Table 3. *Two-hourly output of uric acid in urine*

Time	Vol. (ml.)	Sp. gr.	pH	Uric acid	
				(mg./ 100 ml.)	(mg.)
12 noon	350	1005	6.4	14.8	51.8
2 p.m.	74	1018	7.4	41.7	31.3
4 p.m.	263	1006	5.8	20.0	52.6
6 p.m.	153	1010	6.0	32.7	49.1
8 p.m.	130	1012	5.8	34.5	45.0
10 p.m.	158	1012	6.6	33.0	39.5
12 p.m.	130	1011	6.6	37.9	50.5
2 a.m.	86	1015	5.4	33.0	26.4
4 a.m.	54	1018	5.2	35.4	17.8
6 a.m.	43	1020	5.2	45.4	19.5
8 a.m.	40	1020	5.4	55.5	22.2
10 a.m.	44	1018	5.4	53.5	23.1
Total, 10 a.m. to 10 a.m.					428.8

by comparing the uric acid values of fresh samples with samples kept for 24 hr. in presence of dilute acid and alkali. As the results were almost identical, it appears that uric acid in urine occurs as a simple solute (Table 4).

Table 4. *Uric acid values of urine after treatment with dilute acid or alkali at 18°*

Urine	Uric acid (mg./100 ml.)	
Fresh, pH 5.8	30.5	30.3
After 24 hr., undiluted	30.1	30.3
After 24 hr., diluted with an equal vol. of water	30.3	30.6
After 24 hr., diluted with an equal vol. of N/10-HCl	30.12	29.94
After 24 hr., diluted with an equal vol. of N/10-NaOH	30.5	30.3

DISCUSSION

Validity of the method. In general, the values obtained for urinary uric acid by the chloroimide

method are of the same order, but slightly lower than values obtained by the phosphotungstate reduction methods. Variations, however, often occurred in the results obtained by different clinical workers using different preparations of the phosphotungstate and cyanide reagents, and it was concluded that unless the entire group of methods was checked by the actual isolation and direct estimation of the uric acid present in the samples, conclusions could not be safely established. This procedure was adopted by Myers & Wardell (1928), but present circumstances did not allow of it being attempted with prospect of the same degree of accuracy as that postulated by the American workers. The exhaustive survey by Brøchner-Mortensen (1937) shows that any comparison of values for urinary uric acid depending on colorimetry can be misleading, as the same set of errors may reappear in different methods, notably those involving tungstate reduction, which, according to Folin (1934), 'represents probably the most complex reaction we have in the whole field of practical colorimetry'.

SUMMARY

1. In buffer solutions at pH 9–10, uric acid reacts with 2:6-dichloroquinone-chloroimide, forming a yellow pigment that turns red on addition of Ag⁺.
2. The test can be used for the estimation of uric acid in concentrations down to 1 in 100,000, and has been adapted for urine analysis.

I wish to thank Prof. T. J. Nolan for a pure preparation of 2:6-dichloroquinone-chloroimide. I am indebted to Dr E. Kawerau for having carried out the experiment on the 2-hourly excretion of uric acid, and to Mr J. Harwood for having done most of the estimations.

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Some Hemicellulose Extraction Methods

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Hemicellulose preparations made by methods involving extraction of woody tissues with aqueous sodium hydroxide are mixtures, containing materials resistant to chlorination and others which are

rendered soluble in sodium sulphite solution by this treatment. The two types have tentatively been designated cellulosan and encrusting hemicelluloses (Norman, 1937; Preece, 1941), and their proportions

in a given preparation depend on the actual conditions of extraction. It is clear that, to obtain a useful knowledge of the nature of each, separate extraction is necessary, and since it has been shown that delignification facilitates hemicellulose extraction (see, e.g., Norman & Shrikhande, 1935; Norman, 1937) the suggestion arises that holocellulose would provide the ideal starting material. Thus, Mitchell & Ritter (1940) have successively extracted holocellulose with water, dilute sodium carbonate solution, and sodium hydroxide solution; however, this does not take into account the dual origin of hemicellulose fractions, whilst the use of hot alkaline solutions for extraction is inadmissible owing to the certainty of hemicellulose degradation thereby (Preece, 1940).

A general consideration of the position suggests points which must receive attention in attempting a separation. Preliminary extraction with boiling 0.5% (w/v) ammonium oxalate solution and with water is desirable to remove pectin and water-soluble materials; suitable means of delignification should then be sought, but the procedure must not itself involve any significant loss of furfuraldehyde-yielding material nor must it diminish the yield, or in any way modify the crude cellulose as determined on the wood residue or holocellulose and compared with that from the original wood. In the most favourable case complete extraction of encrusting hemicellulose might then be possible with water, though other solvents (e.g. 1% (w/v) sodium sulphite solution) could be used if it were shown that they have no degradative effect. Again such extraction must not influence the yield or composition of the crude cellulose. Careful control is necessary at all stages, so that the origin and fate of all furfuraldehyde-yielding material is known. The possibility of separately extracting encrusting hemicelluloses following simple delignifying methods, not necessarily leading to holocellulose production, is examined below.

METHODS

Since large-scale working is ultimately to be aimed at, it seems preferable to use a modification of the Norman & Jenkins (1933) hypochlorite technique in those cases where chlorination is to be applied. Comparative determinations require to be carried out on individual samples and not successively on the same sample, since oven-drying apparently increases the solubility of certain of the cellulosic materials present (Norman, 1936). Willow sawdust was used, as this material had proved to be rich in encrusting hemicelluloses (Preece, 1941) and to yield preparations the compositions of which are very susceptible to conditions of working; a new sample of the sawdust being used, results are not directly comparable with those quoted earlier. All determinations were carried out on 2 g. portions of the

sawdust, which had been previously extracted in bulk with boiling 0.5% (w/v) ammonium oxalate solution and with water and air-dried at room temperature; the necessary adjustments for the moisture contents of the original wood and of the air-dried residue are made in the calculations.

Chlorination. The extracted wood is washed into a beaker with 100 ml. of 0.2N- H_2SO_4 , 5 ml. of sodium hypochlorite solution (approx. 12% (w/v) of available chlorine) are added, and the mixture is stirred for 7 min. with the beaker standing in a vessel of water to maintain the temperature within the range 14–18°. The volume of acid is just sufficient to liberate substantially the whole of the chlorine. The wood is then filtered at the pump and well washed with water. If the immediately following treatment is to be with an ethanolic reagent, the residue is further washed with 80% ethanol.

Alkaline treatments and pretreatments. The wood, with or without preceding chlorination, is washed into the beaker with 40 ml. of the ethanolic alkaline reagent, and treated with stirring at controlled temperature for 7 min. as before, except in the cases of some experiments summarized in Tables 3, 4 and 5, where the alkaline treatment was for longer times. After filtration and washing with 80% ethanol, the residue is available for any desired further treatment. After the final alkaline treatment of a series, the ethanolic washing is in some cases followed by washings with ethanolic H_2SO_4 (5 g. of H_2SO_4 /100 ml. of 80% ethanol) and aqueous 0.2N- H_2SO_4 (in that order when both are used).

Aqueous extraction of residue. The residue is twice extracted under reflux for $\frac{1}{2}$ hr. periods with 100 ml. portions of water, extracts and washings being combined and concentrated for further examination.

Sulphite extraction. This normally follows aqueous extraction and is carried out similarly, using 100 ml. portions of boiling 1% (w/v) sodium sulphite ($\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$) solution.

Crude cellulose determination. The procedure for chlorinations is precisely that described above, but the subsequent sulphite extractions are for 20 min. each, with 80 ml. portions of 3% (w/v) sodium sulphite solution in a boiling water-bath.

Lignin determination. Lignin was not determined separately as such, owing to the uncertainty attaching to results obtainable by the usually available methods. However, a measure of the degree of delignification is available for many of the treatments by subtracting the figure for the yield of wood residue from 94.92, the value for wood residue after extractions with ammonium oxalate and water (Table 1). The figure for delignification so obtained will slightly over-estimate the true value, owing to the loss of a small proportion of furfuraldehyde-yielding material (see below); it will, however, serve as a useful guide to the progress of lignin removal.

RESULTS

As a measure of the efficiency of extraction, furfuraldehyde yields were determined on wood residues, aqueous and sulphite extracts and crude cellulose. Further, the yields of wood residue and crude cellulose (both ash-free) are also quoted. For ease of comparison, all results (unless otherwise stated) are calculated to the original, dry, unextracted wood.

Table 1. *Influence of progressive alternation of chlorination and alkaline extraction of willow sawdust on the yield and composition of wood residue and crude cellulose and the solubility of furfuraldehyde-yielding components.*

(Final washing includes use of ethanolic and aqueous H_2SO_4)

No. each of chlorinations and alkaline treatments...	No treatment 0	Treatment with 40 ml. of ethanolic NH_3 *					Treatment with 40 ml. of ethanolic NaOH †		
		2	4	6	8	10	2	4	6
Wood residue (%)	94.92	93.38	87.85	83.45	77.04	74.97	70.84	66.84	66.27
Crude cellulose (%)	57.79	56.32	55.67	54.75	55.03	54.77	56.71	54.01	52.07
Furfuraldehyde from:									
(a) Wood residue (%)	14.42	14.30	14.18	13.95	13.50	13.41	13.62	12.95	13.08
(b) Aqueous extract (%)	0.00	0.70	1.26	1.76	2.33	2.39	3.11	3.95	5.16
(c) Sulphite extract (%)	0.21	2.93	3.46	3.37	2.93	2.92	0.92	1.04	0.96
(d) Crude cellulose (%)	9.18	8.79	8.35	7.85	7.99	7.98	8.63	7.15	6.26
(b + c)	0.21	3.63	4.72	5.13	5.26	5.31	4.03	4.99	6.12
(b + c + d)	9.39	12.42	13.07	12.98	13.25	13.29	12.66	12.14	12.38

(Results as percentage of original dry wood.)

* 10 ml. of ammonium hydroxide (sp. gr. 0.88) and 90 ml. of 80% ethanol.

† 4 g. of NaOH /100 ml. of 80% ethanol.

Table 2. *Changes in yield and composition of crude cellulose, and in the solubility of furfuraldehyde-yielding components of willow sawdust, resulting from various alkaline pretreatments*

(Six chlorinations and six alkaline pretreatments, without final acid washings)

Alkali used ...	NH_3 *		NaOH		Ethanamine	
	In 80% ethanol	In 95% ethanol	4% In 80% ethanol	1% In 80% ethanol	3% In 95% ethanol	5% In 95% ethanol
Crude cellulose (%)	55.76	55.74	53.24	57.21	56.30	56.71
Furfuraldehyde from:						
(b) Aqueous extract (%)	2.05	1.95	5.12	3.11	1.40	1.42
(c) Sulphite extract (%)	2.91	3.03	0.17	0.19	2.70	2.69
(d) Crude cellulose (%)	8.11	7.89	7.14	9.16	8.17	8.35
(b + c)	4.96	4.98	5.29	3.30	4.10	4.11
(b + c + d)	13.07	12.87	12.43	12.46	12.27	12.46

(Results as percentage of original dry wood.)

* 10 ml. of ammonium hydroxide (sp. gr. 0.88) and 90 ml. of 80 or 95% ethanol.

Table 1 shows the progressive delignification and the increasing water-solubility of hemicelluloses which result from increasing numbers of 7 min. treatments with ethanolic NH_3 or ethanolic NaOH as delignifying agents following in alternation with an equal number of chlorinations. With NaOH , delignification is substantially complete after six pairs of treatments, whilst further treatment with NH_3 beyond this number does not increase the combined yields of furfuraldehyde from aqueous and sulphite extracts. Since, however, the partition of furfuraldehyde between the two extracts may be influenced by the final acid washings, the effect of these is shown by Table 2. In obtaining these results NH_3 and NaOH have each been used in two combinations with ethanol, and treatments have also been included using ethanamine in two concentrations.

Ethanolic NaOH itself plays a big part in increasing the water-solubility of hemicelluloses, even though chlorination is not applied, as shown for

single treatments in Tables 3 and 4. The further influences of variations in time of treatment, of different modes of washing prior to aqueous and sulphite extraction, and of changes in NaOH concentration, are also demonstrated.

Further light on the rendering soluble of hemicelluloses is thrown by the series of treatments and pretreatments summarized in Table 5, in all of which the alkaline reagent used was ethanolic NaOH (4 g./100 ml.).

(i) Sawdust was treated for four periods, each of 1 hr., with this reagent, each treatment being followed by washing with ethanol and ethanolic H_2SO_4 only, and by two extractions with boiling water. Four aqueous extraction fractions were thus obtained on each of which furfuraldehyde was determined.

(ii) A single 1 hr. treatment with ethanolic NaOH with washing and water extraction as in (i) was followed by chlorination (7 min.) and treatment with ethanolic (95%) ethanamine (5 ml./100 ml.). Washing with ethanol and ethanolic H_2SO_4 was followed by extraction with boiling

Table 3. *Influence of a single pretreatment with ethanolic NaOH* (without chlorination) on the solubility of furfuraldehyde-yielding components of willow sawdust*

Washing prior to extraction ...	Ethanol, ethanolic H ₂ SO ₄ , aqueous H ₂ SO ₄				Ethanol, ethanolic H ₂ SO ₄				Ethanol only
Treatment time (hr.) ...	1/2	1	2	24	1/2	1	2	24	24
Wood residue (%)	—	—	—	—	—	91.05	—	—	—
Crude cellulose (%)	—	—	—	—	59.27	58.68	57.91	57.44	—
Furfuraldehyde from:									
(a) Wood residue (%)	—	—	—	—	—	14.28	—	—	—
(b) Aqueous extract (%)	1.38	1.90	1.94	2.54	1.98	2.37	2.63	2.87	3.19
(c) Sulphite extract (%)	0.48	0.40	0.71	0.74	0.19	0.18	0.18	0.20	0.00
(d) Crude cellulose (%)	—	—	—	—	10.18	10.04	9.92	9.80	—
(b + c)	1.86	2.30	2.65	3.28	2.17	2.55	2.81	3.07	3.19
(b + c + d)	—	—	—	—	12.35	12.59	12.73	12.87	—

(Results as percentage of original dry wood.)

* 4 g. of NaOH/100 ml. of 80% ethanol.

Table 4. *Influence of a single pretreatment with ethanolic NaOH* (without chlorination) on the solubility of furfuraldehyde-yielding components of willow sawdust*

Washing prior to extraction ...	Ethanol, ethanolic H ₂ SO ₄ , aqueous H ₂ SO ₄			
Treatment time (hr.)...	1	2	4	24
Crude cellulose (%)	59.08	58.12	57.65	57.91
Furfuraldehyde from:				
(b) Aqueous extract (%)	1.20	1.40	1.57	1.40
(c) Sulphite extract (%)	0.65	0.88	0.79	0.76
(d) Crude cellulose (%)	10.24	9.70	9.58	9.91
(b + c)	1.85	2.28	2.36	2.16
(b + c + d)	12.09	11.98	11.94	12.07

(Results as percentage of original dry wood.)

* 1 g. of NaOH/100 ml. of 80% ethanol.

water. Chlorination and ethanalamine treatment were repeated twice more, with water extractions after each pair.

(iii) Six 7 min. treatments with ethanolic NaOH were followed by washings with ethanol, ethanolic H₂SO₄,

aqueous H₂SO₄, and again with ethanol, aqueous and sulphite extracts being then prepared.

(iv) Two 7 min. pretreatments with ethanolic NaOH were followed by six alternations of chlorination and ethanalamine treatment (concentration as in (ii)), the residue then being further treated as in (iii).

(v) As in (iv), except that four 7 min. pretreatments were given.

Finally, there is obvious interest in the action of ethanolic NaOH (4 g./100 ml.) on crude cellulose itself, as also in the solvent action of aqueous N-NaOH on the cellulose. Accordingly, samples of chlorination cellulose were submitted to the treatments shown below.

(vi) Two 7 min. pretreatments with ethanolic NaOH, the residue being washed and extracted with water and sulphite as in (iii), and

(vii) Two extractions, each for 20 min. at room temperature with 50 ml. portions of aqueous N-NaOH.

Results appear in Table 6. The extraction residues were not further chlorinated and extracted.

Table 5. *Pretreatment with ethanolic NaOH*, with or without subsequent treatment involving chlorination, and its influence on the extraction of furfuraldehyde-yielding materials*

(Pretreatment periods as shown; chlorination and ethanalamine treatments 7 min. each)

Final washing ...	Ethanol, ethanolic H ₂ SO ₄		Ethanol, ethanolic H ₂ SO ₄ , aqueous H ₂ SO ₄		
Pretreatment periods ...	4 × 1 hr.	1 hr.	6 × 7 min.	2 × 7 min.	4 × 7 min.
No. each of chlorinations and ethanalamine treatments ...	0	3	0	6	6
Wood residue (%)	—	—	88.69	69.71	66.54
Crude cellulose (%)	54.57	56.07	56.99	58.79	57.86
Furfuraldehyde from:					
(a) Wood residue (%)	—	—	14.23	13.60	12.74
(b ₁) Aqueous extract (%)	2.40	2.38	2.09	1.64	2.07
(b ₂) Aqueous extract (%)	0.85	0.85	—	—	—
(b ₃) Aqueous extract (%)	0.63	0.67	—	—	—
(b ₄) Aqueous extract (%)	0.39	0.46	—	—	—
(c) Sulphite extract (%)	—	—	0.67	0.96	0.89
(d) Crude cellulose (%)	8.17	8.54	9.68	10.12	9.21
Σb or (b ₁ + c)	4.27	4.36	2.76	2.60	2.96
[(Σb) + d] or (b ₁ + c + d)	12.44	12.80	12.44	12.72	12.17

(Results as percentage of original dry wood.)

* 4 g. of NaOH/100 ml. of 80% ethanol.

Table 6. *Extraction of furfuraldehyde-yielding material from crude cellulose*

	I*	II*
Untreated cellulose (%)	57.79	57.79
Residual cellulose (%)	54.83	45.68
Furfuraldehyde from:		
(x) Untreated cellulose (%)	9.18	9.18
(y) Residual cellulose (%)	8.09	2.56
NaOH extract (x - y)	—	6.62
(b) Aqueous extract (%)	0.71	—
(c) Sulphite extract (%)	0.31	—
(b + c)	1.02	—

(Results as percentage of original dry wood.)

* I. Pretreatment was with ethanolic NaOH (2×7 min.); the extracting agents were water and sulphite solution.
 II. No pretreatment was given; extracting agent was aqueous NaOH.

DISCUSSION

It is clear that each of the treatments investigated succeeds in rendering soluble in water a proportion of the hemicelluloses present in the original wood, a further portion—of magnitude often influenced by the type of final washing—usually being soluble in 1% (w/v) sulphite solution. Nevertheless, the condition of complete extraction of encrusting hemicelluloses in water-soluble form without influence on the yield and composition of the subsequently prepared crude cellulose is in no case fulfilled. Analysis of the original wood indicates a furfuraldehyde yield of 5.24% from encrusting hemicelluloses (as tentatively defined in the introduction), as will be seen from the 'no-treatment' column of Table 1 (a-d). From aqueous extracts, this figure is attained only after six alternations of chlorination and treatment with ethanolic NaOH (4 g./100 ml.); if (b + c) in the tables is held to represent furfuraldehyde from encrusting hemicellulose, then the figure of 5.24% is substantially attained with six, eight, and ten NH_3 treatments and with four by NaOH, whilst it is exceeded with six of the latter (Tables 1 and 2). However, in all these cases the cellulose yields are definitely below the standard figure of 57.79%, and the furfuraldehyde from cellulose correspondingly falls short of 9.18%, the figure from cellulose pre-

pared directly. It is worth noting that, with this type of treatment, a roughly linear relationship holds for cellulose yield and furfuraldehyde from cellulose, when both are calculated to the original wood. It is clear, then, that the chlorination-resistant material is contributing soluble hemicellulose to the extracts, and it may well prove that a definite fraction is responsible for this contribution, yielding material of substantially constant composition in amounts dependent on the intensity of the alkaline treatment. That cellulose itself is affected by ethanolic alkali is clear from Table 6. Table 1 shows further that hemicellulose solubility increases with increasing numbers of treatments, though the indications are that NH_3 treatment never gives all the encrusting fraction in water-soluble form.

The summation (b + c + d) in Tables 1-5 should give 14.42%; that the observed figures fall short of this is due to two causes. If extraction from the wood residue is incomplete, furfuraldehyde will be lost in the preparation of crude cellulose; this calls for no further comment. However, loss also occurs in the treatments leading to the production of the wood residue itself; this loss (14.42 - a) can only be due to material passing into solution in the aqueous chlorination reagent and in aqueous H_2SO_4 where this is used for final washing. In Table 7, the losses during the preparation of wood residue are summed with (b + c + d) from Table 1; the extent to which the summation figure falls short of 100% of the total furfuraldehyde reflects the loss during the final cellulose preparation. Since the furfuraldehyde determinations on sulphite extracts are necessarily made in presence of NaCl, the values for c are slightly in error (cf. Preece, 1940), but insufficiently to affect the present argument. It is clear that in all the cases of Table 7 considerably more furfuraldehyde-yielding material passes into solution than corresponds with encrusting hemicelluloses. Figures are available (unquoted) showing that ethanalamine is little better than NH_3 or NaOH in this respect. Increase of ethanol concentration to 95% gives no improvement with NH_3 , reduction of NaOH concentration to 1 g./100 ml. merely gives reduced ex-

Table 7. *Loss of furfuraldehyde during final preparation of crude cellulose*

Alkaline reagent	NH_3^*			NaOH†	
	6	8	10	4	6
No. each of chlorinations and alkaline treatments ...					
Furfuraldehyde as percentage of original dry wood:					
Losses from above treatments (%)	0.47	0.92	1.01	1.47	1.34
(b + c + d) (Table 1) (%)	12.98	13.25	13.29	12.14	12.38
Summation as percentage of total furfuraldehyde:					
Accounted for above (%)	93	98	99	94	95
Loss during crude cellulose preparation (%)	7	2	1	6	5
Chlorinations necessary in preparing crude cellulose	3	2	2	2	1

* 10 ml. of ammonium hydroxide (sp. gr. 0.88) and 90 ml. of 80% ethanol.

† 4 g. of NaOH/100 ml. of 80% ethanol.

traction, whilst the results for 3 and 5 g. of ethanolamine/100 ml. are substantially identical. There is thus no clear-cut distinction between two classes of hemicellulose with regard to the ease with which they can be rendered soluble in water.

Omission of chlorination gives a different type of extraction. Treatment with ethanolic NaOH (4 g./100 ml.) under a variety of conditions renders soluble in water and sulphite material yielding furfuraldehyde which, in the most favourable case (24 hr. treatment, Table 3), is equivalent to approx. 60 % of the encrusting hemicellulose. However, this material is almost certainly contaminated with chlorination-resistant material. Furthermore, both the cellulose yields and compositions are apparently anomalous; yields greater than the standard 57.79 % are frequent (see also Tables 4 and 5), whilst the furfuraldehyde yields from cellulose are consistently much greater than would be expected from the standard figures and from a consideration of the figures of Tables 1 and 2. The explanation is plain; in preparing the cellulose from the extracted wood residues, removal of lignin does not run parallel with removal of hemicellulose. Hence, working to the end-point for lignin does not necessarily ensure complete removal of encrusting hemicellulose. There is, however, a comparatively ready removal of a hemicellulose fraction of low furfuraldehyde yield. The high furfuraldehyde yields and the uncertainty of the end-point are accordingly due to relative persistence of a hemicellulose fraction under conditions which have accelerated removal of lignin. Untreated wood requires 5-6 each of chlorinations and sulphite extractions to remove lignin, as indicated by disappearance of the characteristic colour reaction; wood pretreated for as little as $\frac{1}{2}$ hr. with ethanolic NaOH requires only four of each. At the other end of the scale, much hemicellulose can be removed with but little removal of lignin. Thus, 1 hr. pretreatment with ethanolic NaOH (4 g./100 ml.) renders the equivalent of some 50 % of the encrusting hemicellulose (as measured by furfuraldehyde) soluble in water or sulphite; the lignin removal at this stage may be approx. 3.87 % (i.e. 94.92-91.05 %) of the wood. It is apparent that part of the hemicellulose lends itself to ready extraction; part of the remainder is slowly converted to soluble form by the more concentrated NaOH (Table 3), though this does not apparently happen with the more dilute (Table 4), where a limit of extraction is quickly reached. Initial speed of action and subsequent retardation also appear in Table 1.

The results of Table 5 lend general support to these conclusions. It may be additionally noted that removal of hemicellulose after pretreatment with ethanolic NaOH permits a further substantial removal after subsequent alkaline treatment, so

that four 1 hr. treatments give considerably greater extraction than a single 24 hr. treatment. Also, the simple alkaline treatment is just as effective as alternation of chlorination and ethanolamine treatment, though extraction by the two methods probably does not follow the same course, as is evidenced by the yield and composition of the celluloses.

The conversion of hemicelluloses into a water-soluble condition would accordingly appear to depend primarily on two factors, depolymerization (or disaggregation) and liberation from union with lignin; with some portions of the hemicellulose material both factors are involved, but the former seems to be the more generally important. The varying effects of alkalis of different types and concentrations may depend largely on their influence on molecular size, and there is little doubt that the partial dissolution of chlorination-resistant material is also governed by this factor. However, it requires also to be borne in mind that fractions which are water-soluble in the form of their sodium salts are not necessarily so when converted to the free acid; fractions which have lost water-solubility by washing with acid are nevertheless soluble in 1 % (w/v) sulphite solution, doubtless as a result of double decomposition. It may be remarked that ammonium oxalate can also restore lost water-solubility.

It is clear then that these extraction methods do not rigidly differentiate encrusting and cellulosan hemicelluloses as previously defined; it becomes doubtful whether even the normal chlorination process for production of crude cellulose separates two well-defined types with complete efficiency, though close approximation may be obtained. Nevertheless, recognition of the two groups is probably of utility as a basis for further study along botanical and constitutional lines. In the latter connexion it may be recalled that the xylan of esparto cellulose (presumably wholly or mainly cellulosan material) was stated by Hampton, Haworth & Hirst (1929) to be structurally closely related to cellulose itself, though reason was later given for some modification of this view (Haworth, Hirst & Oliver, 1934); the plant gums, on the other hand, definitely possess branched-chain structures (see, e.g. Hirst, 1942), and it may well be that the encrusting hemicelluloses are analogous to these. Whether this be true or not, it is desirable to transfer definition of the two hemicellulose groups away from dependence on the chlorination technique; ultimate decision on structure must clearly await more precise methods of extraction.

SUMMARY

1. Rigid differentiation of encrusting and cellulosan hemicelluloses is not possible by a variety of methods described.

2. Both fractions show graded ease with which they can be rendered soluble in aqueous solvents, considerable overlapping existing around the end-point of chlorination cellulose preparation.

3. Tentative distinction of two hemicellulose types seems to remain of value as a working hypo-

thesis for further botanical and constitutional investigations.

4. Factors affecting the water-solubility of hemicellulose fractions are discussed; there is only partial parallelism between hemicellulose solubility and degree of delignification.

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Large-scale Extraction of Protein Samples Reasonably Representative of the Whole Proteins in the Leaves of some Plants: the Amide, Tyrosine, Tryptophan, Cystine (plus Cysteine) and Methionine Contents of the Preparations

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Limited experimental evidence supports the statement (Lugg, 1943) that the whole proteins of the somatic, photosynthesizing, gametophyte tissues of bryophytes, and sporophyte tissues of pteridophytes and spermatophytes, appear to be of similar composition. The evidence, though meagre, was held to justify the view that for the purpose of animal nutrition studies, comprehensive analyses of pasture proteins might well be confined in the first instance to those of only a few species belonging to the most important families of pasture plants.

Considerable data already exist concerning the amide, tyrosine, tryptophan, cyst(e)ine (cystine plus cysteine) and methionine contents of the whole-leaf proteins of several spermatophytes, and of numerous protein preparations which, in most instances, may not have been properly representative of the whole protein in the leaves from which they were obtained (Lugg, 1938 *b, c*, 1939, 1943; Chibnall, 1939). The arginine, histidine and lysine contents of various preparations of uncertain representativeness have been estimated (Tristram, 1939), and in some instances the amounts of aspartic and glutamic acid have also been determined (Chibnall, 1939). The proline content of one such preparation has been estimated (Miller, 1936), and the glycine,

alanine, valine, leucine (plus isoleucine and nor-leucine) and phenylalanine contents of another preparation have been estimated only very approximately and uncertainly by Lugg (quoted by Chibnall, 1939, and by Lugg, 1941).

The presence of cellulose, polyuronides and similar contaminants in protein preparations introduces no apparent systematic errors in the estimation of amide, tyrosine, tryptophan, cyst(e)ine and methionine contents when suitable, tested methods are employed. The methods available for the estimation of other amino-acids, however, are known to be subject to systematic errors or are of uncertain reliability, under these conditions. For the most satisfactory estimation of these other amino-acids, it is therefore necessary to work with preparations which are freed as far as possible from such contaminants, and if the estimations are intended to refer to the whole proteins of the leaves, it is necessary also to ensure that the preparations are properly representative of these.

The principles of methods designed to provide representative preparations of reasonable purity have been discussed elsewhere (Lugg, 1939). In the present article there will be described the extraction of large samples of such protein from the leaves of