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(Examination of the Cambium of Trees)
Project Reports (1)

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PROJECT NO. 1702
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REPORT NO. 10
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SIGNED E. O. Dillingham
E. O. Dillingham

CAMBIUM CHEMISTRY

Further Investigation of Microbiological Assay Method for Free Amino Acid Determinations

This study was an extension of the work initiated by Dr. G. E. Wessman on the quantitative and qualitative determination of amino acids in the cambium and associated tissues. In the preliminary work (see project report 1702 dated July 20, 1954) Wessman established the amino acid requirements of the assay organism, Leuconostoc mesenteroides P-60, and set up a standard curve based on twelve concentrations in the range of 0 to 50 γ g./ml. The standard curves thus obtained were quite satisfactory. The unknowns were run at four dilutions: 1/20, 1/40, 1/200 and 1/400. In the work described subsequently, it was found that these dilutions did not satisfactorily define the growth response curve for a quantitative estimation of free amino acids in the aspen cambium extracts studied.

METHODS

The cambium extracts were dried over calcium chloride under reduced pressure and each dissolved in a minimum amount of distilled water with mild warming and agitation on the day that the assay was performed. The assay was set up as follows: The aqueous solution of the unknown was diluted in 18 assay tubes to the following relative concentrations: none, .05, .10, .20, .30, .40, .50, .60, .70, .80, .90, 1.0 (these from the original solution), .20, .10, .05 (these from a 1::10

dilution of the original solution), .02, .01 and .001 (these from a 1.:100 dilution of the original solution). Two ml. of appropriate assay medium was added to each tube, bringing the final volume to 4 ml. The tubes were capped with aluminum caps, autoclaved, cooled, and inoculated with one drop of a washed suspension of cells of L. mesenteroides P-60. The tubes were allowed to incubate at 37°C. for 36 hours at which time they were titrated to neutrality with 0.02N NaOH using bromthymol blue as an indicator. The standard was run in triplicate; the unknown was run but once.

RESULTS

It was found that the growth response of L. mesenteroides P-60 was not uniform in the presence of cambium extract. Several deviations from the theoretical response curve were observed. First, as shown in Figure 1, the lactic acid produced should be linearly related to dilution above a dilution of .05 and should drop away rapidly in a curvilinear manner to the base line or intercept (representing the inherent acid of the assay medium) below a dilution of .05. In only one instance (a lysine assay of T-160-P-male) did the assay results approximate such a theoretical curve. All other assays gave results which would approach the values expected if the linear portion of the curve were extrapolated to the y axis. Seldom was the slope of the experimental curve sufficiently great to be statistically significant and when significant was of an entirely different order of response than that found in the standard curves. Compare figures 2 through 7. A second major deviation was found in the 1::10 and 1::100

dilution points. It will be noted that the .10, .05, and .025 points (relative concentration) were duplicated except that the initial material was diluted 1::10 as an intermediate step in reaching the final dilutions for one set. Although the final concentration of the cambium extract was identical in each set, it can be seen in Figures 3, 5 and 7 that the results of the "two step" dilution deviate drastically from the results obtained when the material was taken to the final concentration in one step. The nature of this deviation seemed to be determined by the individual sample and no general response was evident over-all except that the points obtained by the "two step" dilution were generally higher than those obtained through direct dilution. In each case an inhibition seems to have been relieved by the additional step. It is possible that the intermediate dilution facilitated the precipitation or complexing of toxic compounds, allowing the organism to make a more nearly ideal growth response. The results of "two step" dilution shown in Figure 5 appears to approximate the type of curve expected theoretically, in that range of concentration. The tyrosine assay shown in Figure 3 indicated that the "two step" dilution gave a very decided relief of inhibition. The proline assay, Figure 7, indicated an inverse relationship between growth response and dilution of the cambium extract in addition to whatever effect the "two step" dilution procedure may have had. This inverse relationship was evident in several other assays and tends to support the hypothesis that dilution is in some way removing the inhibitory properties of the cambium extract.

Also of note is the nature of the direct dilution curve for the tyrosine assay shown in Figure 5. The very large growth response shown in the latter part of this curve can not be accounted for in terms of relief of inhibition by dilution.

DISCUSSION

In terms of ml. of lactic acid produced by the organism, the standard curves are best suited for assays involving the production of about 2.5 to 14.0 ml. of 0.02N acid. Hardly any of the assays produced as much as three ml. This fact alone discourages the use of the method for quantitative work. The irratic response of the organism to the crude cambium extract makes the method completely untenable. Further purification of the free amino acids should in all likelihood make them ameanable to this type of quantitation.

CONCLUSIONS

The microbiological assay method using L. mesenteroides P-60 is not a satisfactory method of qualitative and quantitative determination of free amino acids in crude cambial extracts of aspen.

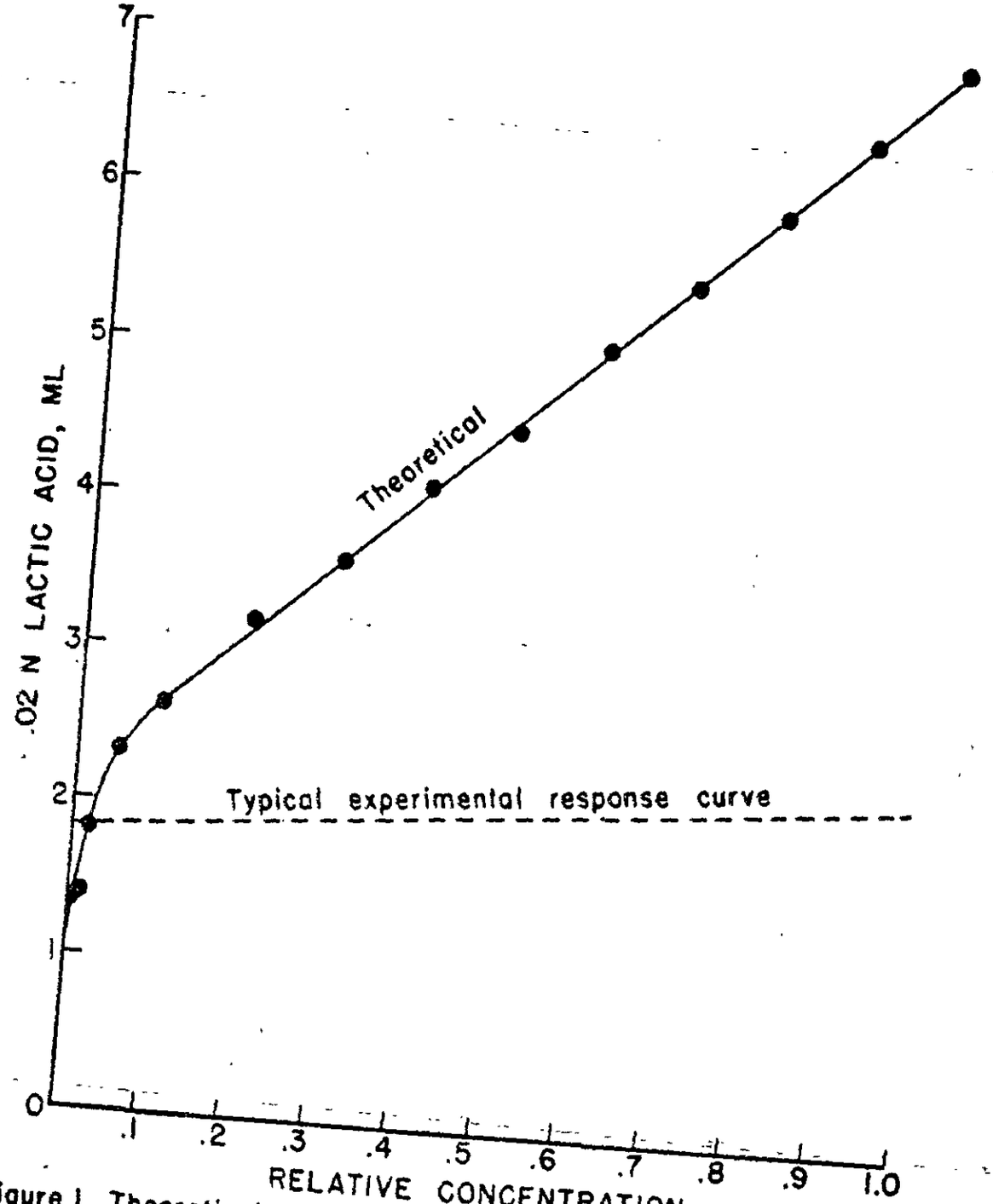

Figure I. Theoretical Growth Response Curve of L. Mesenteroides Plotted From a Phenylalanine Standard Curve and the Typical Experimental Response Curve Obtained with Crude Cambium Extracts

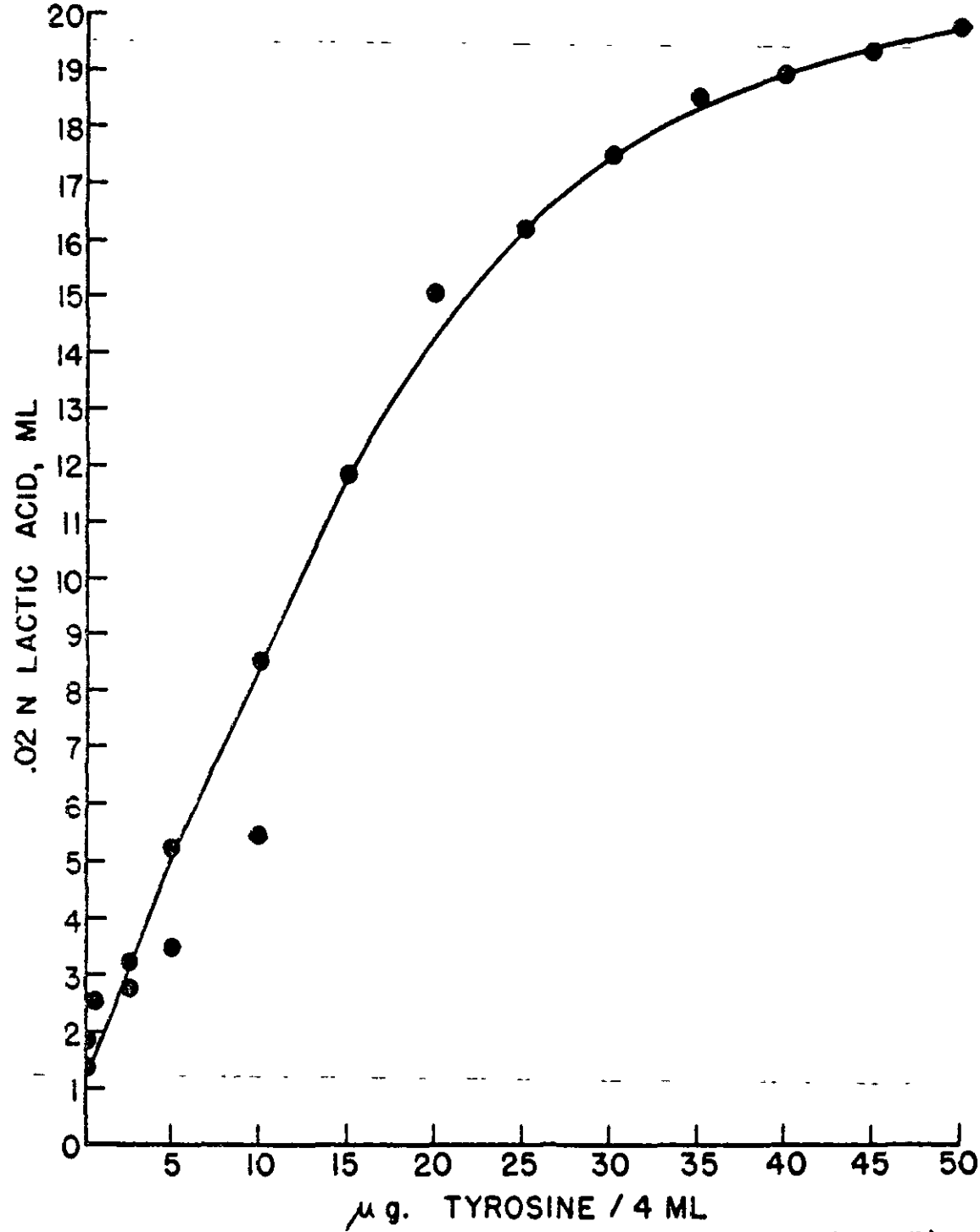

Figure 2. Standard Curve for Tyrosine Assay (Fig. 3)

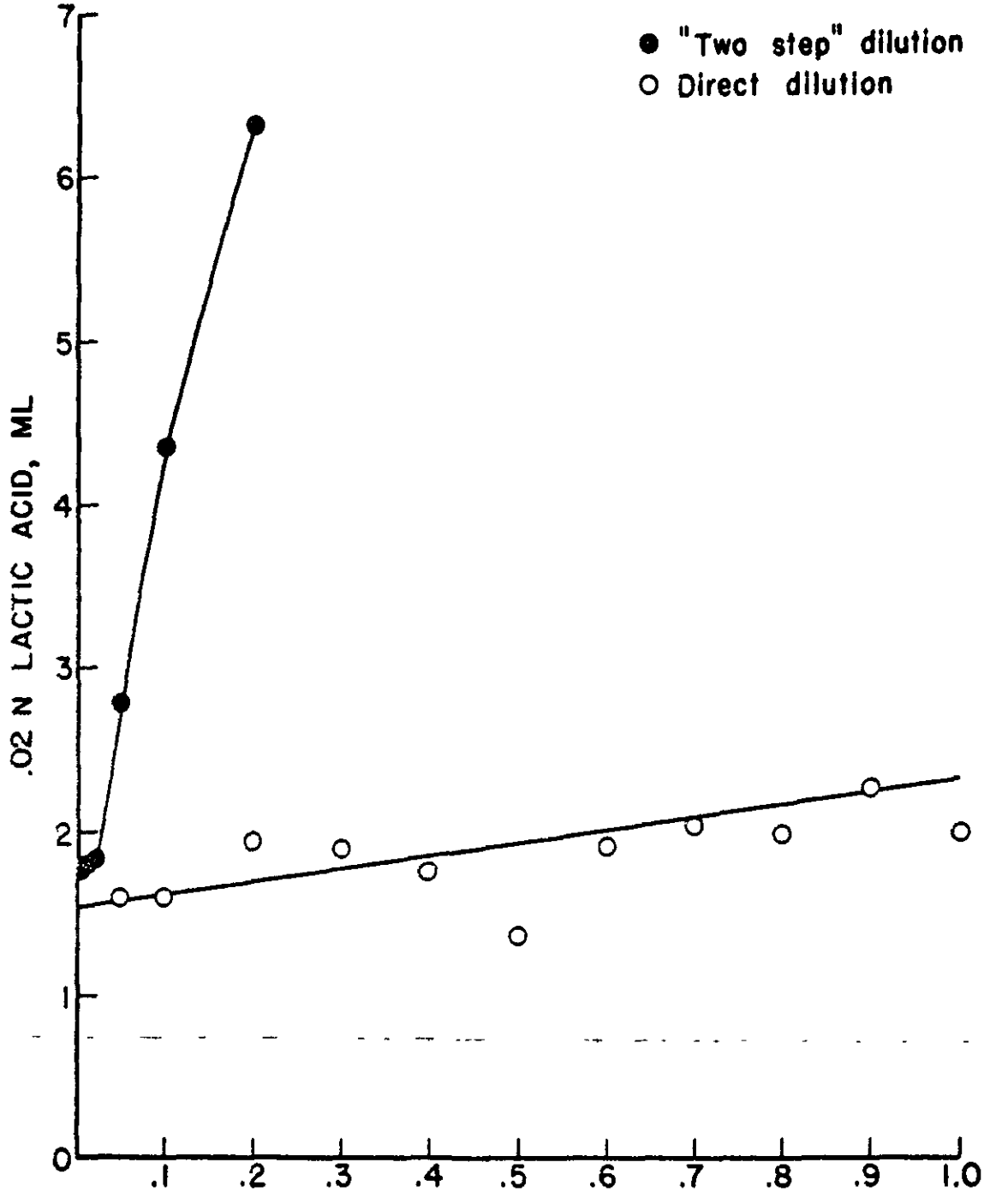

Figure 3. Tyrosine Assay of Crude Phloem Extract From Trembling ~~Big Tooth~~ Aspen (Tremuloides) T-158-P

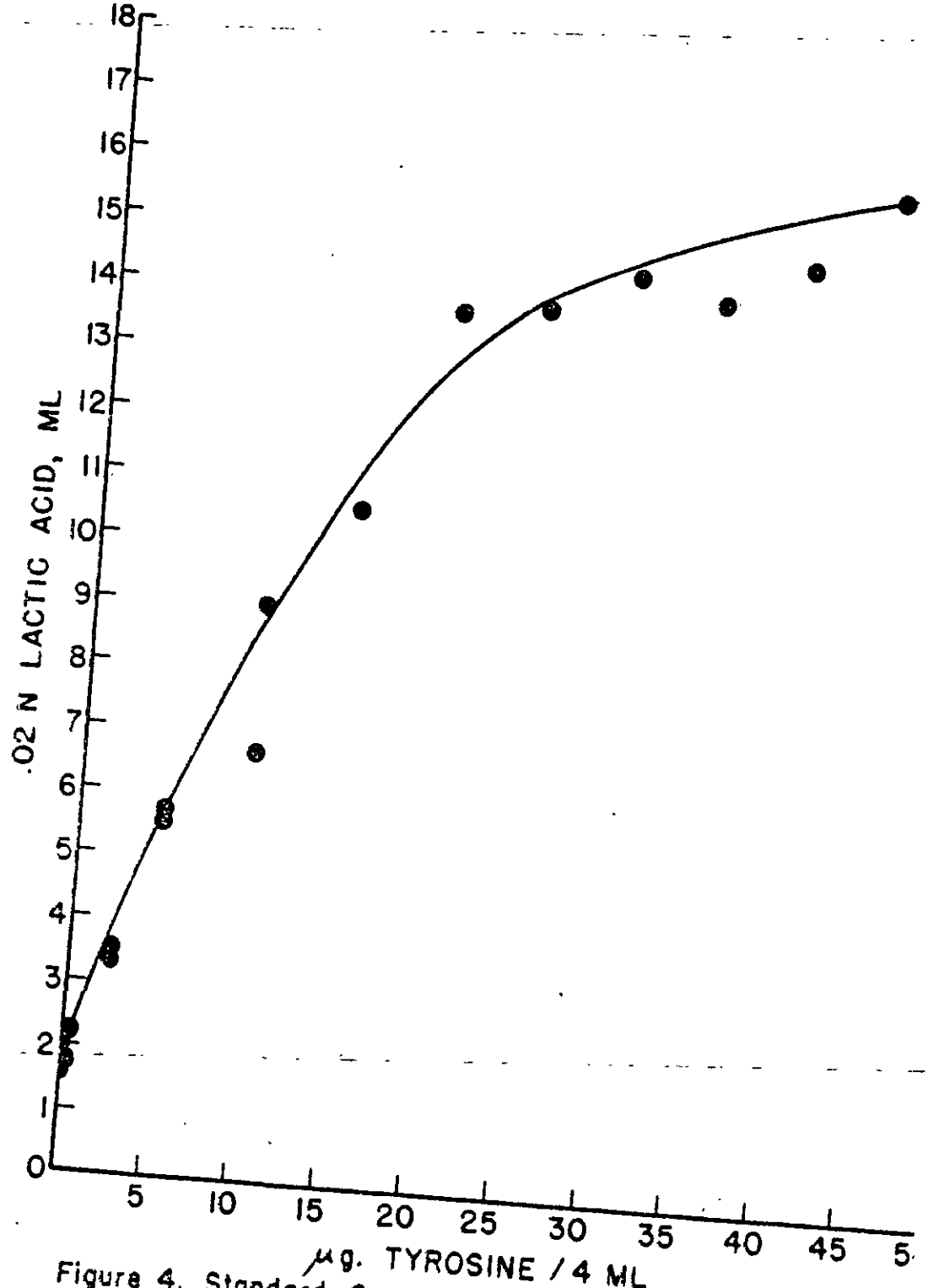

Figure 4. Standard Curve for Tyrosine Assay (Fig. 5)

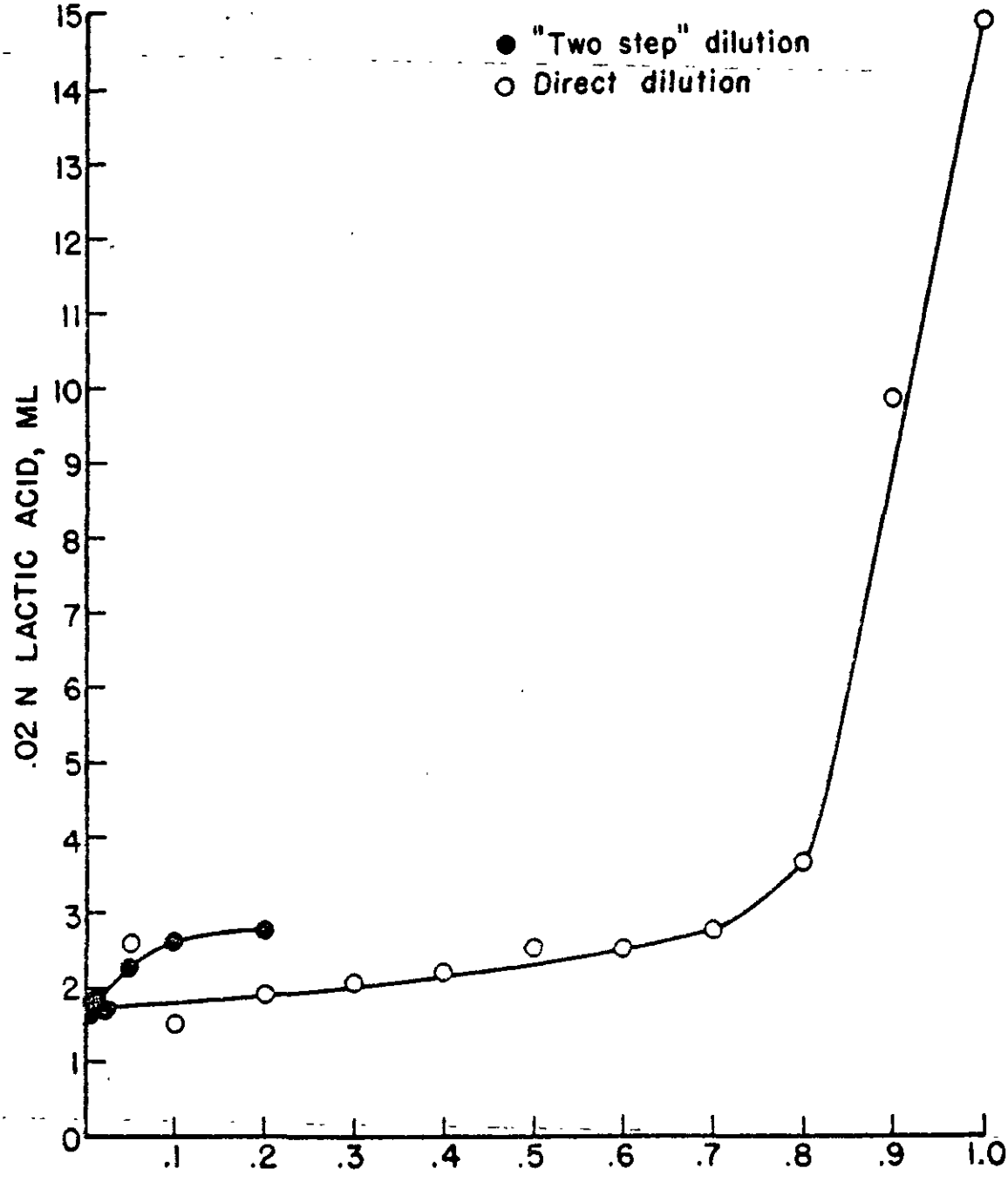

Figure 5. Tyrosine Assay of Crude Cambium Extract From Trembling Aspen (Tremuloides) T-159

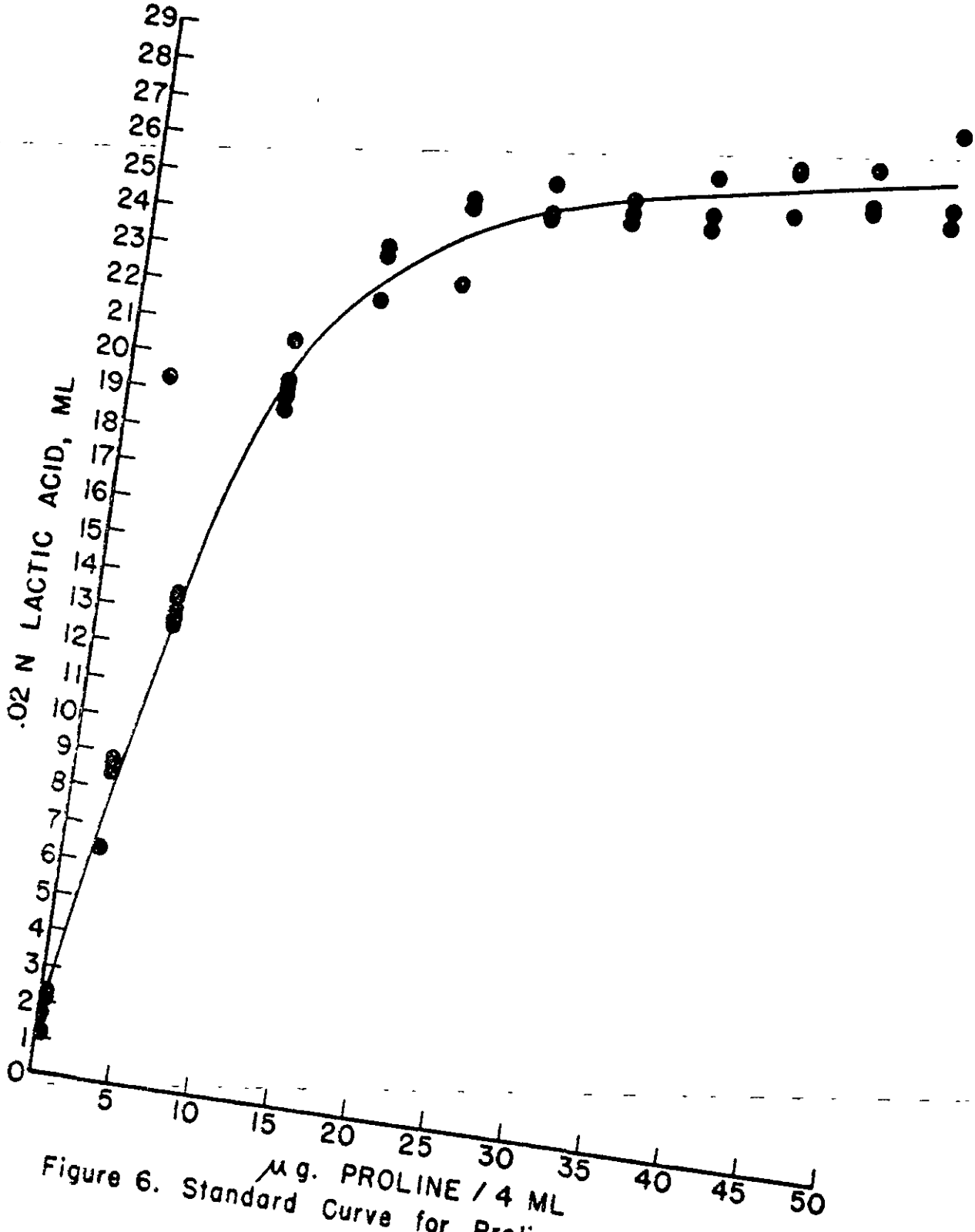

Figure 6. Standard Curve for Proline Assay (Fig. 7)

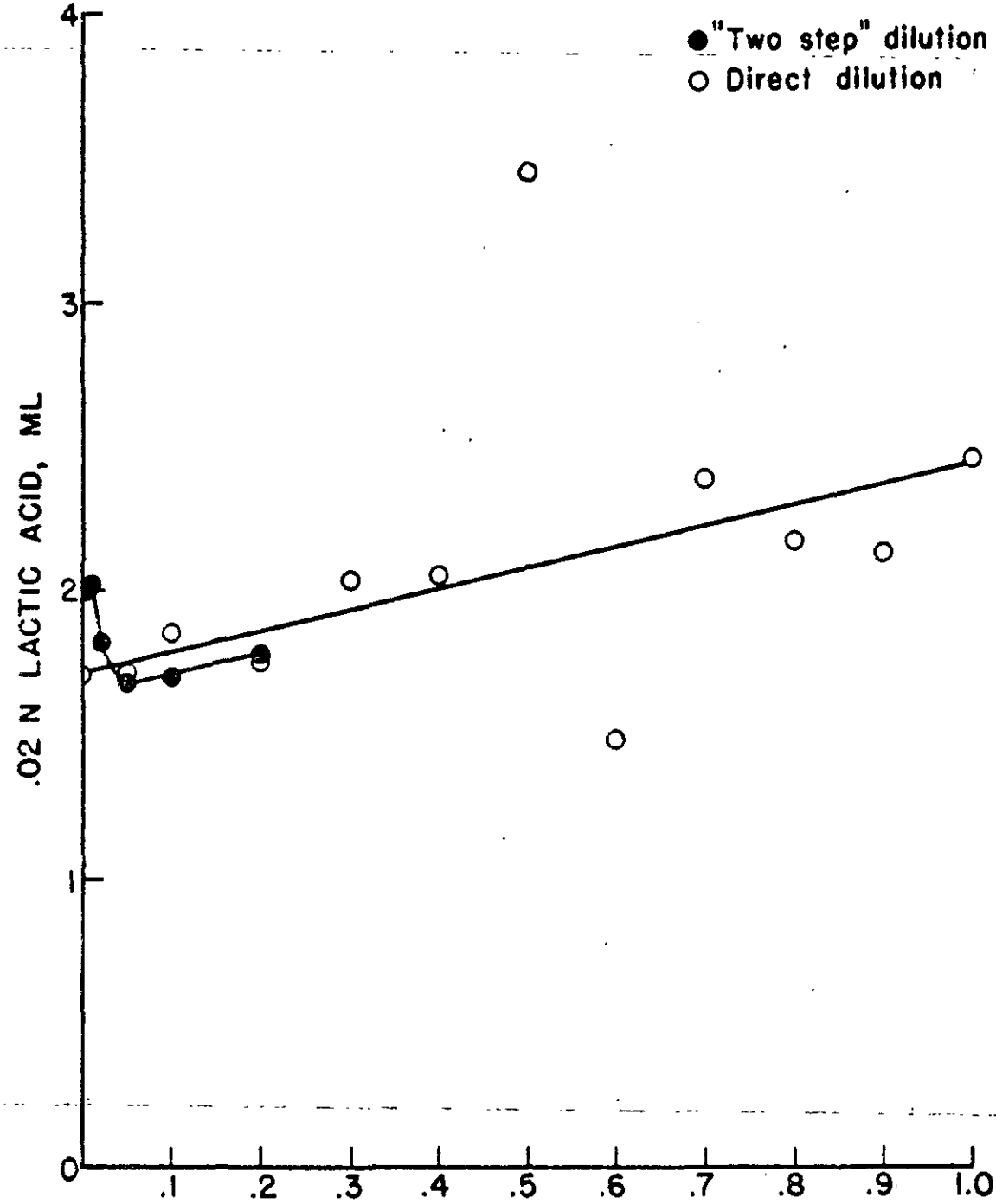

Figure 7. Proline Assay of Crude Phloem Extract From Big Tooth Aspen (Grandidentata) G-104-P

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Barbara Reeder
Barbara Reeder

Donald Worth
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COLOR REACTIONS: A SURVEY OF "CAMBIAL ZONE" TISSUES OF ASPEN, AMERICAN ELM AND EUROPEAN LARCH WITH THE ISENBERG-BUCHANAN, PHLOROGLUCINOL AND MÄULE TESTS

Empirical data have been tabulated for the responses of the above species to the three tests. As previously reported by others, the aspen xylem tissues are consistently negative in the Isenberg-Buchanan test. The phloem gives a variable, but positive response, which is not in accord with the statement that barks do not give colors with similar reagents.

The responses of soft xylem to phloroglucinol-HCl support the assumption that this tissue is only slightly lignified, i.e., that it is in a transition stage and very active biochemically. The Mäule test does not parallel the phloroglucinol test with soft xylem.

COLOR REACTIONS

Chemical tests which develop colors have often been used with wood and offer a means of making quick comparisons. Three such reactions were chosen to make a preliminary survey of the phloems and xylems which had been obtained from bigtooth aspen, quaking aspen, American elm, European larch, Scotch pine, and white spruce. These reactions are: (1) the Isenberg-Buchanan test, which has been used as an aid in identifying

wood species; (2) the phloroglucinol-hydrochloric acid reagent for lignin, which is used as a color test and a stain for microscopy; and (3) the Mäule test, which is used to distinguish between soft- and hard-wood lignins.

The tests were performed in the conventional ways[1] and were applied to the methanol-extracted tissues after they had been dried and ground to pass the 40 mesh screen of a micro-Wiley mill. The results have been tabulated; the minus sign (-) indicates the absence of color formation, the plus sign denotes color formation coupled with an indication of color intensity from very faint (+) to very intense (++++) color. The results of the Isenberg-Buchanan test were read at the end of 20 hours to permit maximum color development; in the other tests 5-10 minutes were deemed sufficient.

In the Isenberg-Buchanan test the color was present in both the solid and the solvent. In the other two tests the color developed only in the solid; however, it was observed that, in some cases of low intensity reaction, the color appeared in some particles, but not others. This suggests that more use be made of these tests, particularly the Mäule test as reagents in microchemistry.

The tabulated data warrant the following statements:

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1. Isenberg, I. H. and Buchanan, M. A., Jour. Forestry 43, 888-90 (1945). Brauns, F. E., Chemistry of Lignin, p. 26, 29, 41 (New York, 1952). The chlorine water-Na_2SO_3 modification of the Mäule test was used.

ASPEN

All xylems of both species are negative with the Isenberg-Buchanan test; this result is consistent with the original report (loc. cit.). With one exception, 6-T, the phloems all show a positive response, though the intensity varies. This result challenges the generalization that all barks are negative to this test [Adler, E., Svensk Paperstid. 54, 445 (1951)].

To the phloroglucinol test all 1953 and 1954 xylems respond with intense color formation. The soft xylem gives a variable, generally weak, response, but the samples taken on June 1 and July 22 (14-G) are exceptions. All the phloems respond positively, but with variable intensity.

As with the phloroglucinol test, the phloem response to the Mäule test is variable, but also is generally weaker and with more instances of atypical color. The 1953 and 1954 xylems are all positive, but with variable intensity. The soft xylems all respond positively to this test and there is no correlation with their response to the phloroglucin test, unless it is in a negative sense.

ELM

The phloem reactions are variable in intensity with all three reagents; they generally turn orange with the Mäule reagent and red-orange with the Isenberg-Buchanan reagent.

The xylems react positively (except for one negative), but with variable intensity, to the Isenberg-Buchanan test. In the phloroglucinol test the outermost (newer) xylem generally gives a faint test in contrast to the intense color given by the mature xylem; the Mäule test does not distinguish these xylems as sharply.

EUROPEAN LARCH

With the Isenberg-Buchanan reagent this species tends to give negative or atypical responses for xylem and intense, but atypical, colors for phloem. The Mäule test usually produces more color with xylem than phloem, but the orange color is atypical.

SCOTCH PINE AND WHITE SPRUCE

The few observations do not warrant comment.

TABLE I

QUALITATIVE TESTS: ASPEN

Date	Sample	Isenberg-Buchanan Phloem	Isenberg-Buchanan 53 Xylem	Isenberg-Buchanan 54 Xylem	Isenberg-Buchanan Soft Xylem	Phloroglucinol-HCl Phloem	Phloroglucinol-HCl 53 Xylem	Phloroglucinol-HCl 54 Xylem	Phloroglucinol-HCl Soft Xylem	Mäule Phloem	Mäule 53 Xylem	Mäule 54 Xylem	Mäule Soft Xylem
2-22-54	G104 ♀	+	-			++	++++			+	+++		
	G106 ♂	+	-			++	++++			+++	++		
	T106 ♂	+	-			+	++++			+	++++		
	T108 ♀	+	-			+	+++			++	++++		
3-23-54	T111 ♀	++	-			+++	++++			+	++		
		+++	-			+++	++++			+	++++		
3-26-54	G107A ♂	++++	-			+++	++++			+	+		
		++++	-			+++	++++			++	+		
		++++	-			+++	++++			+	++		
4-7-54	T113	++++	-			+++	++++			+	+		
		++++	-			+++	++++			+	+		
4-17-54	T115 ♂	++	-			++	++++			+	++		
		+++	-			++	++++			+	+++		
		++	-			+	++++			+	++		
4-26-54	G52 ♂	++++	-			++	++++			+	++		
		++++	-			++	++++			+	++		
	T115 ♂	+++	-			+	++++			+	++		
5-3-54	G52 ♂	+++	-			++	++++			+	++		
	T115 ♂	++	-			++	++++			+	++		
5-14-54	G52 ♂	+++	-			++	++++			++	+++		
		++	-			+++	++++			+++	+++		
6-1-54	G				-				++++				+
	T				-				++++				+
	T				-				+++				+
	T				-				+++				+
6-17-54	1T	+				+++				+			

TABLE I (CONTINUED)

Date	Sample	Isenberg-Buchanan Phloem	Isenberg-Buchanan 53 Xylem	Isenberg-Buchanan 54 Xylem	Isenberg-Buchanan Soft Xylem	Phloroglucinol-HCl Phloem	Phloroglucinol-HCl 53 Xylem	Phloroglucinol-HCl 54 Xylem	Phloroglucinol-HCl Soft Xylem	MBule Phloem	MBule 53 Xylem	MBule 54 Xylem	MBule Soft Xylem
6-18-54	2T	+				+++				+			
	3T												
6-28-54	4G	++	-	-	-	+++	++++	++++	+	++	++++	+++	++
6-29-54	5T	+				+++				++			
	6T	-				++++				+			
6-30-54	8G	+	-	-	-	++++	++++	++++	-	+	+++	++	++++
	7T	++				++++				-			
7-20-54	9G5	++	-	-	-	++++	++++	++++	-	++	++++	++++	++++
	10T	+				++++				+			
7-21-54	12G	++	-	-	-	+++	++++	++++	+	+++	++	++++	++
	11T	+				++++				+			
7-22-54	14G	+	-	-	-	+++	++++	++++	+++	+++	++	++	+
	13T	++++				+++				-			
8-16-54	15T	++++				++++				+			
	16T	+++				+++				+			
8-17-54	17G	++	-	-	-	++++	++++	++++	-	++	++	++++	++++
	18G	++	-	-	-	+++	++++	++++	++	++++	++	+++	++
9-25-54	G-114	++	-			+++	++++			+	+		
	T	++	-			++	++++			+	+		
10-21-54	G-112	+	-			+++	++++			++	++		
	T-122	+	-			++	++++			-	+++		
12-6-54	G-113	+++	-			++++	++++			++	++++		
	T-122	++	-			++	++++			+	+++		

TABLE II

QUALITATIVE TESTS: AMERICAN ELM

Date of Collection	Isenberg-Buchanan Phloem	Isenberg-Buchanan Outermost Xylem	Isenberg-Buchanan Xylem	Phloroglucinol-HCl Phloem	Phloroglucinol-HCl Outermost Xylem	Phloroglucinol-HCl Xylem	Mäule Phloem	Mäule Outermost Xylem	Mäule Xylem
11-12-53 Top	++++ [A]		+++	+++		++++	++++		++++
11-12-53 Middle	++++		++++	++		++++	++++		++++
11-12-53 Base	+++		++++	+++		++++	++++		++++
12-16-53 Top	+++		+++	++		++++	++		++++
12-16-53 Middle	+++		+++	++		++++	+++		++++
12-16-53 Base	+++		+++	++		++++	+++		++++
2-4-54 Top	++		++	++		++++	+		+++
2-4-54 Base	+		++++	++		++++	+		++++
3-3-54	+		++	+		++++	+		++++
3-25-54	++		+++	-		++++	-		+++
4-5-54 Top	+++		+	++		++++	++		++++
4-5-54 Base	+++		+++	+++		++++	+++		++++
4-28-54	++		+	+		++++	++++		++++

[A] Atypical, orange color

TABLE II (CONTINUED)

Date of Collection	Isenberg-Buchanan Phloem	Isenberg-Buchanan Outermost Xylem	Isenberg-Buchanan Xylem	Phloroglucinol-HCl Phloem	Phloroglucinol-HCl Outermost Xylem	Phloroglucinol-HCl Xylem	Mäule Phloem	Mäule Outermost Xylem	Mäule Xylem
5-26-54 Top	+++	++*	+++	+	++++*	++++	++++	++++*	++++
5-26-54 Base	+++		+++	+		++++	++++		++++
6-23-54	+++	+**	+	+++	+**	++++	++++	+**	++++
7-15-54	++	+**	+	+	+**	++++	++	+++**	++++
8-5-54	+++	++**	++	+++	+**	++++	+++	++++**	++++
8-26-54	+++		-	++		++++	++++		++++
9-23-54 Top	++	+*	+	-	+*	++++	+	+++*	++++
9-23-54 Base	+		++	-		++++	-		++
11-4-54 Top	++	+++*	+	-	+*	++++	+	+++*	++++
11-4-54 Base	++		+++	-		++++	-		++
12-15-54	+++		+	++		++++	++++		++++

* Xylem scrapings
** Soft Xylem

TABLE III

QUALITATIVE TESTS: EUROPEAN LARCH

Date of Collection	Isenberg-Buchanan		Phloroglucinol-HCl		Mäule	
	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem
2-4-54	++++	-	+	++++	+	+
3-3-54	+++	-	+	++++	+	++
4-5-54	+++	-	+	++++	++	++
4-28-54	+++	-	+	++++	+	+++
5-18-54	++++	+	+	++++	+	++
6-24-54	++++	+	+	++++	+	++
7-16-54	++++	++++	+	++++	++	+++
8-10-54	+++	+++	+	++++	++	-
8-30-54	+++	-	+	++++	++	+++
9-23-54	+++	+	+	++++	+	++++
11-4-54	+++	++	+	++++	++	++++
12-14-54	++++	+	+	++++	++	+++

TABLE IV

QUALITATIVE TESTS: SCOTCH PINE

Date of Collection	Isenberg-Buchanan		Phloroglucinol-HCl		Mäule	
	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem
3-3-54	++++	-	++	++++	++	+
4-13-54	++++	+	+	++++	+	+
5-8-54	++++	-	+	++++	++	++
7-13-54	++++	+	+	++++	++++	+

TABLE V

QUALITATIVE TESTS: WHITE SPRUCE

Date of Collection	Isenberg-Buchanan		Phloroglucinol-HCl		Mäule	
	Phloem	Xylem	Phloem	Xylem	Phloem	Xylem
4-13-54	+	-	+	++++	-	+
5-8-54	+	-	+	++++	-	+
8-10-54	+	-	+	++++	-	+

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Barbara Reeder
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Notebook: 1266
Pages: 140-147

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CAMBIAL CHEMISTRY: AMERICAN ELM
COLLECTIONS AND ASSAYS, 1954

SUMMARY

Monthly collections were made and assayed according to the established routine.

The work covered by this report is a continuation of that started in November, 1953. (cf. Project 1702, Report No. 2).

Twelve specimens of American elm were taken from the Institute's Pulpwood Plantation during 1954 for sampling and assay according to the routine procedure (cf. Project 1702, Report No. 6). Since the elms in this stand are volunteers, young saplings were cut off near the base; hence the cambial tissues in this instance are derived from stems instead of from branches. In five instances, two ages are shown for a single collection date; this indicates that top and basal sections were cut from the stem, obviously the younger is the top section. Whereas ample material was obtained for assays of phloem and mature xylem from each section, the yield of soft, or immature, xylem sufficed for only one assay.

The data under the headings cambium and starch were taken from the report of the Microscopy Laboratory on the blocks which were submitted for sectioning and staining.

The table for sugar chromatograms has a heading, "Origin", which denotes the presence of carbohydrate material of very low mobility, and probably of higher molecular weight, than glucose. This spot does not appear in the chromatograms for mature xylem. Its presence in those for the soft xylems from the May 23, Sept. 23, and Nov. 24 collections might be due to incomplete separation of phloem and xylem. The histological report that the cambium was still active in the specimens taken Sept. 23 and Nov. 24 merits further study because it implies other criteria of cambial activity than easy bark slippage.

In other respects the data only warrant comments similar to those on the aspens.

TABLE I

BIOMETRIC AND EXTRACTION DATA

American Elm - 1954

				Phloem		Soft Xylem		Xylem	
Collected	Age	Cambium Active	Starch Present	% H_2O	% CH_3OH Sol.Solids D.B.	% H_2O	% CH_3OH Sol.Solids D.B.	% H_2O	% CH_3OH Sol.Solids D.B.
2-4-54	5	No	Yes	57.3	8.9			21.3	3.2
	10			56.2	10.6			31.2	3.6
3-3-54	5	No	Yes	51.7	12.1			21.6	3.9
4-5-54	3	No	Yes	44.7	17.3			23.6	2.7
	6	No	Yes	39.2	15.3			29.7	3.2
4-28-54	13	No	Yes	48.0	12.8			36.0	1.7
5-26-54	5	Yes	Yes	52.3	13.5	76.9	15.7	34.8	0.92
	10			45.7	14.6			35.8	1.3
6-23-54	8	Yes	Yes	53.8	15.6	87.0	39.5	45.9	4.7
7-15-54	11	Yes	Yes	66.3	14.1	87.6	37.1	56.8	4.4
8-5-54	11	Yes	Yes			73.2	19.6	35.8	2.0
8-26-54	8	Yes	Yes	47.1	9.6			38.4	1.6
9-23-54	6	Yes	Yes	51.6	8.2	59.1	21.0	31.9	1.3
	12			53.7	8.2			31.9	1.2
11-4-54	6	Yes	Yes	60.5	22.0	52.4	31.9	35.2	2.9
	12			61.7	15.3			24.9	2.9
12-15-54	12			54.1	17.4			36.2	4.7

TABLE II

SUGAR CHROMATOGRAM DATA

American Elm - 1954

	Phloem				Soft Xylem				Xylem			
Collected	Origin	Sucrose	Glucose	Fructose	Origin	Sucrose	Glucose	Fructose	Origin	Sucrose	Glucose	Fructose
2-4-54	+	++	++++	+						++	++++	+
	+	++	++++	+						++	++++	+
3-3-54	+	+++	++++	+						+++	++++	+
4-5-54	+	+	++	++						++	+	+
	+	+	+++	+						+	+++	+
4-28-54	+	–	+++	++						–	++	–
5-26-54	+	–	++++	–	+	–	++	–		–	++	–
			+++								++	
6-23-54	+	++	+++	+		–	+++	+		++	+	+
7-15-54		+++	++	–		+++	++	+		–	+	–
8-5-54						++	+++	?		++	+	
8-26-54		+++	++							+?	+?	
9-23-54	+	++	–	–	+	++	–	–		+		
	+	++	–	–						+		
11-4-54	+	+++	+++	+	+	+++				++	+	?
	+	+++	+++	+						++	+	?
12-15-54			+++	+						+	+++	+

TABLE III

AMINO ACID CHROMATOGRAM DATA

American Elm - 1954

	Phloem			Soft Xylem			Xylem		
Collected	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
2-4-54	2	1	2				2	1	2
	2	1	2				2	–	2
3-3-54	2	1	2				2	1	2
4-5-54	2	1	1				2	1	1
	2	1	1				2	1	1
4-28-54	2	2	3				2	2	1
5-26-54	2	–	3	3	1	3	2	–	3
	2	–	3				2		1
6-23-54		1	2	2	1	2	1	1	2
7-15-54		1		1	1	2		2	1
8-5-54				1	2	1			
8-26-54		1	2					2	2
9-23-54	1	1	2	1	2	2	1		1
	1	2	2				1		1
11-4-54	1	1	2	1	1	1	1		1
	1	1	2				1		1
12-15-54	1	2	1				1	2	1

PROJECT REPORT FORM

PROJECT NO. 1702
COOPERATOR I.P.C.
REPORT NO 7
DATE February 21, 1955
NOTE BOOK
PAGE TO
SIGNED R. E. Kremers

Notebook: 1285
Pages: 70-129

Copies to: Files
Dr. Kremers (2)
Mr. Weiner

Notebook: 1314
Pages: 9-22, 35-78, 80-84, 86-93, 109-117

Barbara Reeder

CAMBIAL CHEMISTRY: ASPEN COLLECTIONS AND ASSAYS, 1954

SUMMARY

This report lists the aspen materials collected and assayed during 1954. During the growing season, trees were felled to provided larger quantities of materials, especially "soft xylem"; otherwise the procedure for the assay of small samples, described in Report No. 6, was followed.

The variability of the data emphasize the need for a statistical interpretation; hence the results are presented as tables without attempt at interpretation. Subject to confirmation, the following comments may be added:. The free sugars regularly present are glucose and sucrose; fructose is variable and the pentose sugars are absent. The amino acids indicated include a "basic" group, composed of lysine, arginine, and histidine which are associated with important protein functions, and an "amide" group, asparagine and glutamine which are associated with nitrogen transport. The relative absence of phenols in xylem tissues and their relative abundance in phloem was unexpected in view of the abundant lignin formation by xylem cells and the occurrence of native lignin in most woods.

This report lists the aspen materials collected during 1954 and presents data from the extractions with methanol and the chromatographic

inspection of the extracts. The "monthly assays" were made in accordance with the procedure described in Report No. 6 on Cambial Chemistry.

MATERIALS COLLECTED

Materials for this study were collected in each month of 1954, except January and November, and represent both of the aspen species which are important pulp-woods in the Northern Lake States, i.e., bigtooth aspen (P. grandidentata) and quaking aspen (P. tremuloides). Two types of materials were collected: (1) twigs and branches, which were used primarily for analytical comparisons between individual trees; and (2) trunk sections, taken below the crown, which provided the larger amounts needed for the study of chemical composition.

From branches the usual small samples were taken and, since the cambia were "inactive", only phloem (inner bark) and the most recent growth rings of xylem (outer wood) were separated. The June 1 collections are an exception, since some "soft xylem", i.e., presumably a new increment of wood, was removed from the older xylem.

The trunk sections were taken during the growing season for the special purpose of accumulating soft xylem; also, as the season progressed, the xylem formed during 1954 was removed in order to compare it with the xylem formed in 1953. With care, it was possible to detect a slightly yellow colored layer which marked the boundary between the 1954 and 1953 growth rings and thereby to separate the two increments of xylem.

Small "blocks" were cut routinely and submitted to the Microscopy Laboratory for histological examination.

METHODS AND DATA

The preparation and analysis of small samples, e.g., from branches, have been described in a previous report (Report No. 6 Cambial Chemistry). Since the initial operations of preserving and extracting larger amounts of material parallel the corresponding steps in the routine for small samples, analogous extraction and chromatographic data were recorded.

The biometric and analytical data for each species are recorded in the appended tables. In those for quaking aspen, there are no entries in the xylem columns opposite the four collections from June 17 to August 16, inclusive. These materials are being used for a doctoral research; hence the data will be reported separately. Under the headings "cambium" and "starch" (Tables I and IV) the entries are taken from the reports of the Microscopy Laboratory on the "blocks" which were submitted for histological examination.

DISCUSSION

Perhaps the first and dominant impression to be gained from an inspection of the extraction data is the variability of the results. Imperfections in the analytical methods are a contributing factor, but this finding is consistent with the variability which aspens show in their botanical characteristics. In consequence the accumulation and interpretation of data must proceed on a statistical basis before seasonal trends or other

comparisons between the individuals can be stated with finality. At the same time this variability justifies the effort to develop sampling and assay methods which can be used routinely. It also favors the premise that chemical correlations will eventually be useful indices to superior individuals or races of aspen.

The relations between the different tissues collected from the same tree show a more definite pattern and are consistent with the literature*. Taking high water content and methanol-soluble extractives as indices of biochemical activity, the soft xylem rates as the most active and the mature (1953) xylem as the least active tissue. The 1954 xylem appears to be an intermediate between these two stages in activity, just as it is in age and position. The phloem has a water content which is usually a little higher than that of the mature xylem, but it is always much richer in extractives. Accordingly it is also the locus of much biochemical activity, even though it ranks below the soft xylem. Under the microscope, the phloem is seen to be composed of both new and old tissue-elements. Thus the botanical and chemical findings are consistent.

The chromatographic data should be regarded as preliminary, qualitative inspections of the chemical nature of the extractives. Only three classes of compounds--sugars, amino acids, and phenolic substances--have been considered.

* C. M. Stewart, G. L. Amos, and L. G. Harvey: Chemical Studies on Eucalyptus Regnans F. Muell., Australian Jour. Biol. Sci. 6, 21-47 (1953).

The sugar chromatograms indicate only glucose, fructose, and sucrose. Rarely there is an indication of a carbohydrate which does not move from the origin. The free pentose sugars are conspicuously absent. There is an indication that sucrose is the predominant sugar in P. tremuloides. In P. grandidentata this may be true of the phloem with some allowance for seasonal variation, but glucose seems to be more prominent than sucrose in the active xylem tissues. Fructose seems to be the minor sugar in both species at all times and frequently was not detected.

The amino acid chromatograms have been read only in terms of sub-groups. In general the results indicate appreciable amounts of amino acids in the phloem and the more active xylem tissues. The amino acids presumed to constitute groups I and II have considerable biochemical importance. Thus the basic amino acids, lysine, arginine and histidine, are considered "essential" in the animal organism, the dicarboxylic acid amides, asparagine and glutamine, are important in the transport or conservation of amino nitrogen in the plant. One of the inadequacies of the methods used to date is that they do not detect allantoin or other ureides which may also be important in nitrogen transport.* A more effective use of amino acid chromatography is one of the important objectives of this project (Cf. Wessman report).

Very little has been done to interpret the phenol chromatograms because all extracts have given unresolved streaks. One general result, however, is that all phloem extracts give an intense phenolic chromatogram,

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* K. Mothes and L. Engelbrecht. Flora 139, 586-616 (1952).

whereas xylem extracts appear to be practically devoid of phenols (at concentrations spotted). This seems unexpected in view of the quantity of lignin deposited in the cell walls of mature xylem and the reported presence of at least some native lignin in most woods.

TABLE I

BIOMETRIC AND EXTRACTION DATA

Aspen - 1954
Bigtooth Series

Collected	Code	Location	Age	Cambium Active	Starch Present	Phloem		Soft Xylem		1954 Xylem		Xylem	
						% H_2O	% CH_3OH Sol. Solids D.B.	% H_2O	% CH_3OH Sol. Solids D.B.	% H_2O	% CH_3OH Sol. Solids D.B.	% H_2O	% CH_3OH Sol. Solids D.B.
2/18	G104 ♀	W. of Brule				50.1	9.2					50.7	1.8
2/19	G106 ♂	W. of Watersmeet	8	No	Yes	49.3	8.5					34.6	1.3
3/25	G107A ♂	Spring Field Corners		No	Yes	50.0	23.9					40.7	5.4
						48.4	21.7					42.8	2.4
						46.2	19.7					41.6	3.8
4/25	652 ♂	Near Rhinelander	16	No	Yes	44.5	22.4					44.8	3.0
			13			45.9	24.4					46.9	2.5
5/1	652 ♂	Near Rhinelander	13	No	Yes	51.3	13.8					40.9	3.3
5/13	652 ♂	Near Rhinelander	17	No	Yes	45.8	21.2					43.2	3.3
6/1	O_B	Ripco Farm, Heavy Stand No Disease		No	Yes	49.1	13.3		31.8				
6/28	4-G	Ripco Farm	29	Yes	Yes	43.9	10.1	87.4	37.6	50.1	5.4	20.9	1.3
6/30	8-G	Ripco Farm	22	Yes	Yes	49.9	10.2	86.6	37.5	57.4	8.2	51.7	1.4
7/20	9-G	Ripco Farm	29	Yes	Yes	45.9	12.3	85.5	41.2	61.9	5.7	38.4	1.3
7/21	12-G	Ripco Farm	32	Yes	Yes	39.1	12.3	82.0	41.2	51.4	6.3	39.6	0.5
7/22	14-G	Ripco Farm	27	Yes	Yes	28.3	7.8	74.8	26.0	34.3	4.5	49.1	1.7
8/17	17-G	Ripco Farm	31	Yes	Yes	41.5	13.0	78.3	37.6	57.1	4.2	48.5	1.2
	18-G	Ripco Farm	29	Yes	Yes	35.7	11.6	70.3	35.1	51.4	4.1	24.7	1.1
9/23	G114	Ripco Farm	16	No	Yes	42.1	12.4					41.1	2.3
10/21	G113	Ripco Farm	15	No	Yes	48.8	21.6					56.6	3.6
12/4	G113	Ripco Farm	13			46.2	19.6					52.6	4.2

TABLE II

SUGAR-CHROMATOGRAM DATA

Aspen - 1954
Bigtooth Series

Code	Phloem Origin	Phloem Sucrose	Phloem Glucose	Phloem Fructose	Soft Xylem Origin	Soft Xylem Sucrose	Soft Xylem Glucose	Soft Xylem Fructose	1954 Xylem Origin	1954 Xylem Sucrose	1954 Xylem Glucose	1954 Xylem Fructose	Xylem Origin	Xylem Sucrose	Xylem Glucose	Xylem Fructo
G104 ♀	+	++++	+++	+++									+	++++	+++	+++
G106 ♂	+	+++	++	++									+	+++	++	++
G107A ♂	-	+++	++++	+									-	+++	++++	+
	-	+++	++++	+									-	+++	++++	+
	-	+++	++++	+									-	+++	++++	+
G-52 ♂	-	++	++	-									-	++	++	-
		++	++	-										++	++	-
G-52 ♂	+	+++	++	-									-	++	++	-
G-52 ♂	-	+++	++	-									-	++	++	-
GB		+++	+	-	-	+										
4-G		+++	+	-		+	++++	+		+	++++	+		+	+	-
8-G		+++	+	-		+	++++	+		+	++++	+		-	+	-
9-G		+++	++	-		+++	++++	+		++	++++	+		+	+	-
12-G		+++	++			++	++++	+		+++	++++	+		++	+	-
14-G		+++	++			+++	++++	+		+++	+++			-	-	-
17-G		+++	+			+++	+++	-		+++	+++	-		-	-	-
18-G		+++	+	-		+++	+++	-		+++	+++	-		-	-	-
G114		+++	-	-										++	-	-
G113		+++	++	-										++	+	-
G113		+++	++	+										+		-

TABLE II

SUGAR-CHROMATOGRAM DATA

Aspen - 1954
Bigtooth Series

Code	Phloem Origin	Phloem Sucrose	Phloem Glucose	Phloem Fructose	Soft Xylem Origin	Soft Xylem Sucrose	Soft Xylem Glucose	Soft Xylem Fructose	1954 Xylem Origin	1954 Xylem Sucrose	1954 Xylem Glucose	1954 Xylem Fructose	Xylem Origin	Xylem Sucrose	Xylem Glucose	Xylem Fructose
G104 ♀	+	++++	+++	+++									+	++++	+++	+++
G106 ♂	+	+++	++	++									+	+++	++	++
G107A ♂	-	+++	++++	+									-	+++	++++	+
	-	+++	++++	+									-	+++	++++	+
	-	+++	++++	+									-	+++	++++	+
G-52 ♂	-	++	++	-									-	++	++	-
		++	++	-										++	++	-
G-52 ♂	+	+++	++	-									-	++	++	-
G-52 ♂	-	+++	++	-									-	++	++	-
GB		+++	+	-	-	+										
4-G		+++	+	-		+	++++	+		+	++++	+		+	+	-
8-G		+++	+	-		+	++++	+		+	++++	+		-	+	-
9-G		+++	++	-		+++	++++	+		++	++++	+		+	+	-
12-G		+++	++			++	++++	+		+++	++++	+		++	+	-
14-G		+++	++			+++	++++	+		+++	+++			-	-	-
17-G		+++	+			+++	+++	-		+++	+++	-		-	-	-
18-G		+++	+	-		+++	+++	-		+++	+++	-		-	-	-
G114		+++	-	-										++	-	-
G113		+++	++	-										++	+	-
G113		+++	++	+										+		-

TABLE III

AMINO ACID CHROMATOGRAM DATA

Aspen - 1954
Bigtooth Series

Code	Phloem			Soft Xylem			1954 Xylem			Xylem		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
G104 ♀		+++	+++							+++	+++	+++
G106 ♂	++	++	1							?	?	2
G107 ♂	++	++	++							+	+	+
G52 ♂		3	3							2		1
		3	4							?		2
G52 ♂		4	4								2	1
G52 ♂	1	2	2							?		2
G_B	2	3	3	3	2	3						
4-G	1	2	3	2		2	1	2	2	1	2	2
8-G	1	2	3	2		2	1	1	2	1		1
9-G	1	2	2	2		2	1	2	2	1		1
12-G	1	2	2	2		2	1	2	2	1	2	1
14-G	1	2	2	2		2	1	1	2	-	-	-
17-G	1	2	2	1	2	1	1	1	2	1		
18-G	1	2	2	2		3	1	2	2	1		
G-114	-	-	-	-						-	-	-
G-113		1	2								1	
G-113	2	1	1							1		

Group I Lysine to glutamine, including glutamine
Group II Glutamine to alanine, including alanine
Group III Alanine to i-leucine

TABLE IV

BIOMETRIC AND EXTRACTION DATA

Aspen - 1954
Quaking Series

Collected	Code	Location	Age	Cambium Active	Starch Present	Phloem % H_2O	Phloem % CH_3OH Sol. Solids D.B.	Soft Xylem % H_2O	Soft Xylem % CH_3OH Sol. Solids D.B.	Xylem % H_2O	Xylem % Sol. D.
2/17	T106 ♂	Winters		No	Yes	67.5	18.4			39.9	
	T108 ♀	N. of Winters				64.4	15.9			44.0	
3/20	T111	Green Bay Airport	2			45.8	23.3			41.1	
		Brown County	5								
			4			52.8	21.9			44.6	
4/7	T113	Vicinity of Brown Springs Uncompahgre Nat. Forest Colorado	8	No	Yes	60.9	19.4			68.3	
4/16	T115 ♂	Ripco Farm	10			53.7	29.8			47.1	
			13	No	Yes	47.7	34.5			42.1	
			10			53.5	31.8			39.9	
4/25	T115 ♂	Ripco Farm	11	No	Yes	51.8	23.3			49.4	
5/1	T115 ♂	Ripco Farm	11	No	Yes	52.6	20.9			47.0	
5/13	T115 ♂	Ripco Farm	15	No	Yes	51.7	22.7			49.1	
6/1	T_A	Ripco Farm Heavy Stand No Disease		No	Yes	67.6	24.9	80.0	55.5		
	T_C	Ripco Farm, Fairly Open Area				70.8	20.9	73.5	50.5		
	T_D	Ripco Farm, Heavy Stand				64.9	24.0	77.8	60.4		
6/17	1-T	Ripco Farm	29								
	2-T	Ripco Farm	33	Yes	Yes						
6/18	3-T	Ripco Farm	30	Yes	Yes						
6/29	5-T	Ripco Farm	28	Yes	Yes						
	6-T	Ripco Farm	29	Yes	Yes						
6/30	7-T	Ripco Farm	28	Yes	Yes						
7/20	10-T	Ripco Farm	30	Yes	Yes						
7/21	11-T	Ripco Farm	29	Yes	Yes						
7/22	13-T	Ripco Farm	30	Yes	Yes						
8/16	15-T	Ripco Farm	30	No	Yes						
	16-T	Ripco Farm	30	No	Yes						
9/23	Aspen T	Ripco Farm	17	No	Yes	52.8	26.6			34.1	
10/21	T122	Ripco Farm	15	No	Yes	54.9	33.0			50.6	
12/4	T122	Ripco Farm	13			56.6	30.4			54.0	

TABLE V

SUGAR CHROMATOGRAM DATA

Aspen - 1954
Quaking Series

Code	Phloem Origin	Phloem Sucrose	Phloem Glucose	Phloem Fructose	Soft Xylem Origin	Soft Xylem Sucrose	Soft Xylem Glucose	Soft Xylem Fructose	Xylem Origin	Xylem Sucrose	Xylem Glucose	Xylem Fructose
T106 ♂	+	++++	+++	+++					+	++++	++	++
T108 ♀	+	+++	++	++					+	+++	++	++
T111 ♀		+++	+	-						+++	+	-
		+++	+	-						+++	+	-
T113		+	++	-						-	-	-
T115 ♂		+++	+	+						++	+	+
T115 ♂	+	++	+	-						++	+++	-
T115 ♂	+	++++	+	-						++	+++	-
T115 ♂		++	-	-						++	++	-
T_A		+++	+	1/2 +		+++	++	+				
T_C		++++	++	1/2 +		+++	++	+				
T_D		++++	++	1/2 +		+++	++	+				
1-T		+++	+	+								
2-T		+++	+	-								
3-T												
5-T		+++	++	+								
6T		+++	++	+								
7-T		+++	++	-								
10-T		+++	++	+								
11-T		+++	++	+								
13-T		+++	+	-								
15-T		+++	+	-								
16-T		+++	+	-								
Aspen T		+++	-	-						+	-	-
T-122		+++	++	-						++	+	-
T-122		+++	++	+						+	+	-

TABLE VI

AMINO ACID CHROMATOGRAM DATA

Aspen - 1954
Quaking Series

Code	Phloem			Soft Xylem			Xylem		
	Group I	Group II	Group III	Group I	Group II	Group III	Group I	Group II	Group III
T106 ♂	++++	++++	++++				++++	++++	++++
T108 ♀	+++	+++	+++				+++	++	+++
T111 ♀	2	2	6				1	2	4
	1	2	5				1	2	4
T113		3	5					3	5
T115 ♂	++	++	++				-	-	-
T115 ♂	1	2	2					2	
T115 ♂	1	2	5				1	2	1
	Group 1-Group II			Group 1-Group II					
T115 ♂	5		4					2	2
T_A	2	3	3	5	5				
T_C	2	3	3	5	5				
T_D	2	3	3	5	5				
1-T	2	2	2						
2-T	2	1	2						
3-T									
5-T	2	1	1						
6-T	2	1	1						
7-T	2	1	1						
10-T	1	2	2						
11-T	1	2	2						
13-T	1	2	2						
15-T	1	2	2						
16-T	1	2	2						
Aspen T	-	-	-						
T-122		1	2				-	-	-
T-122	2	1	1				-	1	1

PROJECT REPORT FORM

PROJECT NO. 1702
COOPERATOR I.P.C.
REPORT NO 6
DATE February 17, 1955
NOTE BOOK
PAGE TO
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Notebook: 1266 Pages: 30-37

Notebook: 1285 Pages 87, 95

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CAMBIAL CHEMISTRY: ROUTINE PREPARATION OF EXTRACTS FOR ANALYSIS

SUMMARY

A procedure has been evolved to facilitate sampling and assay of tissues from the "cambial zone" at frequent intervals and/or from several trees. The details of separating the tissues and of preparing methanol extracts are described. The procedure appears to be satisfactory mechanically; the assays have given yield figures and preliminary indications of the soluble sugars, amino acids and phenolic substances present in the "cambial zone" tissues.

Most publications on cambial chemistry have reported the examination of larger amounts of material, for which one or more trees were sacrificed.* For the procedure outlined below a sufficient quantity of material is provided by branches which have a diameter of one inch or more. This should facilitate sampling at frequent intervals and/or of several trees without inflicting gross injury. It embodies the experience gained during the past 15 months with the periodic collection of "cambial" materials and their preparation for analysis. The several steps follow the

* For a recent summary, refer to J. M. Stewart, G. L. Amos, and L. J. Harvey: Chemical Studies on Eucalyptus Regnans F. Muell., Australian Jour. Biol. Sci. 6, 21-47 (1953).

usual procedures for arresting enzyme action and for extracting the more soluble constituents, i.e., sugars, amino acids, phenols, lipoids, resins, etc. The less soluble constituents which form, or are associated with, the cellular structures, remain as marcs which have been reserved for analysis by conventional methods of wood chemistry.

The authenticity of the materials was checked by microscopic inspection. To this end, small "blocks," roughly 2x1.5x1 cm. (l. w. th.), were cut through the bark and into the sapwood. One such "block" was taken from each specimen, preserved in 70% ethanol, and submitted to the Microscopy Laboratory for sectioning, staining and identification of tissues.

Methanol was used in place of ethanol because it dissolves more sucrose and because it has a lower boiling point. The hexane extraction to remove fats and associated substances was made from 50% methanol in order to reduce trouble with emulsions which formed with water solutions.

The procedure seems to be satisfactory in a mechanical sense. So far, the final extracts have been used only for yield figures and for chromatographic indications of the sugars, amino acids, and phenols present. It is now pertinent to consider what additional assays should be made of these materials.

SEPARATION OF TISSUES

The details of separating the tissues to be analyzed vary with the species and the season. In general, the outer bark is scraped or sliced off to a depth just inside a layer of green cells which presumably contain chlorophyll; this outer material is discarded. The inner bark (phloem) is then removed, making as clean a separation as possible at the cambial layer. In the dormant seasons the surface of the sapwood is scraped, if necessary, to remove adhering phloem. In the growing season when the inner bark peels easily, the exposed wood surface is lightly scraped or shaved with a sharp blade to remove cellular elements that are (thought to be) in a state of transition. Under favorable circumstances these elements are removed as translucent strands. Otherwise the scrapings seem to be mostly a mixture of fibers and the aqueous contents of ruptured cells. In either case this material has been called soft xylem or soft sapwood. Finally, the branch (or stem) is sawed into short cylindrical sections and the sapwood samples are obtained by cutting off longitudinal-tangential slices. When the annual rings were sufficiently distinct, separate samples have been taken of the 1954 and the 1953 sapwood (xylem).

PREPARATION OF EXTRACTS

Glass-stoppered, 125-cc. Erlenmeyer flasks containing 100 cc. of absolute methanol are coded and weighed in advance. To receive a given sample, the flask is placed on a triple beam balance and the weights set

to correspond with the desired sample weight. For phloem and soft xylem, this is roughly 10 g.; for sapwood, from 12 to 20 g. The fresh tissue is transferred to the flask and immersed in the methanol as rapidly as it is separated from the branch in order to minimize oxidation and to stop enzyme actions. The flask is restoppered and accurately weighed.

To complete the extraction, the methanol is decanted into a soxhlet flask. The bulk of the sample is transferred with the aid of tweezers to a tared thimble and placed in the extractor; the remainder is rinsed into the thimble with 25 to 50 cc. of methanol. The soxhlet extraction is continued for 6 hours.

To obtain the dry weight of the extracted tissues, the thimbles with contents are drained in the extractor, exposed to moving air in a hood, held overnight in an oven at 65°C. under 23 inches of vacuum, cooled in a desiccator and weighed. The marc is ground in a small Wiley mill and reserved for analysis.

The cooled extract is filtered to remove small amounts of sediment or entrained fibrous matter and distilled under reduced pressure to a residue of 1 or 2 cc.

The residue is taken up with portions of 50% methanol totaling 5 to 7 cc. and transferred to a 60 cc. separatory funnel; 10 cc. of hexane are added, the mixture is vigorously shaken and then allowed to separate. The hexane generally dissolves the extractives which precipitated during the concentration and remained insoluble in 50% methanol.

The lower layer, which is usually clear, but occasionally opalescent, is drawn off into a 10 cc. volumetric flask. The hexane layer is washed with about 1 cc. of 50% methanol and the washings are likewise drawn off into the flask; this step is repeated until the solution has been made up to volume.

Total solids in the 50% methanol solution are determined by evaporating a 1.0 ml. aliquot in a tared 5 cc. beaker over a steam bath. The beaker is reweighed after cooling and further drying in a vacuum desiccator.

For chromatographic comparisons, 5 or 10 λ of the volumetric solution are spotted on triplicate strips of No. 1 Whatman filter paper of appropriate width and 24-inch length. In each set of 3 strips, the reference solution on the first contains carbohydrates; on the second, amino acids; and on the third, phenols. The developing solvent is butanol-acetic acid-water (63:10:27), applied by downward flow for 18 to 20 hours. The corresponding indicators are: (1) anisidine-HCl, (2) ninhydrin, and (3) $FeCl_3$-$K_3Fe(CN)_6$.

rek/mb

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Garner E. Wessman

MICROBIOLOGICAL ASSAY OF THE FREE AMINO ACID CONTENT OF THE CAMBIUM

A study was undertaken to test the applicability of microbiological assay of free amino acids in the cambium layer and associated tissues. Qualitative and quantitative determination would supplement and corroborate regular chemical analysis, particularly chromatographic methods.

The technique chosen is that devised by Steele, et al (1). In this method the organism, either Leuconostoc mesenteroides or Leuconostoc citrovorum, is grown on a chemically defined medium of amino acids, inorganic salts, dextrose, purines, pyrimidines, and vitamins. The amino acid to be determined is left out of the medium, and, if that amino acid is present in the material assayed, a growth response by the organism will result.

The method has followed that outlined in the reference using the titrimetric method with a 72-hour incubation period. The organism employed was L. mesenteroides P-60 which is carried as catalog No. 8042 by the American Type Culture Collection.

Initially the eight amino acids chosen for assay were lysine, arginine, histidine, tyrosine, phenylalanine, proline and aspartic and glutamic acids. Standard curves have been established for all of these acids; arginine, lysine, histidine, and tyrosine particularly give excellent curves. A standard curve is run for each assay, set up at 12 levels.

The curve for aspartic acid cannot be obtained unless asparagine is removed from the medium. This compound replaces aspartic acid for the organism, giving maximum growth even when none of the acid is added.

For the assay of glutamic acid, aspartic acid must be removed from the medium. The presence of the latter acid causes a depression of acid production in the lower levels of glutamic acid, resulting in a sigmoidal curve or very erratic results. This is due to an imbalance between the two acids resulting in such a response when aspartic acid is present in over three times the amount of glutamic acid (2). Steele, et al (1) suggest L. citrovorum for the assay of this compound. However, if aspartic acid is eliminated from the medium, but asparagine left in, a regular curve results with L. mesenteroides.

The materials to be assayed were provided normally in the dry state. These were taken up in water, diluted appropriately, and assayed at four to six concentration levels.

The results of the assays are shown in Table I. They are presented here with no attempt at analysis, since only a relatively few determinations have been carried out. However, they indicate the practicability of the method for the determination of amino acids in the cambial layer.

REFERENCES

(1) Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A. "Media for Leuconostoc mesenteroides P. 60 and Leuconostoc citrovorum 8081" J. Biol. Chem. 177 533-544 (1949).

(2) Baumgarten, W., Mather, A. N., and Stone, L. "Glutamic acid content of feed materials" Cereal Chem. 22 514-521 (1945).

TABLE I

FREE AMINO ACID CONTENT

Sample	Amino Acids (μgm per mg. of dry tissue weight)							
	Arginine	Aspartic Acid	Glutamic Acid	Histidine	Lysine	Proline	Phenylalanine	Tyrosine
Elm, New Xylem, (5/26/54)	12.0	---	0.0	1.20	0.0	0.0	0.0	1.60
Aspen T, xylem No. 1 (6/1/54)	1.76			1.20	0.03			2.20
Aspen T No. 4 (6/1/54)	0.44			1.20	0.52	0.77	2.1	0.38
Elm, New xylem (6/23/54)	0.42	0.63	2.91	0.17	0.42	0.16	1.16	0.17
Aspen T No. 3 (6/4/54)	2.50	4.16	6.94	1.05	0.55	1.22	31.66	0.94
Elm, phloem (6/23/54)	0.52	1.04	0.65	0.26	1.30	0.07	8.83	0.32
Elm, phloem, base (5/26/54)	0.52	0.0	1.56	0.97	0.67	0.39	3.11	0.39
Elm, wood, base (5/26/54)	7.50	0.63	5.63	0.94	1.25	3.75	5.25	0.98
Larch, scrapings (6/24/54)	1.11	0.11	0.83	0.49	0.55	0.01	1.11	0.07

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1266	33-34, 82-83

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ELM CAMBIUM III
PROXIMATE ANALYSES OF 1953 MATERIALS

This report assembles the results of proximate analyses of elm materials collected during 1953. Table I contains the values for the moisture contents determined at the time of collection. The values for the June to August samples were determined by oven drying; those for the September to December samples were estimated indirectly from yield data.

The values for the methanol soluble extracts are given in Table II and have been recalculated to a percentage basis from the yield data in Reports 1 and 2. These data are included because they have been used to calculate the values given in Table IV.

In addition to sample moisture values, Table III records figures for the ash content, methoxyl, nitrogen, uronic anhydride, and pentosan contents of the several materials. These analyses were performed by the Analytical Laboratory and reported in Code Office memoranda dated December 29, 1953 and January 28, 1954. All methods used were Institute standards as follows: moisture, no. 3; ash, no. 4; methoxyl, no. 18; uronic anhydride, no. 25; and nitrogen, no. 606. Acknowledgement is made to Dr. Browning and members of the Analytical staff for their interest and assistance.

The designations of the materials analyzed have the following meanings:

The phloem refers to the inner bark which can be stripped from the woody portion after the outer or corky bark has been removed.

Cambium in this instance was soft material scraped from the inner surface of freshly stripped phloem.

New xylem also was soft material, but scraped from the outer surface of the freshly exposed sapwood.

1953 xylem was essentially mature sapwood comprising the outer or current season's growth ring.

DISCUSSION

The tables are obviously incomplete. In part this was due to the time lag involved in developing a proper routine, in part to the need to combine some samples in order to have sufficient material, and also in part to changes in the cambial zone which occurred during the season. Of the latter, the texture of the new xylem may be mentioned. At the beginning of the collections it was quite soft and nonfibrous; by September it had become very tough and fibrous, being removed with some difficulty; thereafter the 1953 xylem was all mature sapwood. It is obvious, also, that these tables record too few observations to permit generalization, but a few trends seem definite enough to warrant watching during another growth cycle.

TABLE I

MOISTURE CONTENT OF ORIGINAL MATERIALS

Collected Material	June 29 %	July 3 %	July 7 %	Aug. 6 %	Sept.10 %	Nov. 12 %	Dec.16 %
Phloem	60	-	53	56	48	50	50
Cambium	82	-	-	-	-	-	-
New xylem	88	-	-	89	53	-	-
1953 xylem	-	-	-	-	33	34	39

TABLE II

METHANOL-SOLUBLE EXTRACTIVES

Collected Material	June 29 % d.b.	July 3 % d.b.	July 7 % d.b.	Aug. 6 % d.b.	Sept.10 % d.b.	Nov.12 % d.b.	Dec.16 % d.b.
Phloem	16	14	10	13	9	14	16
Cambium	-	-	44	-	-	-	-
New xylem	-	-	46	29	16	-	-
1953 xylem	-	-	-	-	2.0	3.5	4.4

TABLE III

VALUES FOR METHANOL EXTRACTED MATERIALS

Collected Assay	PHLOEM June 29 %	PHLOEM July 3 %	PHLOEM June 23 to July 7 to Aug. 6 %	PHLOEM Sept.10 %	CAMBIUM June 23 to July 3 %	NEW XYLEM June 23 to July 7 %	NEW XYLEM Aug.6 %	1953 XYLEM Sept.10 %	1953 XYLEM Sept.10 %
Moisture	6.4	6.5	6.4	6.2	6.0	7.4	7.4	6.0	4.9
Ash, d.b.	11.0	11.1	9.5	10.1	16.1	7.2	7.1	8.9	.70
Methoxyl,d.b.	1.8	1.6	1.5	1.6	1.3	1.8	1.9	2.3	5.5
Nitrogen,d.b.	.98	.70	.94	.88	3.6	3.0	3.8	1.7	.34
Uronic Anhydride,d.b.	10.6	12.7*	9.5	10.8	9.5	12.9	15.0	8.7	6.2
Pentosans,d.b.	16.2	15.7	13.5	14.5	-	-	-	-	20.4

*Averaged value

TABLE IV

VALUES CALCULATED TO ORIGINAL MATERIAL

Collected Assay	PHLOEM June 29 %	PHLOEM July 3 %	PHLOEM Aug.6 %	PHLOEM Sept.10 %	CAMBIUM June 23 to July 3 %	NEW XYLEM June 23 to July 7 %	NEW XYLEM Aug.6 %	1953 XYLEM Sept.10 %	1953 XYLEM Sept.10 %
Ash, d.b.	9.4	9.6	8.7	9.3	11.0	4.8	5.4	7.7	69
Methoxyl,d.b.	1.5	1.4	1.4	1.4	.97	1.2	1.5	2.0	5.4
Nitrogen,d.b.	.84	.61	.86	.77	2.5	2.0	2.9	1.4	.33
Uronic Anhydride,d.b.	9.0	11.0	8.7	9.4	6.5	8.6	11.3	7.5	6.2
Pentosans,d.b.	13.8	13.6	12.3	12.7	-	-	-	-	20.0

The moisture contents of the cambium and the new xylem appear to be very high for the collections from June to August. The new xylem collected in September shows a distinct drop from the previous samples and doubtless reflects the change from a succulent to a fibrous type of tissue, coincident with the approaching end of the growing season. The phloem seems to have a much more nearly uniform moisture content, though a fairly high one of 50 to 60%, whereas the xylem was the least hydrated material with a range of roughly 30 to 40%.

The amount of methanol soluble material roughly followed moisture content. The new xylem and cambium having the highest percentage of these extractives, the phloem being intermediate and the 1953 xylem the least.

Of the constituents reported in Table III, the ash values also have a certain parallelism with moisture contents. Methoxyl values are high only in the matured sapwood from the September collection. Nitrogen values are high only in the cambium and new xylem during the active growing period.

Some difficulty was at first experienced in obtaining consistent values for uronic anhydride in the July 3 phloem sample. This was shown by a spread from 9.8 to 14.3% in six determinations averaging 12.8% It was thought that the difficulty was due to the lack of uniformity in particle size which was evident in the material. Separations were made by analytical

screens and each screen fraction was analyzed for its uronic annydride content. No difficulty was experienced in obtaining checks with the different assays, but the spread was really not enough to account for the original variations. The values for the several fractions were:

on	60 mesh	12.4% uronic anhydride
60	120 "	12.0% " "
120	230 "	12.8% " "
through	230 "	13.2% " "

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CHEMISTRY OF BLACK LOCUST CAMBIUM: GENERAL CONSIDERATIONS AND OUTLINE OF PROJECT

SUMMARY

This report is presented as an introduction to the general problem of Cambial Chemistry. The work has been started as "pure" research, but it should in due time develop information of practical value to the Pulp and Paper Industry.

A review of botanical literature suggested that the tree as a whole be regarded as a converter of energy and matter, and that a comparison of the biochemical reactions which form wood and bark is a good approach to deciphering those conversions which occur in the cambial zone.

A tentative outline has been drafted for a comprehensive study of cambial chemistry. Initial activities include: bibliographic work; the collection, authentication and preservation of materials; and exploratory assays. As more specific analyses lead to the identification of chemical constituents, the location of the latter in the plant structures should be sought by micro-(histo-) chemical methods. By considering the known reactions of the various constituents in relation to their occurrence it should be possible to formulate hypotheses about the chemical transformations which are brought about by the cells of the cambial zone. In turn, such hypotheses should be checked with living materials, i.e., by experiments of a biological nature.

Thus the tentative program for research on cambial chemistry proceeds from available materials and facilities, but it also indicates the many-sided nature of the problem. In order to be really fruitful, the operations should be expanded as warranted by actual progress.

RELATION TO PULP AND PAPER INDUSTRY

As outlined below, cambial chemistry is essentially an inquiry into the reactions whereby living cells are transformed into wood and bark. This project is being undertaken as "pure" research because at this stage there is too little information to establish a rational connection with any specific, practical problem. But this initial situation is no justification for not seeking relations between these researches and the problems of the Pulp and Paper Industry. In general, these researches should increase the industry's understanding of the complex and individual life histories of the trees which produce pulpwood. This in turn should promote a more rational approach to the many production problems which result from the variability of pulpwood. Eventually, knowledge gained by the study of cambial chemistry may benefit other researches on such problems as the rate of tree growth, disease resistance, the selection or improvement of tree-varieties for specific pulp and paper uses, better pulping processes, and so on. In brief, the biochemical reactions of the cambial zone appear to be so fundamental in nature as to seem pertinent to many phases of the broad problem of conserving the industry's natural resources.

BOTANICAL BACKGROUND

Botanists designate as <u>cambium</u> the proliferating tissue which is

located between the bark and the wood on all lateral areas of a tree, i.e., trunk, branches, roots. The function of the cambium is to increase the diameter of the woody parts of the tree. This is accomplished by repeated division of the cambial cells, followed by enlargement and differentiation of the daughter cells. Those new cells which are formed on the inside of the cambium become wood, whereas those on the outside become inner bark.

Like other plant tissues which divide frequently, the cambium consists of thin-walled, simple (elementary) cells. For theoretical (genetic) reasons, some botanists consider that the true cambium consists of a concentric layer only a single cell thick. However, the daughter cells of such a hypothetical cambium are indistinguishable from the mother cells for some time. As seen under the microscope, the cross-section of the cambium is a zone of similar cells, 5 to 12 units wide along the radius. Accordingly other botanists regard the cambium as an aggregate of cells which exhibits a more or less gradual transition into the adjoining tissues, especially during periods of rapid growth.

Since the cambial cells are devoid of chlorophyll, they are dependent on other tissues for their nutrients. According to plant physiologists, organic substances resulting from photosynthesis in the leaves are transported to the trunk and roots through systems of cylindrical cells in the inner bark. Water, inorganic salts and stored materials move upward to the leaves through comparable cell-systems in the wood. The two conducting systems are interrelated by a third cell system, the medullary rays, which provide connections across the cambium.

Thus it is evident that the cambium is well situated to draw upon the resources of the tree to further its own function of cell-division and growth.

APPROACH TO PROBLEM

The botanical relations sketched above suggest that those attributes of the cambium which will determine the nature of a study of its chemistry include:

1. The cambial zone, though extensive in areas, is minute in thickness.

2. During the growing season, when it is most extensive, the cambial zone is composed of fragile cells in all stages of transition into sapwood and inner bark. When it is dormant, the cambium is more sharply defined, the cell walls are stronger, but it is difficult to separate.

3. The diverse cellular elements of wood and bark develop through metabolic influences from structural indistinguishable cambial cells.

Attributes 1 and 2 pose serious obstacles to the direct separation and analyses of cambial tissue.

Attribute 3 may indicate that cell differentiation results from the adaptation of a set of basic reactions common to all cells rather than from specific reactions bestowed upon individual cells predestined to a definite evolution.

Accordingly, it seems expedient to approach cambial chemistry by a comparative and somewhat indirect path. At first, the tissues of the

cambial zone will be separated to whatever extent is feasible and analyzed by the conventional wood-chemistry methods. Roughly speaking, these data pertain chiefly to cellwall materials. The next step will be to apply procedures which identify components of the cell contents, especially those in living tissues. As the various cell-constituents become known, their location in the plant should be sought by histo-(micro-) chemical means. Collaterally, the chemical behavior of all constituents should be considered in order to establish pathways whereby they may enter into, or be formed by, the basic reactions of the cell. Hypotheses formulated through these inquiries should eventually be supported by experiments with living plants or tissues. In this way the problem of cambial chemistry leads into the field of plant physiology. Once initiated, these several phases should be carried on more or less concurrently in the interest of effectiveness.

This approach recognizes the desirability of beginning the problem in a limited way with the means at hand. It also provides a rational basis for expanding the researches in a manner consistent with the scope of the problem. It reflects the viewpoint that the tree is essentially a converter of energy and materials. Obviously the chemical activity of the cambial zone is an important part of the total conversion because it creates the durable structures of the tree.

OUTLINE OF CAMBIAL CHEMISTRY RESEARCH

The following tentative general outline of cambial chemistry research will serve to orient the participants to convey to others some

concept of the nature of the undertaking, and to indicate the ramifications by which the project may be expected to expand.

I. Bibliography.

The study of the literature and the compilation of bibliographic material is obviously a basic and a continuing phase of the project. The first steps will cover the tree species and the materials being worked on in the laboratory. Then collateral information related to the experimental work will be sought. And, finally, the ramifications of the problem into other branches of science will be explored in order to develop significant theoretical interpretations.

Incidentally information about the workers in the field will be assembled. Such a file will be useful in developing contacts, exchanging information, avoiding unnecessary duplication and in using the results.

II. Selection of Tree Species for Experimentation

To date the tree species with which our experiments have been carried on have been selected entirely on the expedient basis that the material was at hand. As the work progresses, other considerations will become more decisive, including:

1. Previous work as recorded in the literature; e.g., the record may show that a certain species is either particularly suitable or unsuitable for the purposes of this project.

2. Availability. This consideration includes abundance, costs, accessability, seasonal variations.

3. Botanical considerations. These comprehend: classification, habitat and range, structure, physiological adaptations.

4. Industry problems. Other things being equal, a preference should be given to working with species that are commercially important.

III. Collection of Material

The amount of material to be collected and, to a degree, the manner of collection, are determined by the intended use. For the isolation and identification of constituents, especially unknowns, large amounts are needed. For comparative analyses or microscopic examinations, small samples suffice. The methods of collection which have been reported or suggested include:

1. Hand Peeling. In the growing season the bark is readily separated from the wood by cleavage of the cambium. After careful hand peeling of the bark, differentiating tissues can be scraped from the inner surface of the bark and the outer surface of the wood. At best, these tissue separations are not entirely satisfactory.

2. Mechanical Abrasion. A motor-driven rotary wire brush has been used to abrade inner bark of black locust. A color and texture difference between bark and wood indicates the limit of abrasion.

3. Perfusion. The sap-soluble solids of maple trees have been collected by perfusing water through branches one to two inches in diameter. This method should be considered for obtaining materials without extensive tissue disruption.

4. Test Blocks. For the microscopic inspection of cambial activity, small blocks are cut from the trunk surface with a knife or chisel. The injury is limited to a few sq. cm., making repeated sampling of the tree possible. With the development of microchemical methods this type of collection will be increasingly important.

5. Microdissection. The precise separation of tissues is possible with a microdissector, but it is a very tedious operation except for purposes of requiring extremely limited amounts of material, e.g., tissue culture.

IV. Preservation of Materials.

Cambial materials are very reactive and generally require stabilization of some kind even when intended for immediate analysis. The usual procedures represent some form of dehydration:

1. Air drying. Various forms of air drying have been used, especially with larger volumes of material. In general this is the least desirable type of stabilization, but eventually flash- or spray-drying could be considered.

2. Solvent drying. Methyl or ethyl alcohol, acetone, etc. are effective when simultaneous partial extraction is not objectionable.

3. Freeze-drying. Quick chilling and storage at temperatures below the freezing point of the tissues is very effective. Alternatively, vacuum drying in the frozen state has additional advantages. Facilities for these operations will be assembled.

V. Authentication of Materials.

It is axiomatic that analytical data are not really valid without a proper description or certification of the material analyzed. Applied to cambial chemistry, this requires an accurate designation of both tree species and the separated tissues. To this end "blocks" and subsamples of materials collected will be submitted routinely to the Microscopy Laboratory for sectioning and identification.

Besides authentication of materials, a record of the state of cambial activity and the presence of visible cell constituents, e.g., starch, will be gained. This routine will also be a preparation for microchemical studies.

VI. Methods of Investigation

The methods of investigation applicable to cambial chemistry are so diverse and so interdependent that little more can be done at this stage than to list some which are obviously pertinent or especially promising. The relative importance of a given procedure is conditioned by the degree of progress which has been achieved in either a specific phase of cambial chemistry or with the problem as a whole. Hence, this summary should be regarded as subject to constant revision. None the less, even a rough inventory is useful in that it is one means of describing the nature of

this undertaking and of the means required to make it fruitful. To serve these considerations, the proposed methods of investigation are divided into three groups:

A. Analytical Procedures

The purpose of analytical procedures is to isolate and characterize the constituents of cambial zone tissues and to assay their content. This group includes:

1. Mechanical separations, e.g., hand peeling, grinding, sieving, flotation, sedimentation, etc.

2. Neutral solvent separations, e.g., percolation, soxhlet extraction, partitioning between immiscible liquids, fractional crystallization, etc.

3. Chemical reagents, e.g., acid or alkali extractions, saponification, esterification, type reactions, etc.

Especially promising procedures include:

4. Chromatography and related techniques for the resolution, purification, and identification of small quantities of material.

5. Liquid-liquid partition (e.g. by Craig apparatus) for resolution of chemically homogeneous systems.

6 Cold room (0 to 5°C.) manipulation of enzymes, proteins and other very unstable systems.

B. Optical Procedures

Arbitrarily grouped as optical procedures are a number of instrumental methods whose function is to locate in cambial tissues the constituents identified by analysis. Inferences about biochemical functions will follow from these observations. Included are:

1. Histochemical methods with ordinary and polarizing microscopes—sectioning, staining, microchemical reactions, etc.

2. Special forms of microscopy, e.g., "phase," ultraviolet light, electron, and x-ray.

C. Biological Procedures

The purpose of biological procedures is to confirm the biochemical inferences drawn from the analytical and optical investigations. This group includes:

1. Enzyme reactions, e.g., kinetics, specificity of substrates, etc.

2. Tracer studies with isotopes

3. Trapping of intermediates

4. Blocking of biochemical reactions

5. Tissue culture

6. Propagation of genetic or clonal types.

rek/rm

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DATE January 21, 1954
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CHEMISTRY OF ELM CAMBIUM II
COLLECTION OF MATERIALS, 1953
(SUPPLEMENTARY)

SUMMARY

Two saplings were cut to provide material for developing procedures suitable for multiple assays. Since the cambium was dormant, only phloem (inner bark) and 1953 sapwood were separated. The usual biometric and yield data are recorded.

INTRODUCTION

The end of the growing season terminated the program of collecting cambial zone materials during 1953. However, it had become evident meanwhile that there was a need for better methods of making multiple analyses. It was thought that chromatographic procedures, which require only small samples, would be suitable. Accordingly two more saplings were cut for material to develop a routine assay. The data in this report pertain to the collection and preparation of the samples; they supplement those of the previous report.

EXPERIMENTS

Collection of Materials

On November 12 and December 16 a sapling was taken from the Institute plot. Cross-sectional disks, about 3/4-inch thick (vertical axis), were cut

from the stem at three elevations, i.e., at the top in the region of young growth as indicated by smooth green bark, at a midsection of intermediate age as indicated by partly fissured corky bark and at the base where all the bark was corky. A small "block" was cut, preserved in methanol and submitted to the Microscopy Laboratory for sectioning.

From each disk the outer bark, including a green layer, was carefully peeled away with a knife and discarded. The phloem (inner bark) was then cut away in narrow strips and immediately placed in methanol. Whereas the phloem was leathery and discolored rapidly when injured, the outer sapwood was hard, light colored, and apparently stable. Taking advantage of this difference, the surface of the sapwood was scraped to free it of discoloring tissue, i.e., of phloem elements. The 1953 increment of sapwood was then split from the disk with a narrow chisel and the splinters were immersed in methanol.

Thus six samples were prepared from each sapling. Each sample was next extracted with methanol in a soxhlet, the methanol extract distilled to dryness in a vacuum, the residue dissolved in H_2O, the solution clarified by suction filtration through a bed of celite and made up to a volume of 10 cc. The marcs were dried first in air and then in a vacuum oven at 60-65°C.

The numerical data pertinent to these manipulations are presented in the accompanying tables.

TABLE I

BIOMETRIC DATA

Date	Diameters* Top cm.	Middle cm.	Base cm.	Growth Rings Top	Middle	Base
November 12	3.2 x 2.8	4.5 x 4.0	6.7 x 6.3	5	6	10
December 16	2.6 x 2.3	4.0 x 4.0	5.5 x 5.0	4	6	8

* The two diameters indicate the elliptical shape of the cross-section.

TABLE II

YIELD DATA, NOV. 12 SAMPLES

No.	Datum	PHLOEM Top gm.	PHLOEM Middle gm.	PHLOEM Base gm.	1953 SAPWOOD Top gm.	1953 SAPWOOD Middle gm.	1953 SAPWOOD Base gm.
1	Wt. of Fresh Material	3.8470	4.4555	9.2253	3.2483	5.5308	9.6129
2	Wt. of CH_3OH Soluble Solids	.216	.291	.856	.071	.121	.237
3	Dry Wt. of Marc	1.6191	1.8797	4.0666	2.1386	3.3999	6.0194
4	Dry Wt. of Material (2 + 3)	1.8351	2.1707	4.9226	2.2096	3.5209	6.2564
5	H_2O in Fresh Material (1-4)	2.0119	2.2848	4.3027	1.0387	2.0099	3.3565
		%	%	%	%	%	%
6	% H_2O in Original Sample (5:1)	52.3	51.3	46.6	32	36.3	34.9
7	% CH_3OH Soluble Solids Dry Basis (2/4)	11.8	13.4	17.4	3.2	3.4	3.8

TABLE III

YIELD DATA, DEC. 16 SAMPLES

No.	Datum	PHLOEM Top gm.	PHLOEM Middle gm.	PHLOEM Base gm.	1953 SAPWOOD Top gm.	1953 SAPWOOD Middle gm.	1953 SAPWOOD Base gm.
1	Wt. of Fresh Material	8.1251	7.8965	9.1049	15.7418	14.8540	15.6671
2	Wt. of CH_3OH Soluble Solids	.466	.686	.913	.408	.407	.406
3	Dry Wt. of Marc	3.3918	3.3080	3.9337	9.5808	8.6921	8.6031
4	Dry Wt. of Material (2 + 3)	3.8578	3.9940	4.8467	9.9888	9.0991	9.0091
5	H_2O in Fresh Material (1-4)	4.2673	3.9025	4.2582	5.7530	5.7549	6.6580
		%	%	%	%	%	%
6	% H_2O Original Sample (5:1)	52.5	49.4	46.8	36.6	38.7	42.5
7	% CH_3OH Soluble Solids Dry Basis (2:4)	12.1	17.2	18.8	4.1	4.5	4.5

PROJECT REPORT FORM

PROJECT NO. 1702 - (A)
COOPERATOR Institute
REPORT NO One
DATE November 1, 1953
NOTE BOOK 871 and 1186
PAGE ____ TO ____
SIGNED R. E. Kremers

R. E. Kremers
Barbara Reeder

Notebook
871: 97-160
1186: 25-99

Copies to: Files
Dr. Kremers (2)

CHEMISTRY OF ELM CAMBIUM I. COLLECTION OF MATERIALS, 1953

This is a report on the collection and preparation of materials for the chemical study of cambial activity in the American elm, Ulmus americana, L. This species was chosen in preference to collecting fresh material of the black locust (project 1702) because it is readily available in the Institute's demonstration plot.

Six saplings were cut in the interval June 23 to September 10, inclusive. Living tissues were separated when possible, e.g. phloem (inner bark), "cambial scrapings", new xylem (from surface of wood), 1953 sapwood, and preserved in methanol. Subsequently the methanol-soluble constituents of these tissues were extracted and recovered for analysis. Analogously the structural and other insoluble constituents were prepared for the conventional "wood analyses" and submitted to the Analytical Laboratory.

To assure the authenticity of tissue designations and to record the state of cambial activity, appropriate "blocks" of bark and wood were submitted to the Biology Laboratory for sectioning.

INTRODUCTION

The problem of collecting fresh material for the study of "cambial chemistry" was discussed with Dr. Isenberg. Black locust trees are not abundant in the vicinity of Appleton, hence do not offer a ready supply for continuing Project 1702. On the other hand, there is an abundance of self sown elm seedlings (Ulmus americana, L.) in the Institute's demonstration plot. They are readily accessible and their removal is a benefit to the evergreens and other systematically planted species. Accordingly, project 1702-A was initiated to provide for the collection and analysis of elm material.

OBJECTIVES

The immediate objectives of this project are:

1. To provide fresh material for the study of Cambial Chemistry.
2. To gain experience with methods appropriate to the study of Cambial Chemistry.
3. To define specific problems within the generic problem.
4. To develop additional knowledge of the chemistry of American elm.

DIGEST OF LITERATURE

A. Other species of Elm.

According to Sargent—Manual of Trees of North America—Appleton is situated within the territorial range of three species of elm:

Ulmus americana, L.	White or American Elm
Ulmus fulva, Michx.	Slippery Elm
Ulmus racemosa, Thomas	Cork Elm

Two other species are not found this far north

Ulmus alata, Michx.	Wahoo, Winged Elm
Ulmus crassifolia, Nutt.	Cedar Elm

One species, Ulmus serotina, Sarg., Red Elm, has become naturalized locally; it is said to be rare.

B. Pulp Uses.

Experimentally U. americana yields a "good pulp" by the semi-chemical neutral sulfite process (Isenberg page 132; quoted from Thidester, F.P.L.), but industrially its use is limited to its incidental inclusion in mixed hardwood cooks.

C. Chemical Composition

An old report, quoted by Wehmer, states that the bark of Ulmus americana L. is rich in tannin and mucilage. Two proximate analyses of the heartwood have been reported (Pulpwoods, Isenberg), the most significant datum being an alpha-cellulose content above 50% (L. E. Wise). A reference to earlier use of elm barks as a source of "dyes and tans" is devoid of scientific information.

Because of its medicinal use, the mucilage from the bark of the slippery elm, Ulmus fulva Michx., has been carefully examined. The procedures for extracting, purifying and hydrolyzing this mucilage (Anderson, Gill, et al.) should be useful in the analysis of material from U. americana. The aldobionic acid obtained from slippery elm affords a specific point of comparison between the two species. Starch and Ca-oxalate are reported present.

For the common European elm, Ulmus sativa, Mill., comparative proximate analyses have been recorded for the "cambium and differentiating xylem," "newly formed wood" and "mature sapwood." Like similar analyses of "cambium" in other tree-species, the figures reveal a high proportion of water, water-soluble cell constituents and protein, which are characteristic of growing tissues, whereas the proportion of cellulose and lignin, the structural elements characteristic of mature tissues is low. (Allsop and Misra).

In another European species, Ulmus campestris galls collected in Turkey were reported to contain a tannin of the catechol type (C.A., 1943). Cambial tissue of this species has been cultivated in vitro (morphology? C.A. 1940). Ulmus effusa contains $CaCO_3$ (4.3%, through Wood Chemistry 2nd ed.).

In the diseased condition known as "wetwood" the sap of elms (American ?, C.A. 1946) contained large amounts of potassium and phosphate. The pH of normal sap and wood was acidic, 6.35 and 5.89 respectively; that of diseased sap and wood was slightly alkaline, 7.67 and 7.39 respectively.

References to structural features of elm cambium are found in a recent book (Esau, 1953). The seasonal activity of the elm cambium has been followed by histological observations (N. Y. S. C. For., Bull. 1928).

Chemical Abstracts refer to a number of publications which have not yet been consulted. They deal with other organs, e.g., leaves, and do not have a direct bearing on cambial chemistry at this time.

At this date (November, 1953) no reference has been found dealing with the chemistry of the cambium and its transformations in the American elm. Accordingly the results of this project should provide new information at least for this species.

An annotated bibliography will be prepared.

COLLECTION OF MATERIALS

The stand of elms in the demonstration plot was inspected with Dr. Isenberg and the first sapling was cut on June 23, 1953. At this date growth was already well advanced; consequently materials representing the early stages of an annual cycle will not be available until 1954. Subsequently

5 more saplings were taken. The procedures used to obtain the different living tissues followed the general pattern set by previous investigators.

After a sapling had been felled and trimmed of branches, it was cut into suitable lengths so as to separate the part with smooth bark from the older part with corky surfaces. From the latter the corky outer bark was largely removed with a draw-shave, avoiding as much as possible slicing off portions of phloem (inner bark). Residual patches of cork and a layer of green cells (phellogen?) were scraped from the outer surface of the phloem. These manipulations injured the latter tissue and caused it to turn reddish brown within a few minutes. The discoloration appeared to be only superficial. The elements removed in this step were set aside to air dry; no extensive investigation of them is planned.

After the phloem had been exposed, annular and longitudinal incisions were made through it into the stem so that strips of phloem roughly 1-2 cm. x 12-16 cm. could be peeled off. In the case of the first three saplings, scrapings were taken with a sharp knife blade from the inner surface of the phloem strips because the material so obtained was supposed to be composed of cambial cells. But this practice was abandoned when it seemed unlikely either to be a valid tissue differentiation or to provide a material which had other significance. The scrapings ("cambium"), scraped phloem, and also some entire (unscraped) phloem were preserved by immediate immersion in absolute methanol.

When the inner surface of the phloem was scraped, it darkened very rapidly. In an effort partly to segregate this oxidized tissue and partly to differentiate between younger and older portions of the phloem, a shallow incision was made on the inner surface near the end of a phloem strip and a layer of tissue pulled off. Since the separation proved to be quite variable, the effort seemed to serve no useful purpose and was abandoned.

The stem surface exposed by the peeling of the phloem was quite different from the phloem surface with which it had contact. It was jucier, less fibrous, softer, and did not darken rapidly. A thin layer could readily be sliced off the surface by running a sharp knife blade, held tangentially, down the stem. The ribbons of tissue so obtained somewhat resembled the texture of young onion scales. This material, designated "new xylem" or "wood scrapings" was immediately transferred to absolute methanol. These characteristics hold for the four saplings felled from June 23 to July 7 inclusive. In the specimen collected August 6 this tissue was drier and more fibrous; however, the phloem still peeled smoothly from the stem. But in the sapling collected September 10, the surface elements remaining on the stem seemed to be essentially phloem; material similar to the "new xylem" of the previous collections was not obtained.

In the case of the September 10 sapling, a number of discs 2-3 cm. thick were cut from the stem. From these the 1953 growth was split off by placing the edge of a small chisel on the porous ring and

tapping the handle with a hammer. This new growth sapwood was preserved in methanol. With this exception, the remainder of the stem was set aside to air dry. No heartwood had formed in any of these saplings.

Except the first sapling, the felling and collection of tissues was accomplished within one workday. Obviously some metabolic changes occurred in this interval, but no attempt has been made to measure them.

After the above operations became somewhat routinized, time was available to cut "blocks" through the phloem, cambium, and new xylem into the sapwood. These were preserved in methanol or 70% ethanol and sent to the Biology Laboratory for the preparation of cross- and radial longitudinal-sections. These slides will be used to determine the stage of cambial activity at the time of collection and to identify the tissues actually obtained by the several manipulations.

The collection of materials is summarized in the accompanying table.

TABLE I

ELM

COLLECTION OF MATERIALS--1953

Code: √ air-dried; 0 preserved in CH_3OH

Date	Stem				Blocks	Bark		Inner Bark				Wood		
	Diam. Base	Diam. Top	Rings Base	Rings Top		Corky	Green	Entire	Scrapings	Scraped	Strips	Scrapings	53 Ring	Sap-wood
June 23	10/4.2 cm.		7/-			√		0	0	0	√	0		√
June 29	7/3.5 cm.		7/5			√			0	0	√	0		√
July 3	7.5/2.8 cm.		8/3			√	√	0	0	0		0		√
July 7	7.5/2.7 cm.		8/3		0	√	√	0				0		√
Aug. 6	9/4 cm.		8/3		0	√	√	0				0		√
Sept. 10	6/1.8 cm.		6/2		0	√	√	0				0	0	√

METHANOL PRESERVED MATERIAL

The materials preserved in methanol were kept at refrigerator temperatures until they could be worked up. Judged by color, they were stable under these conditions.

Since the conditions of preservation constituted partial extraction, it was logical to complete this process. The bulkier materials, i.e. the phloem strips and the 1953 sapwood were transferred to large round-bottom flasks, covered with the methanol solution in which they had been preserved and heated to reflux for one hour. After the hot methanol solution had been decanted, the phloem strips were covered with fresh methanol and refluxed for an hour to give a second extract. A third extraction was made in like manner.

The three methanol extracts from each unit of material were kept separate and their volumes recorded. Duplicate 2.0 cc. aliquots were taken of each fraction, carefully evaporated over a hot-water bath in small tared beakers, heated 30 minutes in an air oven at 105°C., cooled in a desiccator and weighed to estimate the yield of solid extractives. The extracted phloem was exposed under a hood to evaporate retained methanol, then vacuum-dried overnight at 60-65°C., weighed and stored in desiccators. Later the accumulated material was ground in a Wiley mill until it passed through a 40-mesh sieve.

The methanol solutions were distilled under reduced pressure (about mm.) in the reverse order of their preparation. Water bath temperature was kept below 45°C. Since the elm materials contained much water, the residual

concentrates were aqueous solutions. The change from methanol to water as the predominant solvent precipitated a small amount of yellowish, wax-like substance which was filtered out of the concentrate with the aid of suction.

[Residues and extracts obtained by the above procedure are no.: 871-118.2; 871-125.2; 871-126; 1186-29.1, 1186-29.4; 1186-70.1, 1186-69.2, 1186-37.3; 1186-37.1; 1186-50.2, 1186-50.4, 1186-67.2; 1186-66.2.]

The less bulky materials, specifically the scraping from the inner surface of the phloem, originally designated as "cambium" and the scrapings from the stem surface exposed by the peeling of the inner bark, designated as "new xylem", were transferred to soxhlet thimbles and extracted eight hours with fresh methanol. The methanol solutions were concentrated, and the marcs were dried, in the manner described above. All concentrated extracts were kept under refrigeration; the dried marcs were kept in desiccators until ground in either a large or small Wiley mill to pass a 40-mesh screen.

[Residues and extracts obtained in this series are no.:

1186-72.4;	1186-74.3;	1186-76.4;
1186-72.1;	1186-74.1;	1186-76.3.]

TABLE II

ELM--1953

Yield of Methanol Preserved Materials

Collection Date		June 23, g.	June 29, g.	July 3, g.	July 7, g.	Aug. 6, g.	Sept. 10, g.
Phloem:	after extraction	--	49[1]	73	88	300	103
	CH_3OH extract[2]	4.9	9.6	12.3	10	45	10
	combined weight	—	59	85	98	345	113
Inner Phloem:	after extraction	—	--	4[3]			
	CH_3OH extract[2]	—	—	3.1			
	combined weight	—	—	7			
Outer Xylem:	after extraction	—	—	--	8[4]	7	7.5
	CH_3OH extract[2]	—	--	--	6.9	2.9	1.4
	combined weight				15	10	9
Sapwood, 1953 growth:	after extraction						68
	CH_3OH extract						1.2
	combined weight						69

[1] Includes collection of June 23
[2] Estimated yields
[3] Includes collections of June 23 and 29
[4] Includes collections of June 23, 29 and July 3.

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