimation.

Turning to the figures for the hemicelluloses, these in all cases showed distinct losses during the starvation period, but, except in the case of the younger bean leaves, the loss was invariably confined to the water-soluble hemicelluloses (fractions "B" and "C"), where the loss in one case reached 66 % of the total fraction.

The nature of the individual hemicellulose fractions was examined in most cases (see p. 211) and determinations of pentosan and uronic anhydride were carried out. The insoluble hemicelluloses ("A") of the bean and vine leaves proved to be hexosans, no more than a trace of furfuraldehyde being evolved on boiling with 12 % HCl. On the other hand, the soluble ("B"- "C") fractions were found to contain units of pentosan and uronic anhydride. The only other recorded examples of hemicelluloses from leaf material were those of grass [Buston, 1934]. Of these, "A" was found to be composed almost entirely of xylan, giving a furfuraldehyde yield of 55 % approximately, while the "B" and "C" fractions contained a variety of pentosans, hexosans and uronic anhydrides. From maize, the "A" fraction again contained much pentosan, while the other fractions also closely resembled those of grass. Further details of these products are recorded in Table IX.

The soluble hemicelluloses isolated from the leaves after starvation were found to differ considerably from those of the control groups, as judged by furfuraldehyde determinations, the results of which are summarised in Table V. In general there was a very considerable loss of hexosan, together with some increase of uronic anhydride. Some net loss of pentosan also seemed to have occurred, so that the difference was perhaps not due simply to loss of hexose sugar by respiration. In Table V are also included some observations on the changes shown by the hemicelluloses of the bean pods during the final stages of ripening. Here again there is a striking decrease in the hexosan content, probably owing, as suggested by Schulze and Pfenniger, to translocation to the seeds, although possibly partly due also to respiration.

The stability of hemicellulose "A" in the bean and the vine leaves seemed curious. This substance, as stated above, was of hexosan type, so that it might

Table V. *Changes in constitution of hemicelluloses of leaves during starvation.*

Material	Sugars in hydrolysis mixture	Total furfuraldehyde (%)	Uronic anhydride (%)	Pentosan (%)	Hexosan (%)
Bean leaf, 1933, control					
Hemicellulose "A"	Galactose	0	Trace	0	>90.0
Hemicelluloses "B"- "C"	Galactose, arabinose, galacturonic acid	18.4	19.6	22.4	58.0
Bean leaf, 1933, starved					
Hemicellulose "A"	Not examined—assumed to be as above	0	0	0	>90.0
Hemicelluloses "B"- "C"		22.2	29.6	24.5	45.9
Bean leaf, 1932, I					
Control "B", "C"	be as above	18.7		Not determined	
Starved "B"- "C"		24.9		" "	
Maize, 1933, control					
Total hemicellulose	Xylose ? uronic acid	45.7	(<5)	Not determined	
Maize, 1933, starved		48.2	(5-7)	" "	
Maize, 1933, starved	?hexose				
Bean pod, 1933					
Green					
Total hemicellulose	Galactose, arabinose, galacturonic acid	21.0	11.2	30.1	58.7
Brown					
Total hemicellulose		33.9	12.6	50.8	36.6

Table V a. *Net amounts of pentosans, uronic anhydride and hexosan in the hemicellulose fractions of leaves, etc.*

Material	Net wt. of saccharide constituent (g. per 100 g. dry leaf)		
	Pentosan of hemicelluloses "B", "C"	Uronic anhydride of hemicelluloses "B", "C"	Hexosan of hemicelluloses "B", "C"
Bean leaf, 1933			
Control	1.17	1.02	3.03
Starved	0.79	0.95	1.48
	Net wt. of saccharide constituent (g. per 100 pods)		
	Pentosan in total hemicellulose	Uronic anhydride in total hemicellulose	Hexosan in total hemicellulose
Bean pod, 1933, fully-grown			
Green	2.75	1.03	5.39
Brown	3.86	0.96	2.78

have been expected to be open to metabolic change. It was found to yield on hydrolysis, galactose only—not a readily oxidised sugar. On the other hand, no hexose other than galactose could be detected in any of the hemicelluloses, even those which had undoubtedly undergone some change.

It was observed that the hemicelluloses isolated from the leaves of the bean and the vine (fraction "A"), but not of the maize, were always contaminated with considerable amounts of protein, as much as 30 % of the fraction in some cases being of protein nature (as judged by its nitrogen content). The contamination was much less in the case of the starved leaves, and the other hemicelluloses ("B"- "C") were free from protein. It will be recalled that Chibnall [1924] has shown the disappearance of protein from leaves subjected to the starvation process.

Some difficulty was experienced in dealing with the vine hemicelluloses. All the vine extracts were extremely dark in colour and inclined to be viscous, and attempts to estimate the "A" and "B"- "C" fractions separately by the ordinary method were unsuccessful. An estimate of the relative amount of each present was obtained by determining the furfuraldehyde yield of the whole fraction (mixed), and of a small portion of the "B"- "C" fraction isolated from a further batch of material; from these values, knowing that fraction "A" gave no furfuraldehyde, it was possible to calculate the relative amounts of the two types. The vine leaf is known to contain large quantities of organic acids, and the presence of these substances, or some of their degradation products, may have led to an alteration in the physical nature of the hemicelluloses, causing the difficulty. It may be mentioned further that although no difficulty was met with as a rule in identifying the sugars in the hydrolysis mixtures, with the vine products it is possible that some sugars have escaped identification, as the yields of the different derivatives were poor and crystallisation thereof always slow.

In the case of maize, the loss of material was apparently confined to the water-soluble hemicelluloses. Since however in this case the "A" fraction was mainly xylan, it was hardly to be expected that this would show any alteration. Some change in its physical condition had occurred, since it was much less readily precipitated on acidification of the extracts from the starved leaves.

*5. Changes in the cell-wall constituents of leaves
during a process of slow drying.*

Leaves of the bean and the vine were again used, the method of sampling being identical with that adopted in the starvation experiments. Controls were dried in the oven, while the experimental leaves were spread on paper in an ordinary garden frame, the lights of which were removed during fine weather but replaced at night. The leaves were allowed to dry in this manner for 7-10 days, after which they were shaken to remove dust, and drying was completed as usual. Estimations of pectin and of hemicelluloses were carried out as in the previous experiment (see Table VI). Again the pectin remained unchanged by the treatment. The hemicelluloses had undergone modification, but in a manner different from that met with in the starvation experiments. In each case there

Table VI. *Effect of slow drying on cell-wall constituents of leaves.*

Values expressed as % of dry wt. of original tissue.							
Material	Pectin (as Ca pectate)	Hemi- cellulose "A"	% change	Hemi- cellulose "B"- "C"	% change	Hemi- cellulose total	% change
Bean, 1932, I							
Control	6.55	1.09	—	2.14	—	3.23†	
Dried	6.50	2.47	+125	1.72	-20	4.19†	+30
Bean leaf, 1932, II							
Control	7.74	1.06	—	1.59	—	2.65†	
Dried	7.71	N.d.*	—	N.d.	—	3.57	+35
Bean leaf, 1933							
Control	N.d.	1.77	—	5.22	—	6.99†	
Dried	N.d.	2.75	+56	3.51	-33	6.26†	-10
Vine leaf, 1932							
Control	6.96	1.23	—	3.66	—	4.89†	
Dried	7.31	2.92	+137	2.92	-20	5.84†	+19

* Not determined.

† By addition.

was a considerable increase in the insoluble fraction ("A"). Its furfuraldehyde yield however remained *nil*, so that there was no evidence of any production of pentosan. Definite loss had occurred in the soluble hemicelluloses, and again an increase in the proportion of pentosan was observed (see Table VIa). Total

Table VIa. *Changes in constitution of hemicelluloses on drying of leaves.*

Bean leaf, 1932, I Hemicelluloses "B". "C"	Furfuraldehyde yield	Bean leaf, 1933 Hemicelluloses "B". "C"	Furfuraldehyde yield
	%		%
Control	18.7	Control	18.4
Dried	23.7	Dried	23.2

hemicellulose increased in some cases, and the increase was probably due to anhydride formation from hexoses, or simpler soluble hexosans, during the gradual withdrawal of water. Whether the disappearance of some of the soluble hemicelluloses was due to loss by respiration during the early stages of the treatment (which seems hardly likely) or to some alteration in their physical condition could not be judged.

No experiment was made with maize in this case, but examination was made of a sample of hay prepared from a grass previously examined. Some analyses of this grass have already been recorded [Buston, 1934]. The grass (cocksfoot) was fully grown when cut, but still green. The crop from one half of the plot was oven-dried as soon after cutting as possible; the remainder was cut at the same time, spread and raked in the usual manner until quite brown and then oven-dried. This process of hay-making was thus somewhat similar to the slow drying process carried out with the bean and vine leaves. In view of the economic importance of hay-making, it is strange that so little attention has been paid to the chemistry of the process. Fleischmann [1912] records the observation that "crude fibre" remains unchanged, and that there may be a small increase in total dry matter, but little further information seems to have been published. A rather more extended study was therefore made of the cocksfoot hay, the results of which are summarised in Table VII. Unfortunately the figures obtained could not be calculated to "dry weight of original grass," since the relative weights of grass and hay were not determined; values are given as % of dry material, grass or hay. Thus any loss or gain in dry weight by respiration or assimilation is unaccounted for; it may be pointed out, however, that the ash

Table VII. *Comparison of constitutions of grass and hay.*

All figures given as % dry weight of grass or hay.

	Ash	Water soluble material	Reducing substances as glucose	Reducing substances after hydrolysis	Pectin as Ca pectate	
Grass	8.16	21.9	3.54	5.57	1.97	
Hay	8.14	19.5	4.95	5.68	1.95	
	OMe in pectin* %	% total pectin as pectate	Total hemi-cellulose	Cross & Bevan cellulose	Xylan in cellulose	Lignin
Grass	2.8	76	18.31	33.2	8.5	16.85
Hay	1.3	92	18.47	33.2	N.d.	17.05

* Pectin as isolated from large-scale ammonium oxalate extracts.

contents of the two samples were almost identical, so that any considerable loss or gain of dry matter during conversion into hay was not indicated. The methods of analysis (*e.g.* for soluble carbohydrates *etc.*) were those given in the previous paper [1934]. The results showed that the cell-wall material remained practically unchanged, any variation having been confined to the simpler saccharides.

In hay-making, as carried out according to tradition, considerable variations may be expected in the product, depending mainly upon weather conditions during the harvest. It is evident that the rapid drying during hot weather would give a product very different from that obtained when hay-making is carried out under cool or wet conditions. In the present case not only was the weather extremely hot (summer 1933), but the grass crop itself was of unusually low moisture content when cut. Complete drying must therefore have been very rapid, leaving but little opportunity for alteration in the more stable cell-wall components.

6. *Relative distribution of pectins and hemicelluloses in the radish under varying conditions of growth*¹.

In order to examine the relative distribution of the pectins and hemicelluloses in plant tissues of the same type grown under conditions resulting in different rates of development, the radish was chosen on account of its short period of growth and ease of handling. The seed was sown in rows, one foot apart, and in 8–10 days the seedlings were thinned to 3 in. apart. After thinning, alternate rows in the bed were covered with cloches and the plants grown on for 25 days. At the end of this time the plants were carefully pulled and the roots washed free from soil. The “root” portion, including the swollen hypocotyl, was detached and treated separately from the shoot. A second sowing was made somewhat later in the year and grown in the same manner. It was found actually in each case that the total weight of the plants grown in the open was greater than that of the cloche-grown plants; possibly the latter suffered from lack of moisture, or the temperature inside the cloches was too high. The shoots were oven-dried and the usual estimations performed; the fleshy roots were dried by the alcohol method, and allowance was made for loss of alcohol-soluble material in calculating the results of the determinations of the pectin *etc.* (Table VIII). In considering these figures it is to be noted that the data for the batches cannot be compared directly, since the definitely higher temperature during the growth period of the second batch would no doubt have influenced the type of growth.

Broadly speaking, it seemed that a more rapid growth, as measured by the formation of dry matter over a definite period and taking the plant as a whole, favoured the formation of pectin in an increased ratio to hemicellulose, although in absolute quantities both classes of substance showed an increase with more rapid growth. In batch I the total dry solid formed was almost the same with both normal and cloche-grown plants, and little variation in the pectin/hemicellulose ratio was observed. A wide difference was found, however, in the growth rates of batch II, and here the pectin/hemicellulose ratio showed a distinct difference. The shoots, considered separately, showed a similar result. With the roots, considered alone, the greatest discrepancies were to be observed. The formation of polysaccharides in the root, however, differs from that in the leaf in that the process is dependent upon a supply of sugars translocated downward from the shoot, and therefore only represents the final stage of a process having its earlier stages in another part of the plant. Polysaccharide formation actually observed in the root will therefore be influenced by conditions obtaining

¹ The experimental work described in this section was largely carried out by Mr H. Thring.

Table VIII. *Rate of growth and formation of pectin etc. in the radish.*

Batch I, grown at Chelsea, May 1933; 49 plants in each series.

Batch II, grown at Chelsea, July 1933; 74 plants in each series.

	Dry wt. g.	Pectin % dry wt.	Total pectin formed g.	Hemi- cellulose % dry wt.	Total hemi- cellulose g.	Ratio pectin/ hemi- cellulose
<i>A. Whole plants.</i>						
Batch I, normal	32.7	10.30	3.37	5.56	1.82	1.85
„ cloche	28.8	11.25	3.24	6.08	1.75	1.85
Batch II, normal	67.2	11.84	7.96	5.80	3.90	2.04
„ cloche	28.6	9.58	2.74	6.01	1.72	1.60
<i>B. Shoots only.</i>						
Batch I, normal	24.5	10.65	2.60	5.97	1.46	1.78
„ cloche	24.5	11.47	2.81	6.37	1.56	1.80
Batch II, normal	47.4	13.56	6.43	6.12	2.89	2.22
„ cloche	25.1	9.60	2.41	5.86	1.47	1.64
<i>C. Roots only.</i>						
Batch I, normal	8.2	9.45	0.77	4.41	0.36	2.14
„ cloche	4.3	9.92	0.43	4.35	0.19	2.28
Batch II, normal	19.8	7.75	1.53	5.08	1.01	1.53
„ cloche	3.5	9.50	0.33	7.09	0.25	1.32

elsewhere. It seemed therefore best to consider the plants as a whole and to compare the relative formation of the cell-wall constituents in the complete plants. When this is done, the results give support to the suggestion that pectin is formed preferentially during more rapid growth.

7. Notes on the nature of the hemicelluloses of leaves etc.

Reference has already been made to the apparent hexose and pentose contents of certain of the hemicelluloses isolated in the course of these experiments. Since these leaf-hemicelluloses do not appear to have been described hitherto, it was thought desirable to identify their constituents where possible. The usual methods for identification of the sugars *etc.* in the hydrolysis mixtures, as given by Browne [1912], van der Haar [1920] and others in the literature, were employed, and the results are tabulated for the sake of brevity. An important difference, as mentioned above, was noted between the hemicellulose "A" of bean, vine *etc.* and that of grass and maize, the former apparently consisting mainly of galactose units, the latter mainly of xylose. Since it seemed possible that the difference was between typical dicotyledon leaves and monocotyledon leaves (which may be flattened stems rather than true leaves), other monocotyledon leaves, hyacinth and narcissus, were examined; these however gave hemicelluloses resembling those of the bean.

Apart from the leaf products, hemicelluloses from other non-lignified tissues were also examined. Schulze and Pfenniger had already reported that the products isolated from bean and pea pods consisted of galacto-arabans; determination of carbon dioxide in the products showed the presence of considerable amounts of uronic anhydride, identified as that of galacturonic acid.

It was noted, finally, that the hemicelluloses of the radish root (or swollen hypocotyl) were markedly different from those of the leaves, in that fraction "A" contained proportions of pentosan (or uronic anhydride).

The presence of an insoluble hexosan in the insoluble hemicellulose fraction was unexpected, the previously recorded insoluble hemicelluloses having consisted mainly of xylans, with but small amounts of uronic anhydride and no hexosan. It may be suggested that insolubility is characteristic of true sugar

anhydrides of high molecular weight (polysaccharides), whether hexosans or pentosans; such a grouping would include the starches and celluloses, as well as the xylans and the galactans here described. On the other hand, the soluble hemicelluloses may comprise those substances containing a sufficient proportion of uronic acid groups (*i.e.* the acid saccharides or polyuronides) and their solubility may be dependent on the presence of these (polar) carboxyl groups in the molecule. In this connection, it may be observed that the oxycelluloses, which undoubtedly contain glycuronic anhydride units [Kalb, 1927], are much less insoluble than true cellulose. If this conception holds, then the water-soluble ϵ -galactan of Schorger and Smith [1916] should be an acid saccharide, and the fact that Schorger found therein some 6 % of furfuraldehyde but was unable to isolate any pentose sugar may thus be explained. The insolubility of pectic acid, however, with its very high proportion of carboxyl groups, remains unexplained.

Table IX. *Constituents of leaf and other hemicelluloses.*

Tissue	Hemicellulose fraction	Sugars <i>etc.</i> present	Furfuraldehyde yield %	Uronic anhydride content %
Bean leaf	"A"	Galactose	0	0
"	"B"- "C"	Galactose, arabinose, galacturonic acid	18.4	19.6
Vine leaf	"A"	Galactose, ? hexose*	0	0
"	"B"- "C"	?	6.7	15.2
Radish leaf	"A"	Galactose	0	0
"	"B"- "C"	?	21.0	—
Maize blade	"A"	Xylose, uronic acid	45.7†	<5†
"	"B"- "C"	Xylose, arabinose, uronic acid hexose?		
Hyacinth	"A"	Galactose	0	0
Radish root	"A"	?	9.0	—
"	"B"- "C"	?	15.0	—
Bean pods	Mixed	Galactose, arabinose, galacturonic acid	21.0	11.2
Sweet pea pods	Mixed	?	33.0	14.0
Rose shoot	Mixed	?	32.5	16.4

* See p. 207.

† Average for mixed hemicelluloses as isolated.

Table X. *Pectic substances of leaves.*

Leaf	Type of leaf	Total pectin (as Ca pectate) % of dry leaf
Bean	Normal	6.5-8.9
Sycamore*	Normal	5.9
Vine	Acid leaf	6.9
Ivy*	Evergreen	7.2
Maize	Monocotyledon	1.0-1.7
Hyacinth	Monocotyledon	9.2
Narcissus	Monocotyledon	10.3
Cabbage	Inner (etiolated)	7.4

* Cf. figures given by Nanji and Norman [1928].

A summary of the observations on the constituents of these leaf and other hemicelluloses appears in Table IX, while in Table X are given some collected data, arrived at in the course of the present work, concerning the little known subject of the distribution of pectin in leaves.

DISCUSSION.

Dealing in the first place with the observations on pectins and hemicelluloses only, it is evident that the frequently-made statement, that the two classes are complementary, needs some modification, for in some tissues (*e.g.* bean pod) both groups are present in very large amount, the quantities of each being in fact very similar. In this tissue, development of the two classes has been shown to proceed throughout the whole of the period of growth, and it is only at the final stages of ripening that the proportion of the two becomes altered, some of the hemicellulose being lost. In non-lignified tissues in general it seems that pectins and hemicelluloses of the galacto-araban type may exist together in any proportions; for instance, comparing the figures given for pods of beans and sweet peas, the total combined content of the two groups is roughly 30–35 % in each tissue, but whereas the pectin/hemicellulose ratio in the bean is roughly 1/1, in the sweet pea it is approximately 1/9. In lignified tissue, on the other hand, pectin certainly is usually present in but small amounts, and this statement is true both for material of considerable age (timber) and for comparatively young growths (grass, maize), the hemicelluloses greatly predominating, according to the usual conception.

There seems however to be a further distinction between lignified and non-lignified tissues in the nature of their hemicelluloses. The analyses here recorded as well as others given in the literature show that lignified tissues are characterised by the presence of xylan and allied hexo-pentosans of the same series (glucosan—glycuronic anhydride—xylan) while in the non-lignified material galacto-arabans constitute the main bulk of the hemicelluloses. The information to be found in the literature is somewhat indefinite, but in the cases examined this generalisation is found to hold. The distinction is of course not rigid but is sufficiently evident. A brief summary of the available data is given in Table XI.

It would seem best therefore to look upon pectins as comprising a limited number of galacto-arabans of a rather special type, arising in certain circumstances during the course of the transformation of galactans into arabans. With regard to the possible conversion of pectins into bodies of the hemicellulose type, it is evident that from the former only substances of the araban type are likely to arise; this follows from the structural relationships of the various sugars entering into the composition of these substances, if the usually accepted view of the oxidation—decarboxylation mechanism already outlined (p. 197) be followed. Thus the typical major hemicellulose component of lignified tissues, xylan, would not be formed as the result of pectin decarboxylation. Suggestions that hemicelluloses in general may arise thus from pectin have therefore been based on the unfortunate lack of conciseness in the application of the term “hemicellulose,” which has been adopted to include a widely differing collection of substances, with often only certain physical properties in common.

Moreover, from the results of the experiments here recorded, it would seem that the conversion of pectin *in vivo* into other substances of whatever nature is by no means so easy as has been suggested. The persistence of the pectins during the period of development of tissues (bean pods, shoot of rose *etc.*) and under the artificial conditions of starvation and of slow drying points to a marked degree

Table XI. *Collected data concerning distribution of galacto-arabans and xylans in various tissues.*

Material	Authority	Galacto-arabans, including pectins, approx. % of tissue	Xylans, approx. %	Type of tissue
Bean leaf	(This work)	ca. 12	?	Non-lig.
Bean pod	(This work)	ca. 30	?	Non-lig.
Sweet pea	(This work)	ca. 30	?	?
Iceland Moss	Buston and Chambers [1933]	ca. 10	0	Non-lig.
Various fruits	—	15-35	—	Non-lig.
Grass	Buston [1934]	ca. 6	ca. 20	Lig.
Oat-straw	Norman [1929]	(Pectin, 1 %; galactose, arabinose and xylose all present; amounts not stated)		Lig.
Maize	(This work)	(Pectin 1 %)	ca. 20	Lig.
Maize-cobs	Preece [1930]	(Mainly xylan; some araban)		Lig.*
Wheat-bran	Norris and Preece [1930]	(Mainly xylan; some araban)		?
Apple wood	Tottingham <i>et al.</i> [1920]	(Mainly xylan; some galactan)		Lig.
Oak	O'Dwyer [1923]	ca. 3	ca. 15	Lig.
Beech	O'Dwyer [1926]	ca. 2	ca. 5	Lig.
Other woods	—	(amounts isolated on large scale) (Mainly xylan)		Lig.

* 15 % lignin [Schorger, 1926].

of stability; pectin transformation, where it occurs, must therefore normally be a process taking place during a prolonged period. Some interesting figures in this connection are quoted by Schorger [1926, p. 113], which, although the assumptions upon which they are based are open to question, tend to indicate again the comparatively great stability of the pectins.

As the pectic substances in the tissues are considered to be present within the cell-walls, partly as middle lamella substance and partly in definite combination with cellulose, they are to a large extent not readily exposed to the oxidising mechanism of the cell. Alternatively, the stability of the pectins may be due to the fact that, as Sucharipa [1924] has shown, in the case of *Citrus* fruits, the pectins in the cell-wall are esterified, through their carboxyl groups, with cellulose residues, and this association may render the carboxyl groups resistant to attack, although as Norman and Norris [1930] have shown free pectin molecules are extremely easily oxidised *in vitro*.

In contrast to the apparent stability of the pectins, some at least of the hemicelluloses seem open to chemical action, shown partly in intramolecular changes of the substances themselves (as evidenced by the alteration of their apparent pentosan content *etc.*) and partly in their disappearance from the tissues under certain conditions. This disappearance may be due to their mobilisation for purposes of oxidation (respiration) as in the bean leaves during starvation; or to their translocation to another part of the plant, as suggested by Schulze and Pfenniger in the case of bean and pea pods and again indicated in the present work. The changes in structure of the hemicelluloses have been shown to affect mainly the hexosan part of the molecule, although in some cases there also appeared to be a net loss of pentosan (Table Va). The fact that the hemicelluloses of maize, in contrast to those of bean and vine leaves, remained but little affected by the starvation process was perhaps to be expected in view of their much smaller content of hexosan; their chief component, xylan, being unaffected by the experimental conditions.

Returning to the consideration of the relation between pectins and the other galacto-arabans found in the plant tissues, it would be expected that, if all such compounds are the derivatives of a parent galactan (or series of galactans of different molecular weights), the pectins would be formed under conditions conducive to extreme oxidation, without subsequent decarboxylation. That these conditions may be produced during rapid growth in the presence of an abundant water supply (as already suggested by various authors) would seem to be indicated by the known relative distribution of the pectins and of the other galacto-arabans. Examples may be seen, in the present work, in the results of the experiments on radishes, and also in comparing the distribution of the types of compound in the pods of beans and sweet peas respectively—the former having a much more rapid growth rate, together with a greater water supply, than the latter.

More detailed suggestions as to the exact course and nature of the oxidation-decarboxylation process are not possible. Norman and Norris suggest that the latter process is actually a prolonged mild oxidation, but it is difficult to see, if such were the case, why decarboxylation should occur when but a small proportion of galactan units, in some types of molecule, have undergone the primary oxidation; one would rather expect this first type of oxidation to have reached its limit before the presumably slower type of oxidation, leading to decarboxylation, became evident.

With more knowledge of the chemistry of the hemicelluloses and polyuronides generally, it would seem that the time is approaching when the designation of hemicellulose fractions as "A," "B" *etc.* on a solubility basis should be modified. Members of the insoluble ("A") type hitherto described have proved to be mainly pentosans, with at most a low uronic anhydride content. From leaves, another type of substance, which on grounds of solubility would be classed as "hemicellulose A," has now been isolated; chemically, however, it is quite unlike the various hemicelluloses "A" previously recorded. A more satisfactory basis of classification, if it could be applied, would recognise the following types: (I) compounds predominantly hexosan; (II) intermediate compounds comprising hexosan-uronides, pentosan-uronides *etc.*; these might be of any degree of oxidation, from the but slightly oxidised compounds of the type isolated from lichens by Buston and Chambers [1933] to the more complex bodies in which a considerable amount of pentosan formation has occurred; and (III) compounds predominantly pentosan, such as the xylans of maize-cobs, straws *etc.* Further, examples of each of the three types could arise from glucose, galactose or other parent hexoses. Thus pectin would become a particular member of type II of the galactose series. Pectin itself seems to be widely regarded as an entity, the definite formula of Nanji *et al.* [1925] having found acceptance, although there is evidence that pectins from various sources may differ somewhat in their structure. It is agreed however that they are members of the galactose-uronide series and that their characteristic is a high state of oxidation, as evidenced by their high content of galacturonic anhydride; and perhaps it is best to regard them as comprising a sharply defined group of highly oxidised galacto-arabans.

In order to pass from a galactan (type I) to an araban (type III) it would not be necessary to pass through pectin as an intermediate—indeed, direct observations on these compounds *in vivo* suggests that passage into pentosans is actually more easy *via* intermediate compounds of a lower state of oxidation than *via* pectin. Thus the araban isolated by Ehrlich [1930] from beet-pulp is not necessarily derived from the pectin with which it is associated (see also Colin and Chaudin [1934]).

It would seem that the ability to form stable compounds with a very high uronic anhydride content such as the pectins is confined to the galactose series, for no glucose or mannose polyuronides have been isolated having a similar constitution. In the xylan series, in fact, the process seems to tend to pass rapidly through to the pentosan stage, glycuronic anhydrides being of comparatively rare occurrence among plant products and the glucosan-hemicelluloses still more so. The parent hexosan of the xylans is possibly cellulose itself, but this seems hardly likely, for xylan is frequently found in very close association with cellulose and is in fact almost certainly derived from it *via* glycuronic anhydride units; this xylan differs from the xylans isolated as "hemicelluloses" in its extreme resistance to extraction.

Turning to the question of lignin development, in view of the relative lack of knowledge of lignin itself, speculation must necessarily be more tentative. As has been pointed out, the evidence in favour of the theory that lignin arises by a transformation of pectin cannot be ignored. The microchemical evidence put forward by Ritter [1925], that pectin is absent from woody tissues in general and especially from the middle lamella, does not however prove that it is replaced by lignin. The actual isolation of pectins from wood does not altogether accord with the conclusions of Ritter.

It is admitted that, although pectin is remarkably stable in the tissues, over comparatively short periods, there is no reason to suppose that it is completely exempt from the general process of decarboxylation. Its final state is probably to be sought in the small amounts of arabans which are to be found in all woods, and which microchemical tests are not adequate to identify.

The observed possession by pectin and by lignin in common of hydroxyl and methoxyl groups is still less evidence of the supposed transformation, even though the latter constitute similar proportions of the molecule in the two cases. For the methoxyl of pectin is readily removed, *e.g.* by enzyme action, and yet it is supposed to persist in the molecule throughout the admittedly profound changes involved in lignification. In any event, it seems that the first step in pectin transformation consists of a demethylation (as indicated by the relative proportions of pectate-pectin in grass and hay respectively and by the methoxyl contents of the grass and hay pectins as isolated). Further, in his experiments on the extraction of pectin from lemon albedo, Sucharipa [1924] found that only a small amount of pectin was present in the fully esterified form (11.7 % OMe) and that only in the most easily extracted portion. The main bulk of the pectin was present in a form in which much of the methoxyl was apparently replaced by cellulose residues. It seems more likely that the methyl groups are split off from the pectins during the period of transformation and subsequently become combined with the unknown lignin precursor.

With regard to the arguments of Ehrlich [1930] based upon his observations on flax pectins, the reputed presence of xylose in the pectin molecule seems remarkable. No other example of a xylose-containing pectin has been reported elsewhere, so far as can be ascertained. It is true that flax pectin has been considered to be of abnormal type [Henderson, 1928] but the abnormality reported has suggested a simpler structure than usual, and not one more complex as would be required by the inclusion of xylose in the molecule. The question of the constitution of flax pectin has been examined also by Norris [1929] who failed to detect any abnormality. It would seem more likely that the xylan in Ehrlich's product is an accompanying impurity, extracted simultaneously from the tissues—xylans, as already pointed out, being typical of lignified material such as flax stems. The methods of separation of pectins, excluding that of conversion into

calcium pectate, which is not favoured by Ehrlich, are mainly of physical type (based on solubilities *etc.*), and some of the xylans might well have solubilities similar to that of pectin. The lignin-like body separated by Ehrlich may also be but physically associated with pectin, or, if actually in chemical combination, not necessarily derived from pectin. Ehrlich emphasises the facts of its solubility when present in association with the pectin and its loss of solubility on separation and sees in these evidence for its derivation from pectin. The soluble form of cellulose described by Bailey and Norris [1932] and others, which is present in seed mucilages, shows a similar phenomenon in losing its solubility on isolation. There is however no suggestion that it is derived from the other constituents of the mucilage—indeed since the latter consist mainly of pentosans, such an origin is evidently out of the question. Nor has the fact that pectin appears chemically combined with cellulose in protopectin [Sucharipa, 1924] been used as evidence that pectin is a cellulose derivative. Had Ehrlich's product been a link between pectin and lignin, it would probably have been met with elsewhere, in view of the large number of examinations of woods at all stages of growth that have been made.

The experiments recorded in the present paper do not suggest that pectin is the precursor of lignin in the tissues examined. In the case of the rose, considerable formation of lignin occurred with no corresponding loss of pectin, the amount of which indeed increased, though at a slower rate. The main change observed in the rose shoot consisted in a fairly rapid growth, not in the sense of an elongation of the shoot, but in the sense of formation of dry matter, that is, by laying down of fresh material on to the cell-wall structure already present. Of the materials so laid down, lignin accounted for 1.6 g., while the pectin content rose by 0.3 g. It may be argued that the pectin was in a state of continual formation and transformation, while maintaining a fairly constant balance. This would involve a marked lability on the part of the pectin molecule, such as does not appear from the experiments on detached leaves.

It may be pointed out that lignification must be a more rapid process, at least in its first stages, than is sometimes realised; for Mehta [1925] has shown the presence of lignin in quite young pine needles, and there is evidence of its presence in grasses at an early stage. On the other hand, pectin breakdown or alteration, though not excluded, is slow.

While therefore the evidence is not satisfactory in support of the view that pectin is the precursor of lignin, it is evident that the latter must be derived finally from some sugar, and probably through a polysaccharide. The experiments of Hawley and Wiertelak [1931], although possibly of a rather artificial type, suggested that the source of the lignin was to be found in certain pentosans. The amount of pectin and galacto-arabans present in their original ashwood was almost certainly insufficient to account for the amount of "lignin" they obtained.

In view of the predominance of xylans in lignified tissues, these, or closely related bodies, seem more likely to be the precursors of lignin. It has been pointed out that xylan formation seems to take place more rapidly than the corresponding change to araban; this suggests a degree of lability in the precursors of xylan which may bear upon the comparatively rapid rate (in some cases, at least) of lignification. The view that xylan is the main precursor of lignin has been advanced by Klason [1930]. However, as no attempt has been made in the present work to examine these suggestions, it is not appropriate to discuss them here.

Summarising the views here expressed, the following suggestions may be put forward.

- (i) If glucose be regarded as the primary sugar formed in the plant, part of

this becomes converted into galactose, the two sugars thus becoming severally the precursors of the glucosan—xylan and galactan—araban series of polysaccharides. Since the latter are more characteristic of non-lignified tissues, it would seem that there is some connection between formation of galactose or galactans and development of a non-lignified structure. Nevertheless, increasing age, or an alteration in the conditions of the tissue, may result in decreasing galactose formation, together with increasing predominance of xylan production.

(ii) Of the galactan series, the pectins arise under rather exceptional conditions, involving rapid growth, combined with a copious water supply; while the other galacto-arabans, of a lower degree of oxidation, are formed when metabolism is at a different, lower level. Finally, all these bodies may pass into true arabans, but the pectins, either by reason of some innate stability of the molecule (alone or in combination with cellulose), or by reason of their location in the cell-wall, are only slowly transformed. In this limited sense, pectins are the precursors of hemicelluloses, but only of a small range of these substances, *viz.* the arabans.

(iii) The glucosan-xylan series is not apparently typical of rapidly growing tissues, or of tissues with a high water content. The intermediate members of the series are to be found in a less highly oxidised state than the corresponding galactans and much more readily pass into the final products of decarboxylation, the xylans.

(iv) Lignin does not arise from pectin, but more probably from some carbohydrate related to the glucosan-xylan series, with members of which series it is usually associated in the plant.

SUMMARY.

1. The distribution of pectin and of the hemicelluloses in developing pods of beans and sweet peas has been investigated. The proportion of each remains almost constant throughout the greater part of the growth period, but there is a falling off in the content of the latter towards the close of the period. This is possibly due to a translocation of some of the sugar constituents to the seeds.

2. Similar experiments have been made on the distribution of these and other cell-wall constituents during the development of a woody stem (rose). Here, the increase in lignin was found to run parallel with an increase in hemicellulose, but there was no loss of pectin. It is considered that pectin does not constitute the source of lignin during the primary lignification.

3. Detached leaves, submitted to a process of starvation, or of slow drying, showed no appreciable change in their pectin content, but the amount of hemicellulose showed a significant variation; hemicelluloses are therefore considered to be more labile than the pectins. It is possible that they may be in part available for respiration under certain circumstances. The main change during starvation was shown to consist in loss of hexose units, together with some decrease in pentosan.

4. Rapid growth of the plant is conducive to the formation of galacto-arabans, but especially of pectins.

5. The nature of the hemicelluloses of certain leaves has been examined. The "A" type has been shown to differ from those of lignified tissues in consisting almost entirely of hexosan units based on galactose. A modified classification of these bodies is tentatively suggested.

6. The bearing of these experiments on the possible transformation of pectins into hemicelluloses and lignin in the plant, and on certain theories of lignification, is discussed.

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