ANALYSIS OF THE TYPHA SEED HAIRS

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THE ANALYSIS OF THE TYPHA LATIFOLIA LINN. SEED HAIRS

A Thesis

by

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GENERAL INTRODUCTION

The seed hairs of the typha family (cat-tails) are easy to collect and appear to the eye to consist of cellulosic fibers of nearly uniform length radiating from a central stem. The object of the present Thesis was to review the literature concerning the chemistry and possible uses of the typha plant and to supplement existing knowledge concerning the chemical composition and properties of the seed hairs.

It was found that standard methods of wood analyses gave inconsistent results with typha seed hairs and an effort was made to discover why such methods were unsuitable in this particular case.

HISTORICAL INTRODUCTION

As far as the writer, no specialist in botany, can understand botanical articles (1) (2) (3) (4), plants of the cat-tail, reed mace, flag or bulrush family are members of the Typhaceae genus, related to the order of Pandanales. The genus abounds in seventeen different species throughout North and South America (5), Europe, Asia (6) and India (7). Typha elephantina Roxb. is a staple food for the Indian wild elephant. Typha latifolia Linn. and typha angustifolia Linn. are fairly common in North America.

Typha is a water or marsh herb that may appear in almost any wet place and is often the first invader in a newly excavated pool. The underground, perennial stems or rhizomes spread so extensively that a stand of typha an acre in extent may actually consist of but a few plants (2). These rhizomes throw up tall stout, erect shoots surrounded by long sword-shaped leaves and crowned by dense terminal spikes carrying the male and female flowers. As the plant ripens, the female flower develops into a spindle around which the light, feathery seed hairs are symmetrically packed like down. Their dark brown appearance and velvety nature when ripe have led to the term "cat-tails". Figs. 1 to 3 reproduce illustrations of various portions of Typha latifolio Linn., the common American cat-tail.

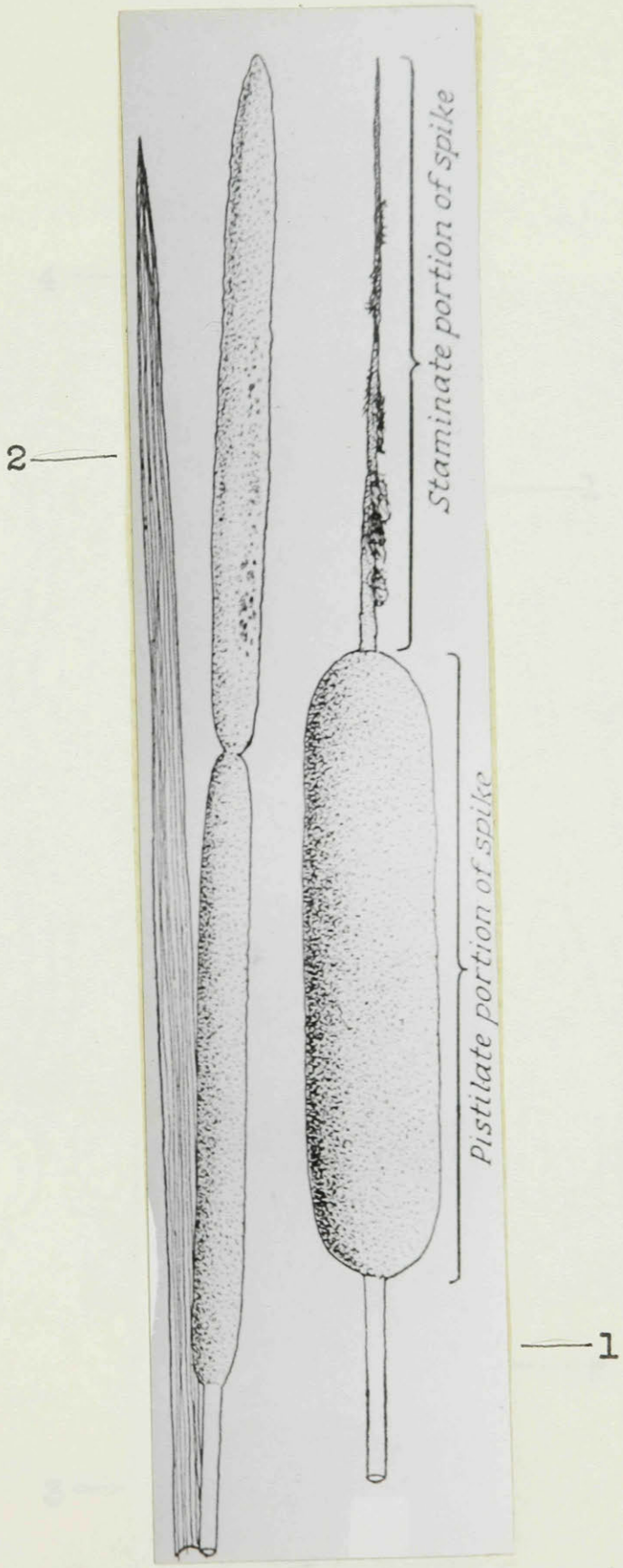

Fig. 1. Typha Latifolia Linn. Pistillate and Staminate

1. Stem 2. Leaves

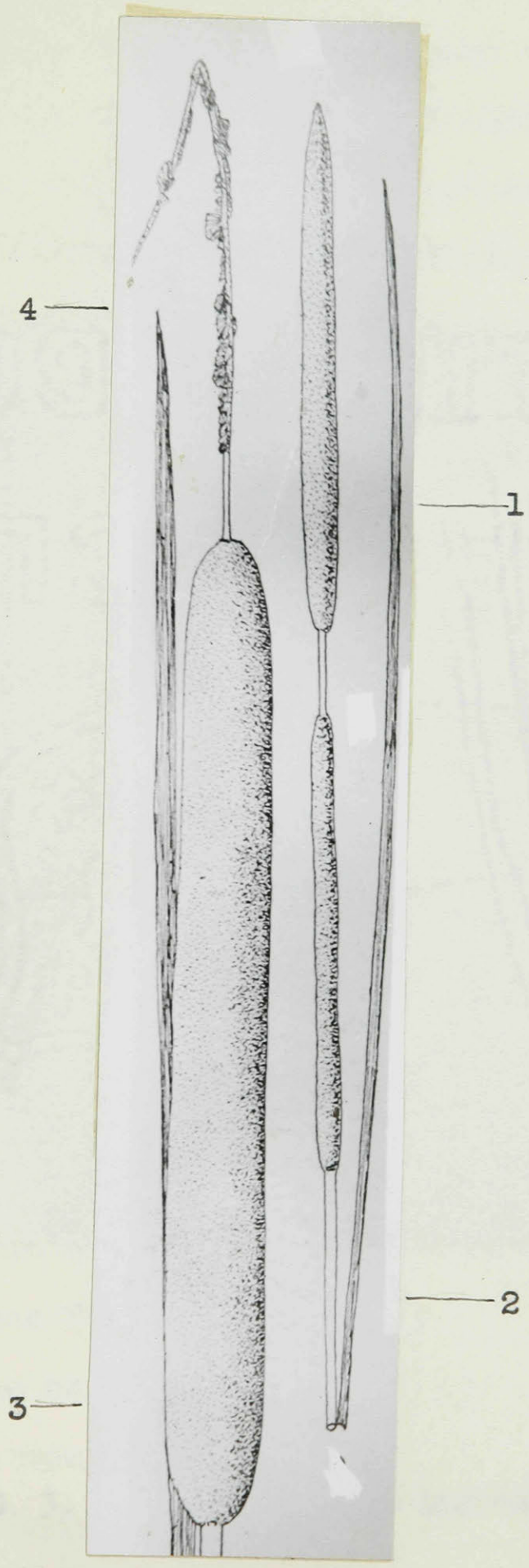

Fig. 2. Typha Angustata Pistillate and Staminate

1. Leaves 2. Stem 3. Pistillate 4. Staminate

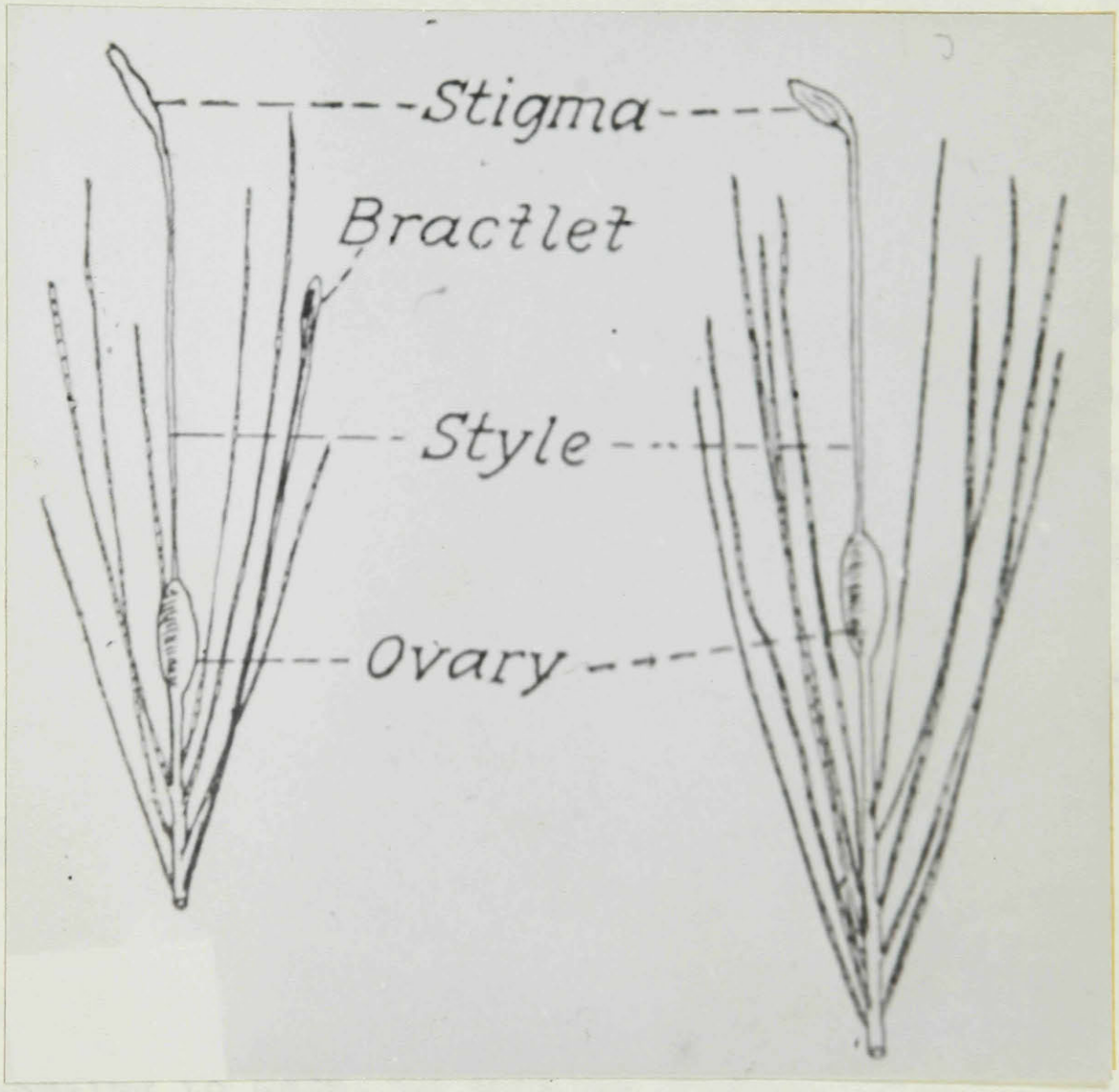

FIG. 3. DETAILS OF TYPHA LATIFOLIA SEED HAIRS

The Typha Plant

The plant has attracted the attention of scientists all over the world. Chardin, in his book of travels, mentions that in Persia typha wadding was mixed with ashes and quicklime to make up a mortar which was as hard as marble (8). When one reflects, modern inventions of sound-proofing and reinforced concrete do not seem to be as original as they appear. The mortar prepared by the Persians would make both a reinforced structure as well as a good sound-proof material. Attempts have been made to combine the fibers with rabbit fur for hat-making and also to weave it along with cotton or silk for making gloves, stockings or fabrics. It has served as a substitute for matches and tinder, since it is very inflammable (9). The down has been used as a dressing for wounds and for upholstering purposes.

According to Collin (8), as far back as 1830 Dart made an effort to use typha for producing a paper resembling Japan paper, but the effort was not a great success. Vertillart and Lecomte did not consider typha as a textile fiber and Höhvel described only the structure of the fiber and suggested no possible uses. According to Geze, in Annales du Ministere de l'Agriculture, a large company was floated in Rumania to cultivate typha for textile fibers at the beginning of this century. Harper and Daniel (10) reported the composition of typha from Oklahoma. They indicated that the nitrogen and phosphorus content was quite high whereas the calcium content was rather low. This fact indicated a rich food value and an absence of objectionable inorganic elements.

Postal (11) in his review of the work done on typha plants from 1765 to 1919, revealed the following interesting facts:- Dr. J.C. Schaeffer, in his sixth volume on paper making without the use of rags, included ten samples of paper made from typha. An article entitled "A Treatise on the Latest Improvements in Paper Manufacture" published by Nurenberg's Commercial Gazette stated "The ordinary reeds would make good paper and merit attention because they are obtainable in quantity". In Norfolk, U.S.A., the American Fiber Co. established a factory and produced pulp from typha according to Layman's patent of 1858. Again in 1909 in Braila, a plant was erected to make cellulose from typha with the aid of the Rumanian government. At the outset of the first world war, when material for papermaking was scarce, a factory was equipped to make paper out of typha in Saxony. In each of these cases, however, although the raw material was cheap and a beautifully white, woolly and absorbent pulp could be produced to make paper and paper board, the attempts were economically ineffectual and impracticable.

Steite (12) reported about the possibility of using typha fibers as a substitute for textile fibers. In the event of an economic shortage of the more common textile fibers, according to him the typha fibers could be successfully used for simple bands and fabrics.

To study the optimum growing conditions of this useful plant in Delaware, U.S.A., Daigh, MacCreary and Stearns (13)

determined the soil salinities required and the optimum pH. For typha angustifolia the optimum salinity was 2950 p.p.m., the maximum pH 6.11 to 6.64 and the minimum value, 2.96 to 3.90.

The prodigious growth of the plant can be economically exploited but at the same time it can also be a nuisance. Thus Prunster (14) noted that owing to its tendency to congest irrigation canals, it had to be destroyed and controlled by bi-monthly spraying with 15 per cent aqueous sodium chlorate (500 gallons per acre) or with 20 per cent crude oil emulsion containing 5 per cent of arsenic (as sodium arsenate).

The different parts of the typha plant have been investigated frequently and research has been done on roots, stalk, leaves, flowers, pollen, seeds and seed hairs.

Roots of the Typha Plant

Thoms (15) who investigated the roots of typha latifolia from the botanical garden in Dahlem with regard to its use as cattle feed, reported that it contains a total of 25 per cent starch of three different grades, 17.67 per cent of crude protein and 52.21 per cent of total carbohydrates calculated on the air-dried roots. His nutritional experiments on mice were very satisfactory. Again Kofler (16) ascertained the starch content of the roots to be 46 per cent, calculated on the weight of the air-dry substance. The starch granules varied from 3.5 μ to 13 μ in diameter and he suggested typha

roots as a possible source of starch. Claassen (17) confirmed the above findings and reported a yield of 7.75 per cent protein, and 81.41 per cent of total carbohydrates. He estimated that 5,500 lb. of flour could be made from an acre of typha roots and thought it would be a good substitute for wheat and corn starch. Jencks (18) also reported the carbohydrate content of the roots to be as high as 81 per cent and nutritional experiments with the rhizomes were successful.

Pathak (19) of India once more suggested the flour from typha roots as a substitute for wheat flour, and the seeds for millet flour. It has a worthwhile product to consider in times of famine as the roots have 68 per cent digestible carbohydrates and 15 per cent protein, while the seeds contain 71 per cent carbohydrates and 11 per cent protein. Thus typha has the two essential constituents of human food, carbohydrates and protein, in considerable quantities and its gregarious growth would guarantee an ample supply.

von Lippmann (20) analyzed the roots for their sugar content and found 1 to 3 per cent, and rarely 3.5 per cent, of sucrose. When the plants had bloomed the roots contained no sucrose at all, which might probably be due to the exhaustion of the reserve material in putting forth a fruit. The amount of reducing sugars, according to von Lippmann, was insignificant, and thus the carbohydrate content reported by previous workers was not sucrose or reducing sugars, but mostly starch.

Freudenthal (21) suggested the flour from typha roots as a substitute for corn flour, which commands a high price. This observation was borne out by his nutritional experiments on hogs. Typha might be an excellent substitute in Fall and Winter when the starchy content of the rhizomes is likely to be at its height.

The evolution of the idea that the roots contain a fair percentage of carbohydrates, which are none too high in sucrose and which can finally be fermented to make alcohol, was brought to a conclusion by Sabalitschka (22). He affirmed that the roots never contained more than 50 per cent cane sugar, generally less, and as such the production of alcohol cannot be as high as has been claimed by other researchers in the field.

Kharitonova of Russia (23) analysed the roots of cattail and reported the following on dry basis: starch, 9.75 to 21.9; crude fiber, 20.7 to 21.79; nitrogenous compounds, 9.05 to 11.73; ash 5.26 to 11.8 per cent. According to him the bulrush roots have a low starch content and a high crude fiber content, and hence are of no economic significance as a foodstuff. But if the crude fibers are considered as purely anhydroglucose units and not necessarily as entirely cellulose, the digestible carbohydrate value is considerably raised. Similarly, if the nitrogenous compounds are interpreted as protein, according to previous workers in this field, the roots also may be assessed as a highly nutritive food. Thus, if he had estimated the total carbohydrate and protein content while analysing the roots, the

values would have thrown a different light on the subject and have been more in accord with the results of other workers.

Kokin (24) of Russia presented data on the chemical composition of typha elephantina roots from middle Asia in its various stages of growth. He determined the content of monosaccharides, disaccharides, polysaccharides, total nitrogen, proteins, fats and ash, foresaw its industrial and food value and its economic development such as manufacture of alcohol, paper and cellulose. According to Kokin, the carbohydrate from the sub-surface organs of the plant is transferred to the above-ground parts (during the maturing period) in the form of monosaccharides and might account for the low carbohydrate content of the root in this period. Supplementary monosaccharides are produced by the hydrolysis of disaccharides, and the latter supplementary saccharides, are produced by the hydrolysis of starch.

TABLE I

Kokin's Table of Soluble Carbohydrates and Starch in Typha Elephantina Roots

Time of Harvesting	Water %	Monosaccharides	Disaccharides		Polysaccharides			Sugar and Starch	Total carbohydrates
			Fructose	Maltose	Starch	Hemicellulose	Cellulose		
Nov. 1st, 1930	11.46	11.74	2.76	0.80	27.91	11.61	18.66	43.21	73.48

Freise of Rio de Janeiro (25) conducted a more detailed analysis of the roots of typha to investigate their medecinal potentialities and reports the following values.

TABLE II

Friese's Analyses of Typha Rhizomes

Starch	11.55%	Tannin	5.45%
Albumin	0.35	Glucose	1.22
Fatty oils	0.29	Inorganic salts	1.73
Resin	0.85	Organic acids	0.25
Essential oils	0.13	Cellulose	8.68
Gummy substance	0.88	Water	68.6

When recalculated on a dry basis, the starch content became about 35 per cent, in agreement with most other workers, but albumin was still low at 1 per cent. He found that the fatty oil consisted principally of palmitic and oleic acids and an unidentified toxic principle which had purgative and emetic properties. The essential oil (d_{20} 0.9065-0.9165, n_{20} 1.4885, $[\alpha]_{20}$ -5° 21' to $+7^{\circ}$ 45') consisted chiefly of thymol together with alpha-pinene, a phenol $C_8H_{11}O_2$ and a lactone $C_{14}H_{22}O_2$, which acted (in vitro) as a medium anthelmintic. The resin (density 1.06 to 1.11, acid no. 22 to 36, sapon. no. 60) had diuretic properties. The ash consisted principally of 35 per cent of potassium oxide and 20 to 28 per cent of silicon oxide. His conclusions were that when the infusions were empirically prepared they proved efficacious in cases of ascites and of considerable value in the treatment of rheumatism

eczema and verminosis.

Novikova (26) reviewed the literature, especially Russian, on the analysis of typha roots and his own results are summarized in Table III.

TABLE III

Novikova's Analyses of Typha Rhizomes

Wet basis		Dry basis	
Water	66.5%	Carbohydrates	52%
Crude protein	6	Starch	46
Pure protein	2	Sugar	7.8 (max.)
Carbohydrates	17.5		
Starch	15.4		
Fat	0.29		
Crude cellulose	7.3		
Ash	2.54		

He affirms that the roots can be utilized as a source of starch but have not been exploited industrially.

Meissner (27) however, outlined a method of not only obtaining the soluble starch but also the crude fiber. He suggested crushing the roots, combing and suspending them to reclaim the fibers for the spinning of textiles, and removing the starch in the usual manner by rinsing with water.

An article in a different vein by Liang (28) described some respiration studies of the roots of typha latifolia. They transpired anaerobically over long periods without injury.

Carbon dioxide was evolved and the rate of evolution of ethyl alcohol was inversely related to the oxygen concentration up to 3 per cent. The more rapid respiration of the smaller growing points was taken to indicate the rapid formation and storage of starch.

Stalks of the Typha Plant

Haller (29) described the morphology of the stalks of the typha plant and investigated the action of various reagents on the fibers. The commercial possibilities of these fibers were also outlined and their prospects, according to Haller, were poor. Ground (30) reported a method of cooking the stalks and obtaining the fibers for paper making. He also mentioned that the fibers had been used in the manufacture of mats, cordage, baskets, wicker-work, etc. The yield of long, regular fibers from the stalks was about 44 per cent.

Gierisch, Krais and Waentig (31) produced single fibers for raw threads and raw fabrics from bast fiber bundles with simultaneous disintegration and removal of the woody particles and incrustants. The treatment was in the usual manner: steeping, bleaching with chlorine, treating with 5 per cent aqueous sodium hydroxide followed by a sour and then drying.

Steinhilber (32), on the other hand, produced pulp for paper manufacture without the use of chemicals. The process he adopted was steaming, passing through crushing rollers, and removing the parenchyma cells. The incrusting substances could

be removed by dissolution with water, leaving a pure cellulose fiber. This process is very similar to the groundwood newsprint process of today. Klien (33) gave photomicrographs of fibers made out of typha stalks and reported on the properties of the paper they made. He was of the opinion that the long typha fibers knot together and are difficult to work with, although they have a linen-like quality. Working along the same lines, Heuser and Haugerod (5) made some cooks of typha domingensis stalks. The analysis reported is in Table IV.

TABLE IV

Analyses of Heuser's Chemical Pulps from Typha Stalks

Constituents	Yield %	Lime cook Halfstuff %	Sulfite cook Halfstuff %
Crude cellulose	45.10	....	....
α-cellulose[a]	....	83.50[a]	93.24[a]
Ash	4.09	7.59	6.74
Silicon oxide	0.112	0.072	0.05
Calcium oxide	....	6.45	....
Fats and waxes	9.47	1.74	3.12
Wood Gums	44.31	....	....
Pentosans	18.25	....	15.72
Copper Number	....	....	1.695

[a] Percentage yield on crude cellulose yield

Heuser obtained a halfstuff after the lime digestion, which could not be bleached and, as such, was suitable only for boards. Nevertheless, when mixed with sulfite pulp this halfstuff could be used for packing papers. A sulfite cook of the same stalks was bleachable and gave a material similar to the above. Owing to the difficulty of getting perfectly bleached fibers, Heuser was of the opinion that the prospects for typha were not very promising, although he suggested mixing typha with sulfite pulp for making paper. Uhlemann (34), contrary to Heuser, reported that typha suitable for the manufacture of a satisfactory paper could be obtained by carrying out the digestion in the manner used for straw or wood.

Similarly von Karawajew and Kriwowjas (35) experimented with cooks on typha stalks and found that the resulting fibers were of fair physical quality.

TABLE V

Karawajew and Kriwowjas' analyses of Typha Stalks digested for 8-9 hours at 6 atmosphere pressure with 10 per cent sodium hydroxide solution

Constituents	Air-dry stalks %	Pulp %
Moisture	8.5	..
Ash	5.6	..
Cellulose	42	90
Lignin	28	1.5
Pentosans	..	7

Postal (11) has described the cooking process of typha stalks and discussed its economic possibilities for paper making. Remezzano (36) prepared activated charcoal from the stalks of cat-tails, using zinc chloride and magnesium chloride as activating agents.

Leaves of the Typha Plant

The leaves of the typha plant have received considerable attention because of their length, from which it was thought that it would be possible to produce long, fine fibers. Heuser and Haugerod (5) carried out some lime as well as sulfite cooks on the leaves but thought they had no great economic possibilities, as a raw material for pulps.

In New Zealand (37) the leaves of typha angustifolia Linn. were found to consist of 17.7 per cent water, 3.0 per cent ash and 38.3 per cent cellulose, the latter figure corresponding to 43.8 per cent on a dry basis.

Alkaline cooking yielded 32 per cent dry, unbleached pulp and 29 per cent of dry bleached pulp. The paper produced from this pulp was pale brown, hard, rather rattly, opaque and strong. For making rayon pulp out of these fibers the workers suggested removing the pitch first from the leaves; however, this process would further reduce the pulp yield and the economic possibilities would be poor.

Similarly, Rinman (38) in Switzerland carried out some digestion experiments on the leaves of typha. The process of

digestion appeared simple and unattended with difficulties, the lignin complex being of such a nature that it was easily removed by alkali under fairly mild conditions. The glutins present were not destroyed by this treatment.

It is reported from Australia (39) that the leaves could be digested easily in alkali to make bleached and unbleached pulp similar to the kraft pulp of today. The following values are reported from the analysis of the typha latifolia pulp: water, 16.1 per cent; ash, 7.4 per cent; cellulose 36.1, the latter figure corresponding to 43.0 per cent on a dry basis. The yield was 30.4 per cent or 36.2 per cent on a dry basis, with mild pulping conditions. Once more the paper was of low wet strength, but tough, somewhat rattly, opaque, of good dry strength and showing slight shrinkage on drying. Moderate digestion separated fibers which were readily broken down in the beaters, yielding a "wet pulp" which furnished a fairly tough, flexible board. The pulp yield was, however, 22.5 per cent, corresponding to 26.3 per cent on a dry basis.

Ludke (40) gave the cellulose content of the leaves as 62 per cent of which only 20 to 35 per cent was alpha-cellulose. The small cell elements which were produced from the long fibers were only 0.1 to 0.3 mm. in length and hence were too short for paper but usable in the paper-board industry. The fermentable sugar content was small.

Meissner (27) produced spinnable fibers not only from

the roots of typhaceae, but also from the leaves. Further, in the process of winning the fibers, the soluble starch in the leaves was recoverable from the rinsing water, in the usual manner. Working on the idea of taking advantage of the long, slender and strong fibers, Opitz (41) discussed favourably the possibility of utilizing the leaves of typha in the textile industry.

Liang's (42) studies on the internal atmosphere and photosynthetical activity of the leaves would be of interest to botanists. During four days of anaerobic respiration, the rate of respiration of the young leaves decreased 50 per cent and they produced ethyl alcohol in concentrations of 3 per cent or less of the oxygen in the atmosphere.

Flowers of the Typha Plant

It is remarkable that the flowers of typha have also been investigated, not only from the point of view of their cellulosic constituents but also regarding their oxidizing reaction. Kihara (43) analyzed the fibers isolated from the flowers as follows.

TABLE VI

Kihara's Analyses of the Fibers from the Flowers of the Typha Plant

Moisture		12.87%
Total cellulose		41.45
alpha-cellulose	69.04	
beta-cellulose	14.64	
gamma-cellulose	16.32	
Pentosans		22.11
Total soluble carbohydrates		23.88
Alcohol-benzene extract		1.55
Ether extract		1.76
Total nitrogen		0.92
Ash		6.87

He was of the opinion that the carbohydrates consisted of a kind of hemicellulose, which on hydrolysis with hot dilute acid broke down to arabinose. The preparation of the appropriate benzyl-phenylhydrazone and phenylosazone confirmed the above observation. The flowers could be successfully cooked by the soda process and yielded a pulp of 83.14 per cent alpha-cellulose content easily bleachable and containing no lignin. The naphtha resorcinol reaction was negative.

Sosa-Bourdouil (44) tested the oxidizing power of the blossoms of typha toward ascorbic acid solution. Different parts of the blossoms exhibited considerable oxidizing power and contained ascorbic acid in varying degrees.

Pollen of the Typha Plant

Several investigators were attracted towards the chemical constituents and the possibilities of the pollen of

the typha flowers. Thus Fukuda (45) identified one of the constituents in typha angustata as isorhamnetin. Other components were palmitic acid and d-glucose. Sumi (46) working on the pollen of typha Japonica Miq., isolated and identified sitosterols. The ergosterol content, which was heterogeneous was determined by the ultraspectroscopic method. Kimura (47) isolated (yield 10 per cent) an oil from typha angustata. The two acids identified were palmitic and stearic acid; the unsaponifiable fraction yielded alpha-typhasterol $C_{27}H_{40}O_6$, m.p. 133-134°, $[\alpha]_D^{15}$ -59.83°

Kuwada and Morimoto (48) characterized these sterols unequivocally. They have identified alpha-typhasterols as $C_{27}H_{46}O$, or $C_{29}H_{50}O$, m.p. 138°, $[\alpha]_D^{18}$ -33.5° in carbon tetrachloride. The alpha-typhastanol they have isolated is $C_{27}H_{48}O_6 \cdot 0.25\ H_2O$ or $C_{29}H_{52}O_6 \cdot 0.25\ H_2O$, m.p. 137° which on oxidation gave alpha-typhastanone $C_{27}H_{48}O$, or $C_{29}H_{52}O$, m.p. 154-155°, the oxime of which yielded alpha-typhastane $C_{27}H_{48}$ or $C_{29}H_{52}$, m.p. 52°. The sitosterol isolated from the extract of rice polishings had similar properties to the alpha-typhasterol.

Hattori and Nakamura (49) extended the study of typhasterol to its oxidation with chromic acid. The oxidation product isolated was trans-dehydroandrosterone (I) from the neutral fraction. The acid fraction yielded 3-hydroxynorcholenic acid (II). Such oxidation studies established the fact that typhasterol belongs to the group of sitosterols.

Sosa-Bourdouil (50), in addition to recognizing the high nitrogen content, isolated and characterised vitamin C from the pollen of typha blossoms. The yield was 180 mg. of vitamin C per 100 g. of pollen and was determined by the methylene blue and the 2,6-dichlorophenolindophenol methods.

The inorganic constituents of the pollen, investigated by Tischer and Antoni (51) were considerable. Taken in conjunction with the high nitrogen content, they indicate the possibility of value as food.

TABLE VII

Tischer and Antoni's Analyses of the Inorganic Constituents of the Pollen from the Typha Plant

Water	9.7%
Nitrogen	3.83
Ash	3.80
Potassium	1.24
Sodium	0.13
Calcium	0.1
Magnesium	0.28
Iron	0.025
Manganese	0.007
Phosphorus	0.41
Sulphur	0.24
Silicon	0.001
Chlorine	0.05

Seeds of the Typha Plant

After the pollination, the plant is by no means without interest for the seeds and seed hairs have been investigated by various workers from different angles.

Some nutritional studies were made on the whole millets of the typha blossoms by French (52) in Africa, before and after germination and he recommends the former as cattle feed.

TABLE VIII

Composition of the Ungerminated Typha Seeds

Crude protein		14.1%
Ether extract		4.0
Nitrogen-free extract		75.5
Crude fiber		4.4
Ash		2.0
SiO_2	0.5	
SiO_2-free ash	1.5	

The fiber content is higher in the sprouts of typha than in English pasturage but the digestibility coefficients are lower and hence the typha sprouts are inferior cattle feed.

Sinozaki and Takumi (53) analyzed the oil and seeds of typha and gave the following analyses. The yield of crude oil was 20.3 per cent and the oil belonged to the semi-drying group. The chemical constituents identified were palmitic, stearic,

arachidonic, oleic and linoleic acids. The unsaponifiable matter contained pentacosane, m.p. 63° and typhasterol. The constants of the oil were: d_{15}^{25} 0.9256, n^{25} 1.4740, saponification value 193.96, acid value 19.1, iodine value 130.8, Reichert-Meissl value 0.22, Polenske value 0.42 and unsaponifiable matter 3.64 per cent.

Clopton and von Korff (6) reported the analyses of the typha seed furnished by Hamley of Burgess-Manning Co., Chicago, Ill. It is of interest to note that the analyses of Hamley and French (52) do not agree.

TABLE IX

Hamley's Analyses of the Typha Seed

Constituents	%
Moisture and volatile matter at 100°	9.0
Crude protein (N x 6.25)	19.8
Crude fat	17.2
Ash	3.5
Crude fiber	15.5
Nitrogen-free extract	35.0
Total carbohydrates	50.5

Clopton and von Korff (6) have also examined the oil expressed from the typha seeds. The constants of the oil were: n at 29.5° 1.4730, acid number 30.7, saponification number 186.0, iodine number, 141.6, acetyl value 10.8, thiocyanogen number 79.8 and unsaponifiables 2.52 per cent. The chem-

ical constituents identified were: lineolic acid 69.2 per cent, linolenic acid 0.12 per cent and oleic acid 14.2 per cent. The possibilities of this oil are being exploited in Minnesota and Wisconsin and a kind of drying oil is produced which could be used in the manufacture of alkyd resins. One and a half million pounds of raw seeds are being processed annually and from them 100,000 lb. of the oil could be recovered. This industry sprang up as a subsidiary to the typha fiber industry where the seed hairs are utilized as a filling material and the seeds are cast out. Thus the waste product of one industry has given birth to another thriving industry.

Other results of Clopton and von Korff (6) bear a close resemblance to Kimura's (47) and Kuwada and Morimoto's (48) work on typhasterol. Golberg and Thorp (54) carried out vitamin assays on the typha seeds which were found to have 310) units of ascorbic acid (average of four samples). These results parallel the work of Sosa-Bourdouil (50) on the pollen.

Seed Hairs of the Typha Plant

Just as the other parts of the typha plant, the seed hairs have also attracted much attention from different points of view. As far back as 1917 Jata-Werk für Pflanzliche Fullstoffe, G.M.B.H. (55) took out a patent for processing the seed hairs for the textile industry. The seed hairs were cooked in alkali-salt solutions to overcome the water resistance of the fibers which were then readily bleached, dyed and prepared for spinning.

Collin (8) reported that attempts to make textile or paper fiber economically out of typha fibers had been futile, the principal reason being the difficult and costly process of removal of seeds from seed hairs. One important observation he made was that the composition of the typha seed hair is not uniform because of the presence of the small flower stalk in the middle of each tuft along with the seed. (See Fig. 3, page 5.)

Haller (29) was of the same opinion as Collin (8), but his reasons were that the fibers have relatively low tensile strength and are brittle. However, since these fibers are long, slender and strong, they can be used to make certain grades of paper which do not require high tensile strength and flexibility.

In Japan, in the nineteen twenties, fibers were extracted out of typha seed hairs for textile purposes. The process is of some interest as it embodies certain principles which are practiced even today. The method given in Azuma's (56) patent consisted of impregnating the seed hairs with a warm saturated solution of soda-ash and then arranging them in a layer on which powdered lime, then another layer of the pretreated seed hairs was spread, and again covered thickly with lime. After cooling, the fibers were collected and bleached. The chemical reaction between the soda-ash and lime produced enough heat to do a "cooking", and the weight of the seed hairs and lime keep the system under enough "pressure". By such a simple and inexpensive treatment the lignin and also the non-carbohydrate mat-

Collin (8) reported that attempts to make textile or paper fiber economically out of typha fibers had been futile, the principal reason being the difficult and costly process of removal of seeds from seed hairs. One important observation he made was that the composition of the typha seed hair is not uniform because of the presence of the small flower stalk in the middle of each tuft along with the seed. (See Fig. 3, page 5.)

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ter were removed, leaving the purified cellulose fibers.

Heuser (57) extracted a crude cellulose from seed hairs, chiefly for cattle feed and also as a substitute in paper manufacture. The composition of the crude cellulose was: cellulose 58.70, pentosans 28.05, lignin 9.45 and ash 3.80 per cent.

The estimation of lignin was by difference and not by direct determination.

The fermentation of the aqueous extract of the seed hairs yielded 9.28 litres of alcohol from 100 kg. of dry substance, a result which signified the presence of fermentable sugars in the water extract of the seed hairs. The other interesting point was the high percentage of pentosan reported.

Bokor (58) of Budapest found the seed hairs were of 10 to 12 mm. length and consisted of cellulose of "good quality". He proposed a unique method of processing these fibers for textile purposes which consisted of extraction in a mixture of methylacetate - benzene, pressing out--presumably to remove the water-resistant fats and waxes--drying and then swelling by heating in a solution of sodium thiosulfate. Sodium chloride was added, the mixture cooled, with stirring, to 60.5°, and sulfuric acid added to precipitate the sulfur on to the fibers in order to strengthen them, and to produce a wavy form after drying. After washing and pressing, the fibers were dried at a maximum temperature of 70 to 80°. The resultant fibers, accord-

ing to Bokor, could be used as such in upholstery; when mixed with long textile fibers they were suitable for textiles.

The Chemurgic Digest (59) reported that at the modern Burgess-Manning Company the seed hairs are converted into a filling material which compares favourably with Kapok. It is mentioned that experiments are afoot to improve the growth of spike and quality of the fluff. Clopton and von Korff (6) stated that the industrial plants in Minnesota and Wisconsin process the seed hairs for uses such as shock-proofing and insulating. They estimate the seed as being about 35 to 45 per cent of the seed hairs.

Gilchrist (60) discussed the advent of typha fibers into the commercial fibers field along the same lines. He stated that the fibers are less than half an inch in length and occur in clusters of thirty-six. The raw spikes of the cat-tails are broken up by a hammer-type disintegrator and the stems, leaves, pollen and other parts are removed from the seed fibers by means of a series of chambers and air filters. Because of their remarkable buoyancy, the seed fibers can be put to various uses such as filler for life preservers, beach balls, ring buoys and buoyant cushions, etc. A cushion of typha could support twenty times its own weight in water for 265 days. This extreme water resistance can be ascribed to its natural fats and waxes. Its thermal and sound insulating properties were good and it could be blended with wool, cotton and rayon. The chemical analysis reported by Gilchrist

is as follows: ether extract (fats and cutin) 3.85, ash 0.46, lignin 19.16, cellulose and hemicellulose 76.53 per cent.

The methods of analysis were not given but one or other of the constituents was probably determined by difference since the total is 100.00 per cent. Further, the hemicellulose was merely mentioned and was said to be linked with the cellulose, a statement that is rather vague.

Identification and Physical Properties of Typha Fibers

Besides obtaining the fiber and studying its constitution and possible uses, a few scientists tested the physical properties of the fibers and have proposed tests to identify it. Thus, Ruchlemann (61) prepared micrographs of papers produced from typha fibers in 1770. These micrographs give an idea of the physical form of the fibers.

Möller (62) and Opitz (63) devised means of identifying typha fibers. Opitz adopted the rapid method developed by Viviani-Herzog and discussed the preparation and characteristics of the fiber.

Maillard and Szymanek (64) measured the "dynamometric resistance" of the fiber by noting the longest span capable of supporting its own weight. The dynamometric resistance was found to be an inverse function of the pentosan content of the fiber.

The above references are difficult to classify in stalks, leaves or seed hair fibers, as they are not mentioned unequivocally by the investigators, hence they are grouped under a general heading as typha fibers.

It is of interest to note that out of many investigators less than ten have worked on the seed hairs, and not all of these were interested in the cellulosic constituents. The analyses conducted on these fibers are either incomplete or show a gross variation, and in many cases the methods of analysis are omitted. More than half of the scientists devoted their attention to the possible value of the seed hairs as filling material.

RESULTS AND DISCUSSION

Refining the Typha Seed Hairs

The cat-tails were gathered in October 1946 from a marsh at the south end of the Mercier Bridge near Montreal. Most or all of the plants were of the typha latifolia species. The seed hairs with the white or brown seed attached at the outer ends, were very readily rubbed away from the air-dried central spindle and were stored in bags until a suitable mechanical method of separating the seeds was developed. Gilchrist's principle (60) of blowing the down violently against a vertical screen was adopted but the description he furnished was incomplete. After preliminary experimentation, the box shown in Figs. 4, 5, 6, pages 58, 59 and 60, and photographs 1 and 2, page 61, was constructed and the optimum positions of the screen and baffles were found by trial and error. The crude down was fed into the equipment through the air blower and the fine, white, practically seed-free product floated upward in the air-flow to the collection chamber. The successful operation of this equipment demanded considerable experience and judgement, but failure in any run could be retrieved by re-cycling the product through the blower.

Analyses of the Crude and Refined Seed Hairs

The investigations set forth were, for the most part, carried out according to the standard methods commonly used in testing plant materials. Needless to say, some modifications

were necessary in certain phases of the experiments and in other cases the common methods of wood analysis failed entirely to give the desired results. Further, since the seed hair samples were not entirely free of seeds and of tough but small flower stalks the composition of the seed hairs was not quite uniform (8). During the analysis of the seed hairs some drawbacks were borne in mind, such as, their extremely large volume compared to weight, and the possibility of stray air currents blowing the seed hairs away during weighings and transfers.

Table X compares the analytical data collected for the crude seed hairs plus attached seed with those of the refined seed hairs. In all cases the results of the analyses are based on the weight of the original air-dry sample.

The ash content of the refined seed hairs was considerably higher than in unpurified cotton linters, which have 1 to 1.5 per cent (65). Because the ash content was high, the ash alkalinity was determined to indicate the presence or otherwise of sodium, potassium, calcium and magnesium which usually appear as oxides, phosphates or carbonates. Remarkably enough, the ash alkalinity was nil before refining but afterwards it was 0.49 ml. Comparison with the recorded value of 13 to 16 ml. for raw cotton (66) shows that the observed figure was minute enough to suggest the above basic oxides were practically absent. Since the ash alkalinity is closely connected with the number of carboxyl groups in the sample and with its affinity for methylene

TABLE X

Composition of Crude and Refined Typha Seed Hairs

	Crude %	Refined %
Moisture	6.39 - 6.41	5.94 - 5.95
Ash	3.33 - 3.56	2.47 - 2.63
Ash Alkalinity	...	0.49 ml.(a)
Hot-water Solubles	12.63	16.67
Fats, Waxes and Resin Acids	14.55 - 14.56	3.60 - 3.61
Nitrogen	1.37	0.35
as protein (x 6.25)	8.58	2.2
Pentosan	15.72 - 15.73	28.18 - 28.59
Klason Lignin (70)	17.25 - 17.42	11.47 - 11.66
Ross and Potter Lignin (71)	9.52 - 9.92	...
Hot 7.14 per cent NaOH Solubles	26.68 - 26.80	29.96

(a) ml. of N sulfuric acid required to neutralize one gram of ash.

blue and other basic dyestuffs (67)(68) the result suggested silica was the principal constituent of the ash and that carboxyl-containing gums or pectic materials were probably absent.

Heuser (57) reported the ash content of typha seed hairs as 3.80 per cent, a value which is somewhat higher than the results obtained in this investigation. The ash content reported by Gilchrist (60) was low and only slightly higher than that of purified cotton linters (about 0.1 per cent). The variation in values may be ascribed to the soil in which the plant has grown, the time of harvesting and to the degree of freedom from impurities.

Fats, waxes and resin acids are normally soluble in ether, benzene, ethyl alcohol, etc. The above substances are intimately associated with cellulose in its natural state and hence their removal forms the first step in an accurate chemical examination of the fiber (69). In the case of the seed hairs their removal is a prerequisite as their presence in fairly large amount makes the fibers extremely resistant to aqueous reagents.

To have a stock of extractive-free fibers on hand, the purified fibers were extracted in batches of 250 g. in a big Soxhlet extractor, first with alcohol-benzene and then with hot water, washed with water and air-dried. To avoid discrepancy in weighing, the bottle containing the fibers was stored in the weighing room, to attain equilibrium with the atmosphere there.

There was a remarkable drop from 14.6 to 3.6 per cent in the fats and waxes content of the fibers before and after refining. Since in the process only the seeds had been removed, they must have contributed a large percentage of fats and waxes. This observation is borne out by the oil extracted from seeds by Burgess-Manning Company in U.S.A. and put on the market (6).

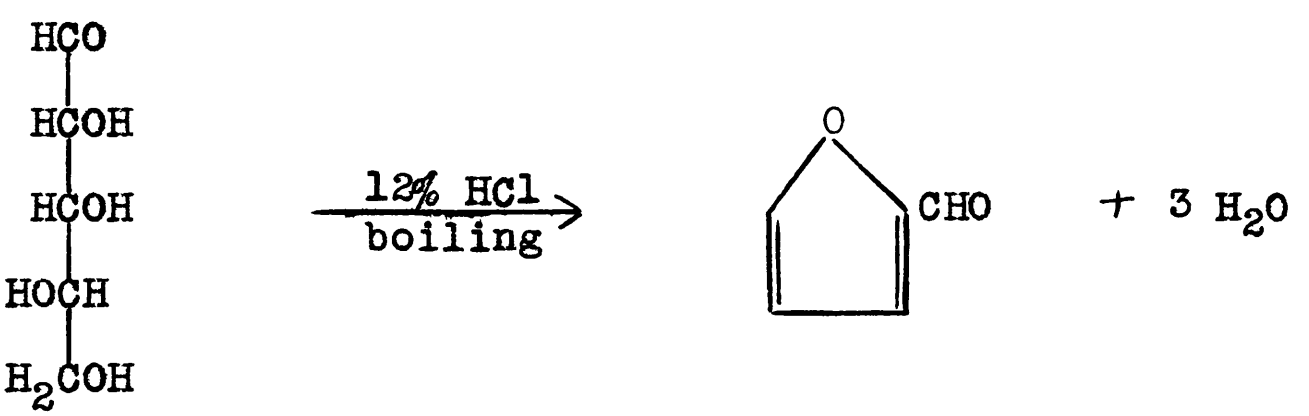
The lignin content of these seed hairs has rarely been reported, and when reported it has been calculated by difference and not by direct determination, which is none too satisfactory or accurate. Therefore the determination of lignin was carried out by two methods and at a later stage by removing the carbohydrate material by oxidation with periodate. The determinations by the Ritter, Seborg and Mitchell 72 per cent sulfuric acid method (70) and the Ross and Potter method using formaldehyde (71) were not entirely satisfactory for the short flower stalks remain undigested and interfered in the analyses.

It is therefore a misstatement to designate all the material, whether digested or undigested, which remains on the Gooch crucible as "lignin".

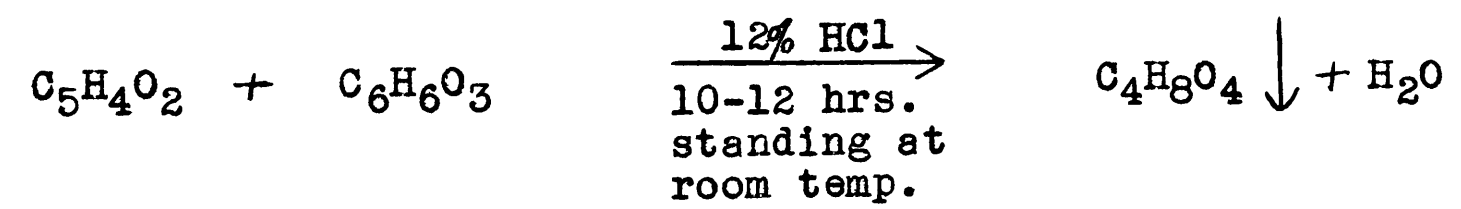
Heuser (57) reported the lignin content as 9.45 per cent, which value he deduced by difference and not by actual determination. The only other instance where there is mention of lignin content is by Gilchrist (60). He reports the value as 19.16 per cent, but Huddle (72) says that this value "was supplied by an outside laboratory".

The methoxyl content of the Klason lignin from the purified fibre was 6.8 per cent or much lower than that in woods (73).

When cellulosic material is boiled with dilute mineral acid, the pentose sugars or pentosans form furfural, which is quantitatively determined by precipitation with phloroglucinol, and from which the percentage of pentosans can be found.



Pentose Furfural



Furfural Phloro-glucinol Furfuralphloro-glucide

The pentosan content before and after refining was 15.7 and 28.4 per cent and presumably the weight of seeds that were present did not contribute any pentoses. The weight was 35 to 45 per cent of the seed hairs (6). If we consider that

only pentoses of seed hairs contributed toward the furfural formation, then the percentage of pentosan in the unpurified seed hairs becomes about 26.2 per cent, which is slightly below 28.39 per cent. This result suggests that the refining operation had been fairly efficient. The pentosan content reported by Heuser (57), 28.05 per cent, formed the only instance where the present analytical results checked with those reported by previous investigators.

The treatment of cellulose fibers with alkali is one of the oldest and most important commercial reactions of cellulose. The two main objectives served by treating cellulose with alkali are the modification of the physical and chemical properties of natural fibers and the production of alkali cellulose as an intermediate in the preparation of chemical derivatives.

Alkali hydroxides act as swelling agents for all forms of cellulose and as solvents for modified or degraded forms of cellulose and hemicellulose (74). Lilienfeld (75) has found that 8 per cent sodium hydroxide scarcely dissolves mercerized cellulose at 0^{o}. Hence the action of 7.14 per cent sodium hydroxide on typha seed hairs would swell the fibers, dissolve cellulose below a certain degree of polymerization, and dissolve much of the huge amount of pentosans present. The solubility of cellulose depends largely upon the concentration of the alkali and the temperature used (76)(77) and conditions applied to cotton may be a little too drastic for typha seed hairs.

The amount of material soluble in 7.14 per cent sodium

hydroxide at boiling temperature increased substantially when the seeds were absent and it accounts for most of the lignin plus pentosans present. The result also compares favourably with the amount of lignin plus hemicellulose, or beta- and gamma-cellulose fractions, as will be discussed later. There is no mention of this analysis in the literature and hence these figures are of special interest.

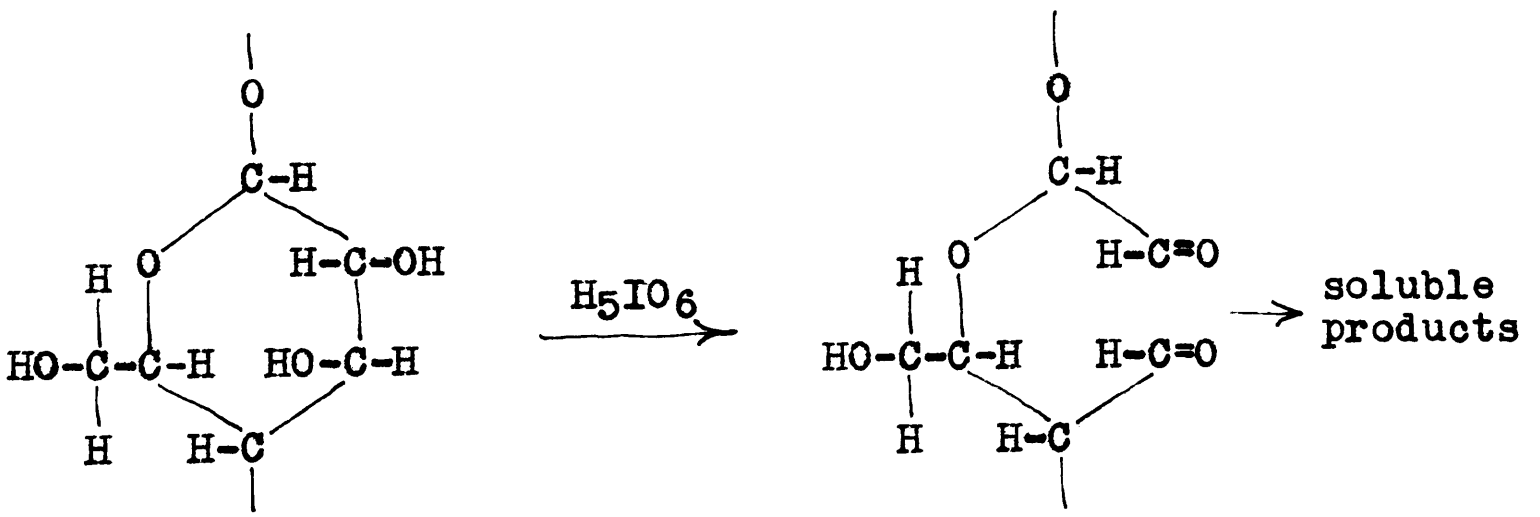
Plant tissue often contains two types of nitrogen, one protein and the other alkaloid. The proteins are often water-soluble and with acids are hydrolyzed to a mixture of amino acids; alkaloids are often insoluble in water but are soluble in alcohol. Much nitrogen is therefore usually removed by extractions with alcohol-benzene and water. The Kjeldahl nitrogen values reported in Table X, carried out prior to such extractions, reveal by indirection the high content in the seeds. Although the nitrogen values have been recalculated as protein by multiplying them by the standard factor 6.25, this procedure was not justified because no work was done to show that proteins were the only nitrogenous substances present. The result, 2.2 per cent of the refined fibers, is the first reported, and may be compared to the value of 0.69 per cent found for spruce (78).

Additional Estimations on the Solvent-Extracted, Refined Fibers

The most interesting aspect of the above analyses was the observation that the standard Klason lignin estimation left a residue, even from the refined seed hairs that obviously

was not all lignin, and that a trial with the Ross and Potter modification of this method on the crude fiber promised no better results, even although this modification is sometimes more efficacious than the original method. (71) In these circumstances the decision was made to prepare periodate lignin from the seed hairs.

Jackson and Hudson (79) (80) (81) have shown that the oxidation of cellulose by periodic acid proceeds by the cleavage of the glucose ring of the cellulose chain between carbon atoms two and three with the conversion of the secondary alcohol groups to aldehydes, according to the following scheme.



cellulose periodate oxycellulose

Davidson (82)(83) did further work on oxidation with periodic acid and suggested that the cellulose is more uniformly attacked by the periodic acid than by chromic acid. Grangaad, Gladding and Purves (84) actually estimated the dialdehyde, in the case of cellulose oxidized by periodic acid, and found that properly prepared samples had about 90 per cent of the dialde-

hyde-type of oxidation. They showed that the side-oxidations of the cellulose were at a minimum at pH 4. Rutherford, Minor Martin and Harris (85) found that in the early stages, the reaction was confined to the oxidation of the secondary hydroxyl groups to aldehyde groups, almost quantitatively, with concomitant rupture of the bond between the second and third carbon atoms of the glucose units of the cellulose chain, thereby confirming the above findings. The aldehyde groups were readily oxidized to carboxyl groups which could be estimated.

Jayme and co-workers (86)(87)(88) extended the reaction to xylans, starch and other carbohydrates found in plant material. The cleavage products were soluble in hot water. On the other hand, according to Freudenberg and collaborators (89), lignin was apparently unchanged by periodate. Oxidation with dilute aqueous periodate has also been employed by Pennington and Ritter (90) to free certain lignin sulfonic acids from traces of polysaccharides. Their research clearly showed that a methoxyl-containing fragment was cleaved from a lignin sulfonic acid during the oxidation. Wald, Ritchie and Purves (91) isolated periodate lignin from spruce and pine woods and later work by Ritchie and Purves (92) showed that it was closely similar to the original lignin in the woods. The main difference was that the methoxyl content of the isolated lignins was lower than the calculated values.

The oxidations of the seed hairs were carried out on the same lines as Ritchie and Purves (92) used with spruce wood.

Northern pine wood, according to Wald (93) consumed about 0.5 x 10^{-3} mole of 0.5 per cent aqueous periodate in three hours at 20° at pH 4.1, but further consumption was minute. The woods did not suffer any appreciable decrease in weight owing to the oxidation, but a sharp decrease occurred when the products were boiled in water at pH 7. It was observed that the undissolved residues were again sensitive to periodate oxidation and that the oxidation-extraction cycle could be repeated until all the readily oxidizable matter was completely removed. Ritchie and Purves (92) have prepared black spruce periodate lignin after five or six oxidation-extraction cycles.

The first oxidation of the seed hairs proceeded along the lines expected. When the curve had levelled off, the residues were washed in a continuous washer and were later hydrolyzed in boiling water near pH 7. The residue was much more sensitive to periodate in the second and third oxidations than in the first. A sudden drop in the consumption occurred in the fourth and fifth oxidations but in the sixth there was a sharp rise in the consumption of periodate. To account for this anomalous behaviour the residue was examined under the microscope before the sixth and after the seventh oxidations. Before the sixth oxidation, entire fibers were conspicuous in the residue, but after the seventh hardly any fibers were visible. The consumption of periodate after the seventh was much smaller and hence the process was stopped at this stage. The course of oxidation is plotted in graphs 1 and 2, pages 88 and 89.

The residue was light yellow in colour, amorphous and contaminated with silica from the glassware. An ash determination was carried out on the residue and the yield of periodate lignin corrected for it. The result of 12.84 per cent (a single determination) was apparently in fair agreement with values 11.47 to 11.66 per cent obtained in a parallel determination by the Klason method. Determinations of methoxyl content, however, showed the two lignins to be different, the periodate and Klason products having 8.24 and 6.80 per cent, respectively. Moreover, when the periodate sample was estimated by the Klason procedure, only 46.1 per cent was recovered as Klason lignin. Periodate spruce and pine lignins, by contrast, analyzed about 90 per cent Klason lignin and had methoxy contents of 12 to 15 per cent (92). The substance analyzing for "lignin" in typha seed hairs not only had a lower methoxyl content than those in woods, but perhaps a different chemical structure, since the periodate was obviously and markedly different from the Klason product when treated with 72 per cent sulfuric acid. When the refined fibers were boiled for three hours with 2.5 per cent sulfuric acid, as in Schorger's (94) estimation, all but 53.4 per cent (oven-dry, 55.9 per cent air-dry) dissolved. As expected, this treatment reduced the pentosan content of the fibers, the change being from 28.4 to 11.59 per cent, and increased their lignin content from 11.57 to 26.3 per cent. When calculated to 100 parts by weight of original fiber, 28.4 - 11.59 x 0.53 or about 22 parts of pentosan were lost during the hydrolysis, while the corresponding figure for Klason lignin,

11.57 - 26.3 x 0.53, represented a gain of about 2.4 parts. Boiling in dilute acid therefore caused a slight apparent increase of 2.4 per cent in the lignin content, a phenomenon sometimes encountered in similar analyses of woods (95).

After soluble substances have been removed from woods by extraction with suitable inert solvents, the residue is supposed to consist almost entirely of cellulose, hemicellulose and lignin. The removal of the latter by suitable means, leaves the "skelettsubstanzen" or the holocellulose in practically quantitative yield.

Table XI summarizes the results of preparing by the Ritter and Kurth (96), the van Beckum and Ritter (97) and the Wise, Murphy and D'Addieco methods (98). The first two of these methods involve similar brief chlorinations and extractions, but differ in the composition of the extraction liquid. The third method differs radically in employing a continuous extraction of the lignin by aqueous sodium chlorite. With softwoods the three methods give closely similar yields of nearly lignin-free holocellulose, while with hardwoods the van Beckum and Ritter method sometimes gave slightly lower yields owing to the removal of small amounts of carbohydrate. When the lignin content of the sodium chlorite holocellulose is recalculated to the basis of the original typha fiber, (3.09 x 0.918) and is subtracted from the original value of 11.57 per cent, the result, 8.8 per cent, represents the substance removed by the sodium chlorite. The calculated holocellulose yield is there-

TABLE XI

Comparison of Methods Used in the Holocellulose Preparation

Preparation	Holocellulose Yield(a) %	Holocellulose Klason Lignin %	Holocellulose Pentosans %
A. Wise, Murphy and D'Addieco	91.79	3.09	...
Sodium chlorite - water	91.79	3.12	
B. van Beckum and Ritter	66.42	2.91	...
Alcohol - monoethanol-amine (b)	66.45	2.95	
C. Ritter and Kurth	81.10	11.95	30.5
Alcohol - pyridine (c)	81.23	11.96	30.6
C. followed by B.	71.9	3.12	34.32
	72.2	3.22	34.88
D. Original fibers	100.	11.47	28.18
		11.66	28.56
D. extracted with Alcohol - pyridine	99.87	...	...

(a) Based on original crude fiber throughout.
(b) Three chlorination - extraction cycles.
(c) Five chlorination - extraction cycles.

fore (100 - 8.8) or 91.2 per cent, in good agreement with the observed value of 91.8 per cent. It thus appears that the sodium chlorite method of preparing holocellulose functions as well with typha fibers as it does with woods. The product was a beautiful creamy white pulp in its native morphology.

On the other hand, a similar calculation shows that the alcohol-pyridine method removed hardly any of the substances analyzing as Klason lignin, but almost 17 per cent of other fiber constituents were missing. The alcohol-monoethanolamine method was also quite unsatisfactory since it extracted much of the non-lignin substances as well as most of the lignin.

The inefficient removal of lignin by the alcohol - pyridine method aroused interest since the observation seems to be the only recorded instance where the delignification was so poor. To ascertain this particularity a few experiments were conducted.

The extract from the alcohol - pyridine holocellulose preparation was concentrated at reduced pressure on a steam bath, and was recovered, after drying <u>in vacuo</u> in a yield of 14.56 per cent. A Klason lignin determination carried out on this residue however, showed that it contained only 0.76 per cent of lignin, thereby confirming the fact that almost all of the lignin had been retained in the holocellulose and some other material had been extracted to an extent of nearly 14 per cent.

Another sample of the seed hairs was then extracted

for 7.5 hours in a Soxhlet extractor, without chlorination, with the alcohol-pyridine solution used in the delignification treatment but the amount of material extracted was only 0.13 per cent. The chlorination was therefore necessary for the removal of substances other than lignin.

The alcohol-monoethanolamine treatment when superimposed on the alcohol-pyridine treatment removed the lignin and the resulting "holocellulose" was analysed. Lignin content had dropped down to 3.17 per cent and the pentosan content increased to 34.6 per cent. When recalculated to the basis of the original fibers, the alcohol-pyridine holocellulose accounted for 30.55 x 81.15 or 24.8 per cent of the original pentosan, and it was interesting to note that the same amount 34.6 x 72, or 24.9 per cent, remained after the superimposed alcohol-monoethanolamine holocellulose treatment. The latter therefore in this particular case removed little save lignin. Reverting to the alcohol-pyridine holocellulose, this product retained 24.8 per cent of pentosan and 81.15 x 11.95 or 9.7 per cent of lignin on the original fiber basis. Comparison with the data in Table XI, line D, shows that about 3.6 per cent of pentosan and 2.3 per cent of lignin had been removed, thereby accounting for a loss in yield of about 5.9 per cent. Since the actual loss was 100 - 81.1 or 18.9 per cent, the alcohol-pyridine holocellulose treatment had removed about 13 per cent of non-pentosan, non-lignin substance from the fiber.

In an endeavour to determine the nature of this mater-

ial, the pyridine-alcohol extract was concentrated to dryness, finally in a vacuum oven at 55°. It was noted that small amounts of white crystals sublimed from the viscous dark brown tar that formed the bulk of the residue and amounted to 14.56 per cent of the original fiber. The crystals could not be recrystallized from the several liquids tried, but sublimation from a special apparatus (Fig. 7, page 90) and in a nitrogen atmosphere was found to be fairly a satisfactory method of purification. The pure white crystals were very hygroscopic and deliquesced in air to a brown oil. These properties made their quantitative analyses difficult, but after considerable trouble the empirical formula $C_4H_5N.HCl$ was assigned to them.

A blank experiment was then run in which a mixture of the same concentration of alcohol-pyridine was treated with chlorine gas under conditions as similar as possible to those existing during the holocellulose preparation. The only product isolated, however, was a small amount of pyridine hydrochloride, which melted 82° instead of at the much higher temperature of 133° to 135° found for the unknown. Anderson (99) passed chlorine through aqueous pyridine and recovered pyridine hydrochloride as well as another compound which was not analyzed and investigated fully. Anderson's pyridine contained some picoline and its isomers; in consequence a trichloropicoline was also isolated. This compound had 54.2 per cent of chlorine, whereas the present compound had only 33.4 per cent, entirely present as a very readily ionizable hydrochloride. Qualitative tests suggested the hydrochloride pertained to a rather volatile amine

which was of a secondary nature since it yielded an amorphous benzene sulfonamide insoluble in alkali, and failed to evolve nitrogen gas when treated with sodium nitrite. Although the formula, $C_4H_5N \cdot HCl$, and general properties were those to be expected of pyrrole hydrochloride, pyrrole is such a weak base that its hydrochloride remains unknown in a crystalline state. It was unfortunate that the inaccessibility of reasonable amounts of the unknown crystals, together with their tendency toward deliquescence and discoloration when exposed to the atmosphere, forced the deferment of further attempts to identify them with precision.

The yield of the crystals was 14.56 per cent, based on air-dry fibers. Since the crystals contained 13.5 per cent nitrogen, they accounted for 1.6 per cent nitrogen in the fibers, wherein only 0.35 per cent was found, Table X. It was obvious therefore that the crystals obtained most or all of their nitrogen from the pyridine used.

Further Study of the Chlorite Holocellulose

As already mentioned, the only method of preparing holocellulose from the typha seed hairs that gave satisfactory results was the sodium chlorite treatments of Wise, Murphy and D'Addieco (98) and the following experiments were carried out on such products.

Alpha-cellulose is that portion of industrial cellulose pulps that fails to dissolve in 17.5 per cent caustic soda

used under exact conditions. When the alkaline extract is neutralized, the fraction precipitated is denoted by the term beta-cellulose. Gamma-cellulose is that portion which remains in solution even on acidification. The latter includes cellulose chains ranging in length up to about 10 glucose units and those in the beta-cellulose fraction have a degree of polymerization increasing to about 140-200 (100). It follows that the insoluble alpha-cellulose consists predominantly of macromolecules of a degree of polymerization of 200 and more. Beta- and gamma-celluloses are not necessarily true celluloses in the sense that they contain only polymers of glucose anhydride units. They may consist of polymers of other sugars such as the xylans, oxidation products such as oxycelluloses, or true cellulose compounds of low molecular weight (101).

The alpha cellulose was isolated by filtration, dried and found by direct weighing to be 43.6 per cent of the typha sodium hypochlorite holocellulose. A standard oxidation with chromic acid revealed the sum of the beta- plus gamma-cellulose in an aliquot of the mother liquor, and a similar oxidation, carried out on another aliquot from which the beta fraction had been precipitated, gave the amount of the gamma-cellulose alone. The percentage of beta-cellulose was then found by difference. Since the sum of the alpha-, beta- and gamma-celluloses (Table XII, 43.6 + 1.86 + 4.65) accounted for only 50 per cent of the holocellulose, a check analysis was carried out on a sample of commercial bleached sulphite pulp. The results, 83.3, 84.06

per cent alpha-, 6.01, 6.38 per cent beta-, and 11.09, 11.42 per cent gamma-cellulose, accounted for 100.4, 101.8 per cent of the pulp on an air dry basis and were good enough to establish confidence in the technique used. Evaporation of the mother liquor from the precipitation of the typha gamma-cellulose showed that it contained 25.3 per cent of organic material. Although this estimation was probably of low accuracy, it established the fact that typha holocellulose contained a large amount of material that became unusually soluble after treatment with the 17.5 per cent caustic soda used in the alpha-cellulose determination.

TABLE XII

Fractionation of Chlorite Holocellulose after removing the Alpha Fraction (a)

Fraction	Yield % Chromic Acid Method (b)	Yield % Solubility Method (c)
Beta-cellulose	1.86	0.18
Gamma-cellulose	4.65	8.08
Mother liquor	25.31	....

(a) 43.6 to 43.94 per cent of holocellulose.
(b) Becker's Method (103)
(c) O'Dwyer's Method (102)

The yields of the beta- and gamma-celluloses were also determined by direct weighing, but a large correction

for associated ash made the results (Table XII) of doubtful value. They were, however, of the same order of magnitude as those found by oxidation.

The difficulties encountered in making the sum of the alpha-, beta- and gamma-cellulose determinations approximate to the proper value of 100 per cent made it desirable to study the action of alkali on the chlorite holocellulose in greater detail. The fibers were therefore extracted with carbonate-free sodium hydroxide solutions of the concentrations given in Table XIII.

TABLE XIII

Extraction of Hemicelluloses with Alkali

NaOH %	Dissolved %	Notes
1	42.28	Overnight, at room temperature in nitrogen atmosphere.
5	55.56	Overnight, at room temperature in nitrogen atmosphere.
7.14	67.99	3 hours, boiling under controlled conditions.
17.5	53.06	45 minutes at 20° in air.

From the data it was clear that an extensive separation of hemicellulose could be conveniently carried out with a concentration of 5 per cent sodium hydroxide, at room temperature

in a nitrogen atmosphere. An extraction with 5 per cent caustic soda under nitrogen was then carried out on a larger scale, and after a similar re-extraction, the undissolved residue of cellulose amounted to 44.44 per cent of the holocellulose. Neutralization of the filtrate should have yielded the hemicellulose fraction A, but the amount was negligible. Two volumes of ethanol were then added to the neutral, concentrated liquors to recover the hemicellulose B in 24.84 yield. Further concentration of the mother liquors, followed by the addition of two volumes of acetone, gave hemicellulose C in 25.32 per cent. This method of extracting and fractionating hemicelluloses followed the O'Dwyer method (102) the theoretical background for which was carefully discussed in the book by Norman (105).

Hemicellulose fractions B and C were highly contaminated with sodium sulfate and were repeatedly dissolved in water and precipitated with 95 per cent ethyl alcohol in order to purify it. Attempts to remove the salt by dialysis through cellophane involved loss.

It was felt that the best way to avoid the interference of the inorganic solids was to keep them in solution and precipitate the hemicellulose fraction only. According to Akerlof, Teare and Turck (106) the solubility of sodium chloride in aqueous ethyl alcohol solution at 20° is considerably higher than sodium sulfate. Therefore an original alkali extract containing the hemicelluloses was neutralized with 20 per cent hydrochloric acid, and the hemicellulose A, B and C

fractions were precipitated as already described.

The purity of the hemicelluloses isolated was tested by ash determinations, and several reprecipitations from aqueous alcohol or acetone were required to remove inorganic salts.

The final result was that the original holocellulose analyzed 44.44 per cent of "cellulose" insoluble in 5 per cent caustic soda, a negligible amount of hemicellulose A, 24.84 per cent of hemicellulose B and 25.32 per cent of hemicellulose C. Only 94.60 per cent of the holocellulose were recovered and the remaining 6.4 per cent was therefore soluble in the aqueous acetone used to precipitate the C fraction.

In order to investigate the solubles contained in the final aqueous acetone mother liquor, a sample containing sodium sulfate was repeatedly concentrated and made up with two volumes of ethanol until inorganic material had been eliminated. Evaporation, finally to dryness in a vacuum at a low temperature left a thick syrup amounting to 7.16 per cent of the holocellulose. This amount corresponded approximately to the deficiency of 6.4 per cent found in the hemicellulose A B C total. The dark, dried, syrup was insoluble in water but soluble in aqueous ethanol in concentrations of about 50 to 95 per cent and also dissolved in acetone. Unfortunately, the solutions were too dark in colour for optical observations to be made.

The reducing sugars which were likely to be in the syrup were estimated by the modified method of Shaffer and Hartmann (107). A 50:50 aqueous alcohol solution was used and trial showed that the calibration curve of glucose in this mixture, as in water, was a straight line starting very near the origin. The syrup did not register any reducing value before and after hydrolysis in 1 N hydrochloric acid for 60 minutes; on the other hand, hydrolysis in 1 N hydrochloric acid for 180 minutes gave a solution apparently containing 26.5 per cent reducing sugars calculated as glucose, as determined from Graph 3, page 60. Too little substance remained to make a more extended study of the apparent contradiction between the results of hydrolyzing for 60 and 180 minutes and the same difficulty made it impossible to confirm a negligible pentosan content found for the syrup.

A preliminary methylation of the syrup with dimethyl sulfate and caustic soda was also carried out, with the result that two fractions of a very dark viscous gum were obtained. The first fraction, soluble in chloroform, had a methoxyl content of 21.60 per cent, while that of the second, water-soluble fraction, was 15.36 per cent. Unfortunately, the amounts were insufficient for further study.

Since an araban was found in typha seeds (43), and since Ehrlich and Schubert (108) showed that the araban associated with pectic acid was soluble in 70 per cent ethanol, the presence of an araban in the above syrup was considered likely. The complete solubility of the syrup in 95 per cent

ethanol and in acetone and its insolubility in water however, made it unlikely that any considerable portion was of a polysaccharide nature, and this conclusion was supported by the dubious reducing power found after acid hydrolysis and by the low pentosan content. The chemical nature of the syrup therefore remained unknown.

EXPERIMENTAL PROCEDURES

Separation of the Seed hairs from the Seeds

(a) Blowing Equipment

The equipment consisted of a rectangular deal-wood box with a motor linked to a blower at the bottom. The open top of the box was covered with a wrapping-paper hood, the end of which was connected to a cloth bag. Inside the box a series of galvanized iron baffles were arranged at regular intervals (see Figs. 4, 5 and 6). At right angles to the direction of the air current of the blower, a stove pipe by-pass was located in such a manner that the heavy lumps of seed hairs which settled at the bottom of the box by gravity were recirculated continuously by the suction created by the blower fan. At the bottom of the box a quarter-inch mesh screen, which had been fixed on to a cardboard-backed frame, was placed facing the blower current. On the wall opposite the by-pass a door was provided to remove the settled seeds and to facilitate the cleaning of the equipment.

Dimensional drawings of the equipment and the specification of the materials used are shown on pages 58, 59 and 60. Two photographic views on page 61 illustrate the special features of this equipment.

(b) Rough Blowing

The pistillate was removed manually from the long

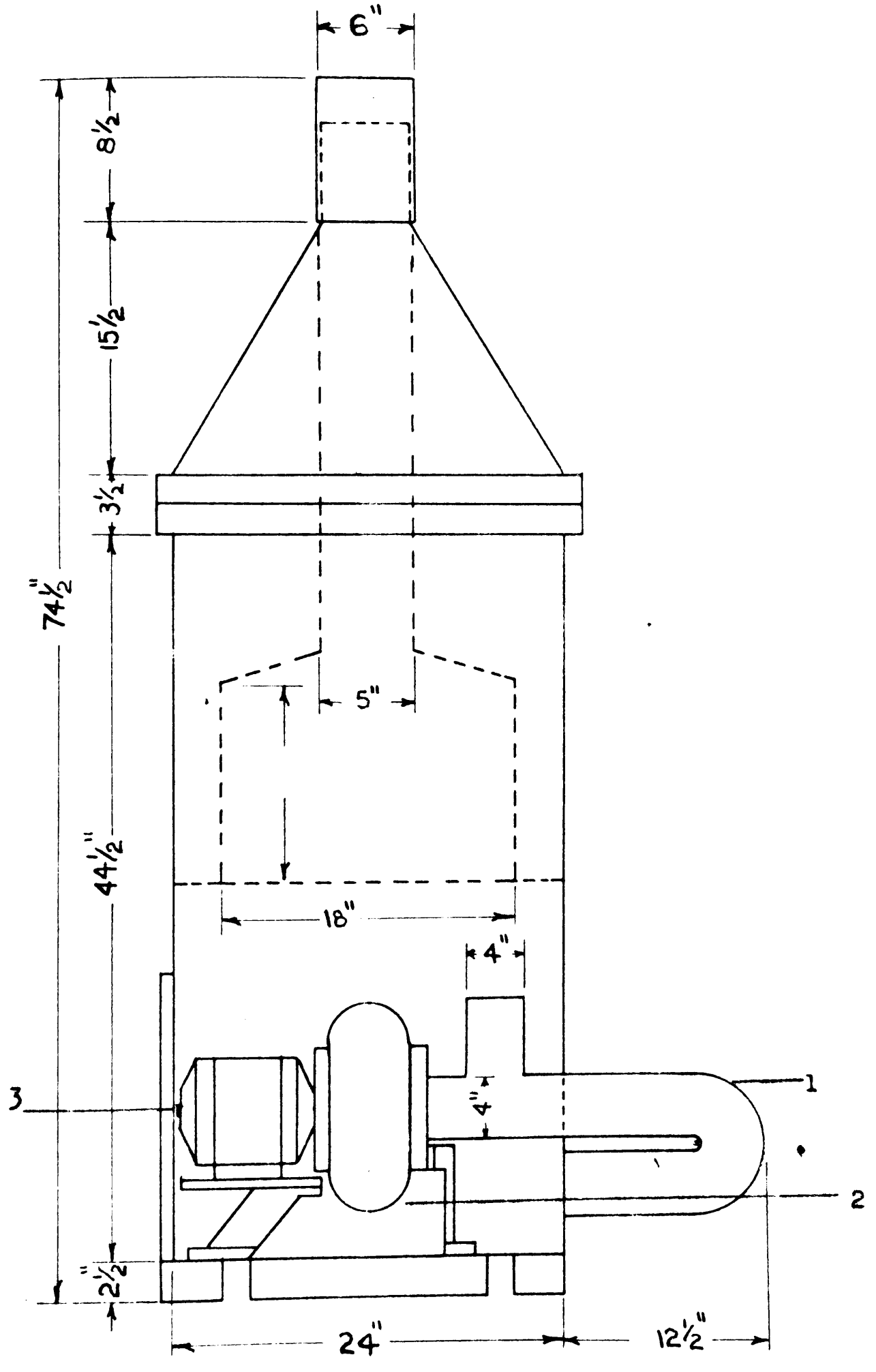


FIG.4 LEFT HAND VIEW OF THE REFINING EQUIPMENT

(1) Stovepipe 4" dia. by-pass
(2) Blower 9" dia.
(3) Motor A.C., phase 1, 1/4 H.P., 24 hour 50°C., 110 volts, 4.9 amp., 60 cycles, 1720 r.p.m.

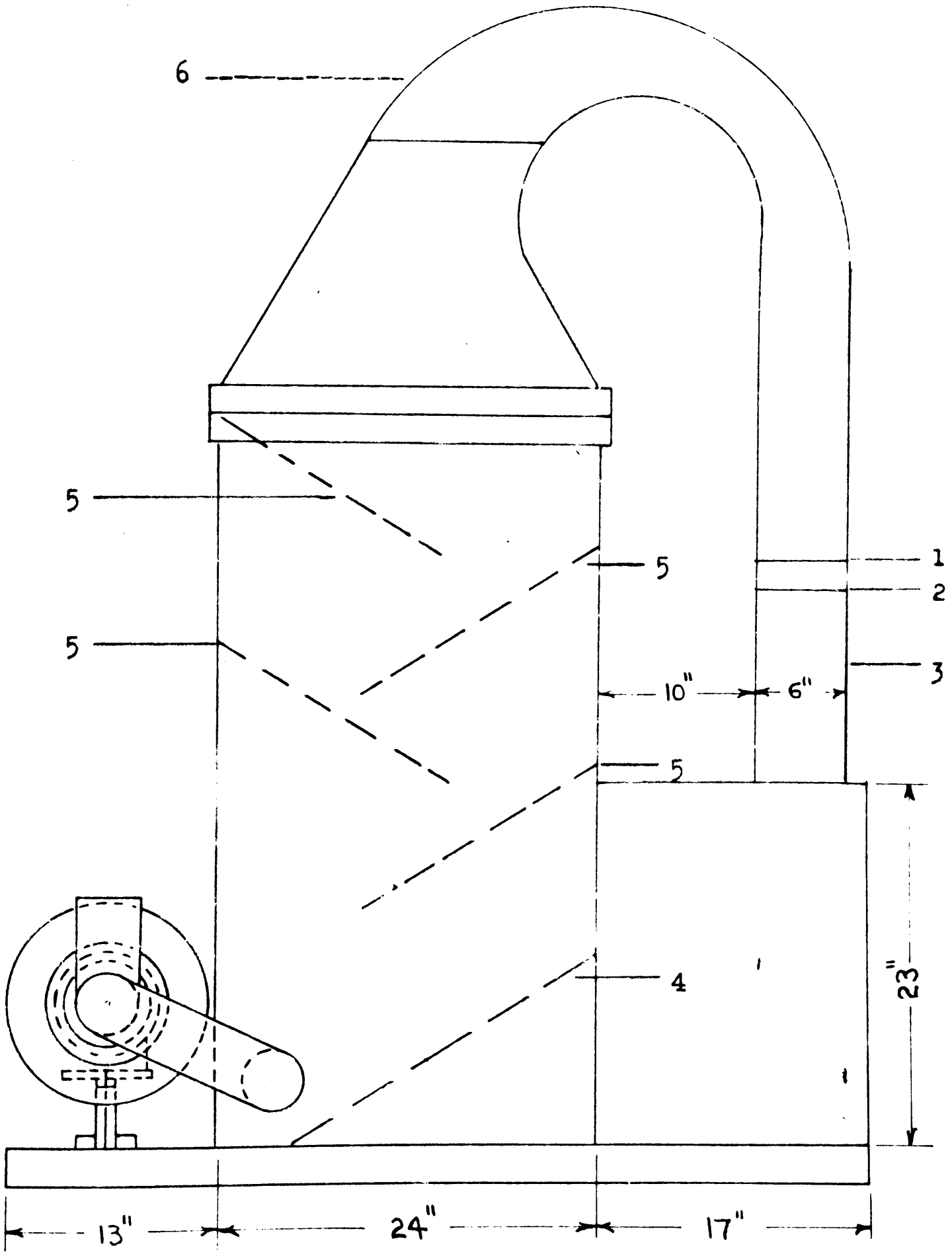


FIG.5 FRONT VIEW OF THE REFINING EQUIPMENT

(1) Cardboard stiffener
(2) Elastic bands
(3) Cloth bag 100-120 holes per sq. inch
(4) Screen, 1/2 mesh, cardboard backed, set 2" deep
(5) Galvanized iron 0.032 sheet baffles
(6) Wrapping paper hood, stappled togethe

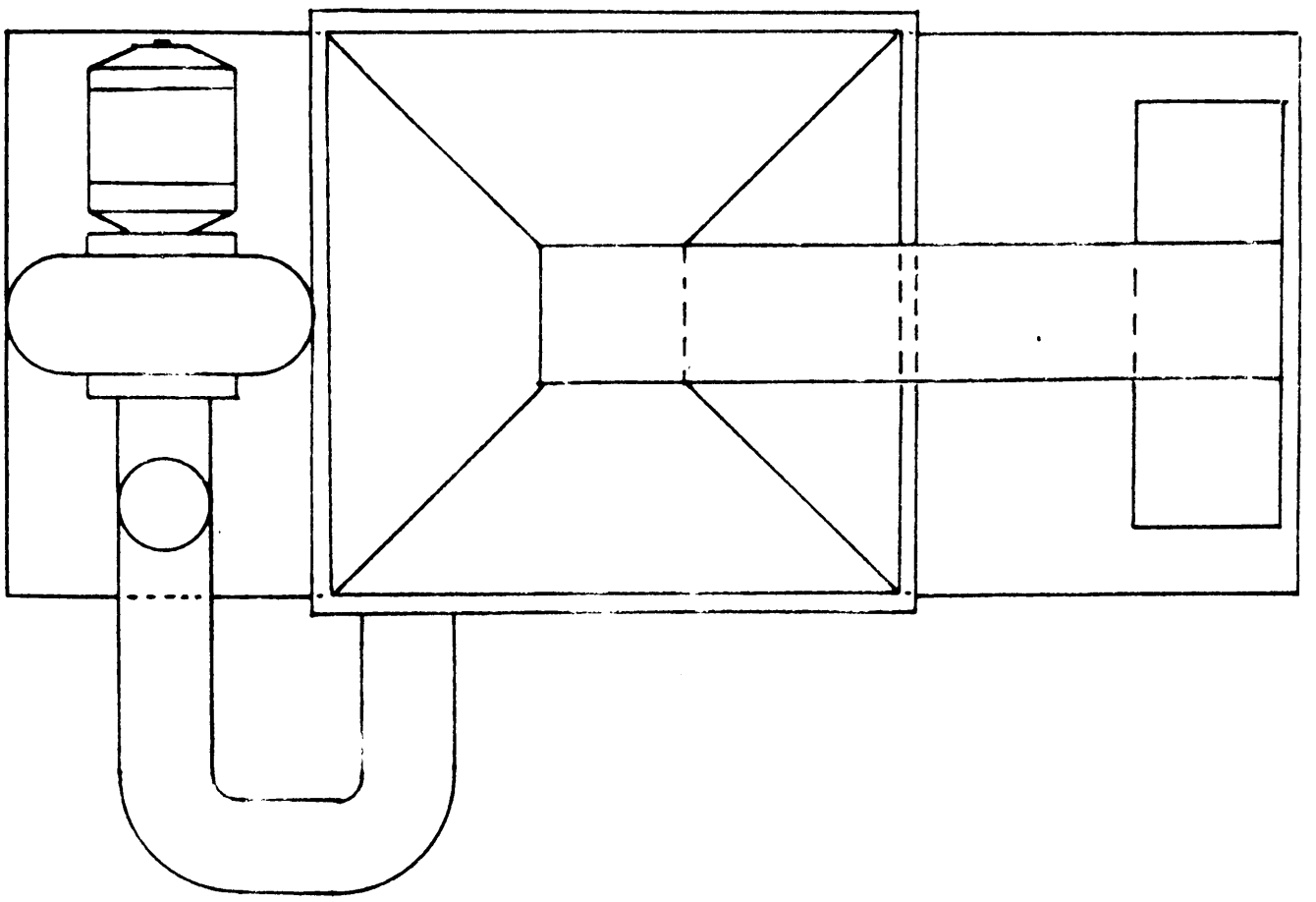
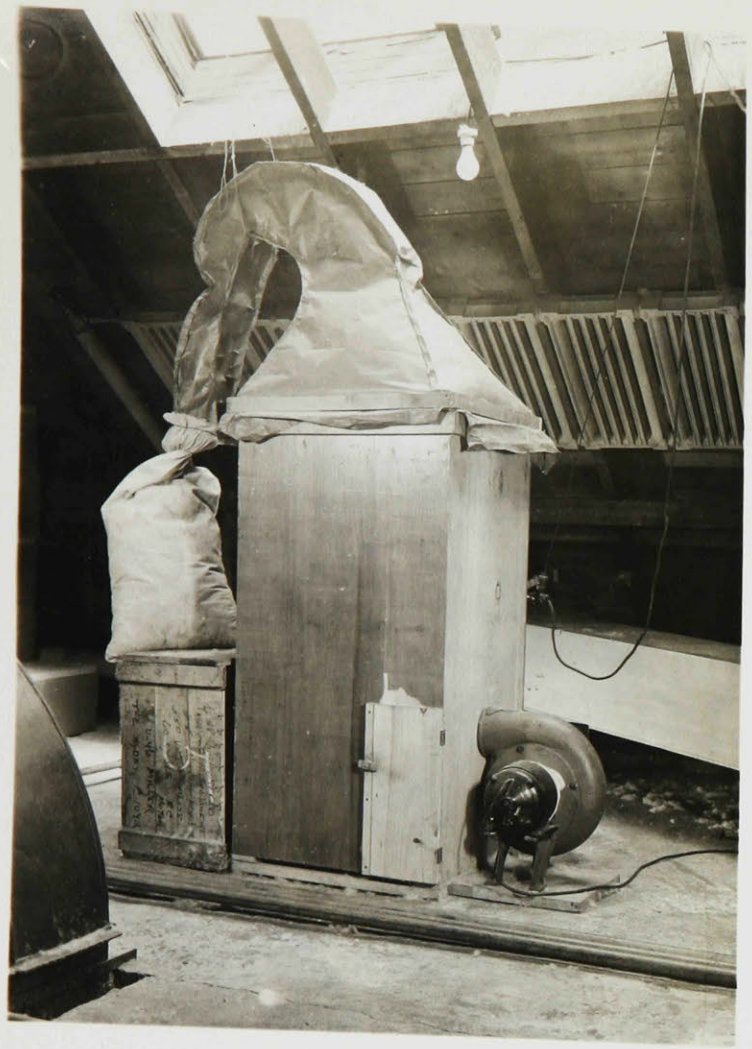
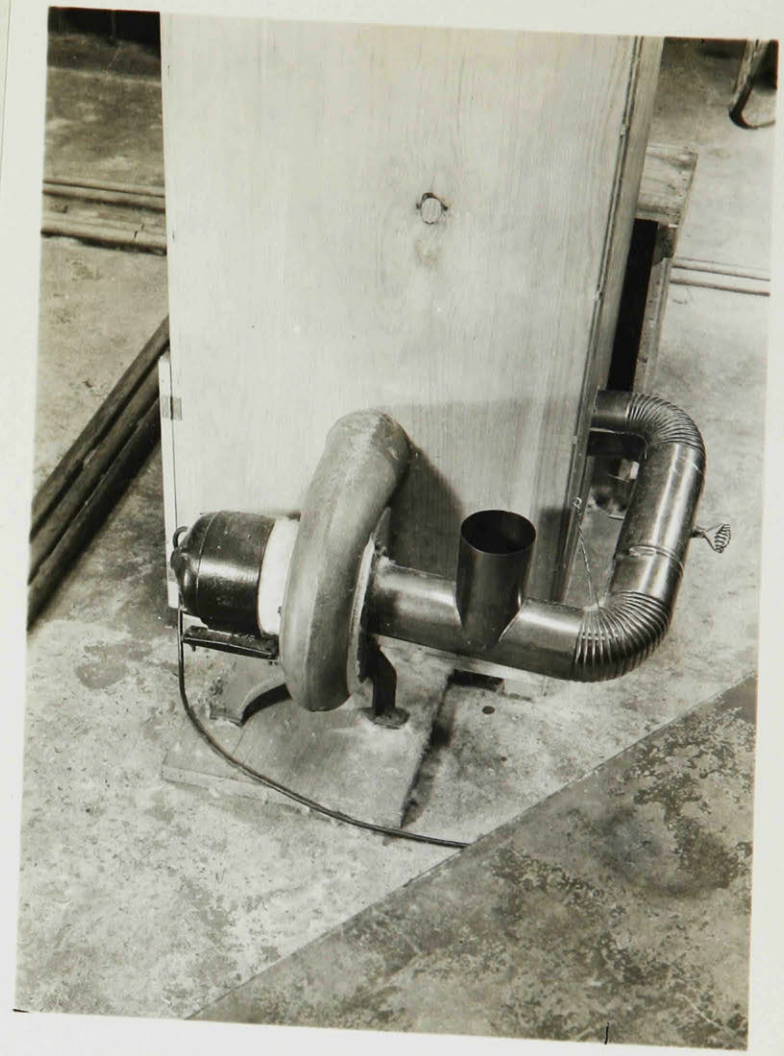


FIG. 6. PLAN VIEW OF REFINING EQUIPMENT



PHOTOGRAPH 1.

Side-view showing the motor and blower, the door in the side used for cleaning, the hood and the bag used for collecting the refined fibers.



PHOTOGRAPH 2.

Close-up showing the details of the blower and by-pass assembly.

stalk by rubbing off into a container. The fibers and seeds thus separated from the stalk were put through the blowing equipment described above. The initial rough blowing was done by feeding the fibers and seeds into the blower inlet, a small quantity at a time, and the refined fibers syphoned into the cloth bag. Then the by-pass circulation was continued for an additional quarter of an hour, following which the screens were taken out and cleaned. The seeds which had settled at the bottom were then removed. The seed hairs in the bag were designated as the first fraction, and the rough fibers that had collected on the top two baffles were designated as the second fraction.

(c) Fine Blowing

Before the fine blowing operation, the equipment was cleaned thoroughly and a fresh cloth bag attached to the hood. Small quantities of the first fraction were passed through the equipment until all the material of the first fraction had been used up. The time required for the same operation, on the second fraction was about double. The seed hairs from this process were taken for subsequent analyses.

Moisture Content

The moisture content of the fibers was determined by the gravimetric method as per the American Standard of Testing Materials. (109). A sample of fibers was weighed in a weighing bottle and dried at 100 to 105^{o} for eight hours or until the weight decreased to a value that remained constant. The stopper

of the weighing bottle was replaced before removal from the oven and quickly transferred to a desiccator and cooled to room temperature. After loosening the stopper for an instant, to equalize the air pressure within and without the container, the final weighing was carried out.

Ash Content

The ash content of the fibers was determined as per TAPPI Standard T. 413m-40 (110). The sample of fiber (recovered from the moisture content determination) was weighed into a porcelain crucible to which a lid was fitted. The incineration was carried out in a muffle furnace maintained at 500 to 600°. The crucible lid was removed to complete the oxidation of the residual carbon, after which the crucible and contents were cooled in a desiccator to room temperature and then re-weighed.

The ash alkalinity (111) was determined by adding 10 ml. of 0.01 N sulfuric acid, warming on a steam bath for one hour, diluting to 70 ml. with distilled water and then titrating to a methyl red end-point with 0.01 N sodium hydroxide.

Hot-Water Solubles

The hot-water solubles were determined as per TAPPI Standard T. 207m-41 (112). A sample of fibers was immersed with 100 ml. of distilled water in a 250 ml. Erlenmeyer flask provided with a reflux condenser. The flask was placed in a boiling water bath maintained at a constant level just above the solution in the flask. After boiling gently for three hours, the contents of the flask were filtered into a weighed Gooch

crucible and washed with hot water. The crucible was dried at 100 to 105°, cooled in a desiccator to room temperature, and weighed in a weighing bottle.

Fats, Waxes and Resin Acids

Fats and waxes were determined as per TAPPI Standard T. 6m-41 (113). A sample of fibers was extracted for eight hours in a Soxhlet with a constant boiling mixture composed of one volume of ethanol and two volumes of benzene. The loss in weight before and after extraction represented the fats, waxes and resin acids soluble in ethanol-benzene solution.

Lignin

(a) Klason Lignin

Klason lignin was determined by a slight modification of the Ritter, Seborg and Mitchell method (70). A sample of extractive-free fibers was triturated with 25 ml. of 72 ± 0.1 per cent sulfuric acid, at 20°. After two hours the acidity was reduced to 3 per cent by dilution with distilled water, and the hydrolysis of the carbohydrates was completed by boiling under a reflux condenser for three hours. The lignin was recovered on a weighed Gooch crucible, thoroughly washed with hot water, dried in an air oven at 100 to 105°, cooled in a desiccator and weighed. A considerable portion of the fibers was not digested and the whole of the undigested residue is reported as "lignin".

(b) Formaldehyde and Sulfuric Acid Lignin

Lignin was also determined by the Ross and Potter method (71). A sample of extractive-free fiber was well wetted in a 100 ml. beaker with 4 ml. of commercial formaldehyde solution. After five minutes the moistened meal was mixed with 4 ml. of 72 per cent sulfuric acid, and after a further five minutes, the carbohydrate portion was finally dissolved by stirring with an additional 6 ml. of 72 per cent sulfuric acid. Ten minutes later, 50 ml. of a reagent, consisting of 6 volumes of glacial acetic acid to 1 volume of chloroform, was stirred into the mixture.

The meal was then quantitatively transferred to a 600 ml. beaker containing from 350 to 400 ml. of distilled water. The beaker was placed over a moderate flame and the chloroform evaporated. After fifteen minutes boiling, the liquid was diluted to 500 ml. with distilled water and maintained hot for a further fifteen minutes.

The granular lignin so precipitated was collected in a weighed Gooch crucible and washed with 200 ml. of 5 per cent hydrochloric acid. The lignin was then dried for two hours at 120°, cooled in a desiccator to room temperature, and weighed in a weighing bottle. The "lignin" again contained non-digested fibers.

Pentosans

Pentosans were determined by the method of the Associa-

tion of Official Government Chemists (114). The apparatus consisted of (1) a 300 ml. ordinary round-bottom flask of the wash bottle type, to the glass stopper of which were fused an outlet tube and a separatory funnel; (2) a water-cooled glass condenser and (3) a graduated cylinder provided with a funnel.

Solutions required were (1) a 12 per cent, by weight, solution of hydrochloric acid, sp. gr. 1.06; (2) phloroglucinol solution: 0.30 g. of pure phloroglucinol was dissolved in 40 ml. of 12 per cent hydrochloric acid, filtered and preserved for use.

A sample of material was placed in the 300 ml. reaction flask provided with a separatory funnel and attached to a condenser. A small piece of paraffin and a few glass beads were placed in the flask, to which was added 100 ml. of 12 per cent chemically pure hydrochloric acid. The contents of the flask were distilled at the maximum rate of 30 ml. in ten minutes. The distillate was passed through a small filter before entering the receiver. As soon as 30 ml. of distillate was collected, 30 ml. of 12 per cent hydrochloric acid was added to the distillation flask and the distillation was continued in this manner until a total of 360 ml. of distillate was collected. Forty ml. of phloroglucinol solution was added, upon which the distillate quickly turned greenish black if furfural were present. After sixteen hours the furfural phloroglucide had settled to the bottom of the flask. A drop of the supernatant liquid gave

no pink colour with aniline hydrochloride paper, and thus indicated the complete precipitation of the phloroglucide.

The furfural phloroglucide suspension was recovered on a previously weighed Gooch crucible, and the cake was washed with exactly 150 ml. of cold distilled water. The crucible was dried for two and one-half hours in an air oven at 100 to 105°, cooled in a stoppered weighing bottle in a desiccator to room temperature, and weighed.

From the weight of the furfural phloroglucide obtained, the weight of the pentosans present in the sample was found from the Kröber and Tollens tables, or according to Kröber the following calculation can be made.

Weight of phloroglucide	Weight of phloroglucide	Weight of phloroglucide
less than 0.030 g.	0.030 to 0.300 g.	exceeds 0.030 g.
Pentosans		
$(a + 0.0052) \times 0.8949$	$(a + 0.0052) \times 0.8866$	$(a + 0.0052) \times 0.8824$

where a = the oven-dry weight in grams of furfural phloroglucide.

The pentosan content was expressed as a percentage of the original oven-dry material.

"KOH Solubles"

The 10 per cent "KOH solubility" was determined by

the method of Bray (115).

Since an equivalent strength of the cheaper sodium hydroxide solution, namely 7.14 ± 0.1 per cent, may be substituted for the costly 10 per cent potassium hydroxide solution, the determination has been carried out in this investigation with 7.14 ± 0.1 per cent sodium hydroxide solution, and the result designated as "KOH Solubles".

A sample of fibers was placed in a 250 ml. Erlenmeyer flask provided with a reflux air condenser, with 100 ml. of 7.14 ± 0.1 per cent sodium hydroxide. The flask was heated for three hours in an oil bath maintained at 100°, care being taken to avoid undue oxidation of the fibers by air. After the heating was completed, the contents were poured into a beaker containing a liter of distilled water and the alkali was neutralized with an excess of glacial acetic acid, which might cause the precipitation of some of the beta-cellulose. The undissolved material, together with the precipitated beta-cellulose was then filtered on to a weighed crucible, and thoroughly washed successively with hot water, alcohol and ether. It was then dried at 100 to 105°, cooled in a desiccator to room temperature and weighed to a constant weight in a weighing bottle.

Holocellulose

(a) Ritter and Kurth

Holocellulose was prepared from the fibers by the Ritter and Kurth method (96). A sample of extractive-free

fibers in a glass extractor was moistened with distilled water and the excess moisture drawn off by suction. Using moderate suction, chlorine gas was passed through the sample for four minutes, and then exhausted for an additional minute. Suction was broken and a few ml. of alcohol-pyridine solution--15 ml. of pyridine with enough 95 per cent ethyl alcohol to make up 100 ml. of solution--was added to the extractor to neutralize the acids formed during chlorination. The system was allowed to stand for one minute, then the excess solution was drawn off by suction. The extractor was transferred to a Soxhlet extractor and extracted with the alcohol-pyridine solution for two and one-half hours. The extractor was removed and the contents washed with cold distilled water. The chlorination and extraction treatment was repeated three times, at the end of which there was no noticeable darkening of the pulp upon further chlorination and addition of alcohol-pyridine solution. The holocellulose was washed with cold distilled water, twice with alcohol, twice with cold distilled water, and again with alcohol until the residue was neutral to litmus. Finally, it was washed thoroughly with ether to remove all of the alcohol and to facilitate drying.

The holocellulose was air-dried to remove excess ether and then dried at 100 to 105° in an oven for eight hours, cooled in a desiccator to room temperature and weighed.

The liquor extracted was set aside for future examination.

(b) van Beckum and Ritter

Holocellulose was again prepared by the van Beckum and Ritter method (97). A sample of extractive-free fibers was placed in a Gooch crucible, moistened with water, and suction applied to remove the excess moisture. The sample was chlorinated for three minutes, then stirred, and chlorinated for an additional two minutes. Suction was broken and 95 per cent alcohol was added to soak and dissolve excess chlorine and hydrogen chloride. After four minutes, suction was again applied and released. Hot alcohol - monoethanolamine of not less than 75°, enough to cover the fibers completely, was added and stirred thoroughly. The alcohol - monoethanolamine solution consisted of 3 ml. of monoethanolamine in 97 ml. of 95 per cent ethyl alcohol. The fibers were soaked for two minutes and the extractives removed by suction. The solvent treatment was repeated and the final wash was with 95 per cent ethyl alcohol and twice with cold distilled water. After removing excess water by suction, chlorination and extraction treatments were repeated until the residue became white following chlorination and was no longer coloured by the addition of the hot solvent for lignin chlorides. At this point the material was freed of alcohol-monoethanolamine by washing twice with alcohol, twice with cold distilled water, and again with alcohol until the residue was neutral to litmus, and finally with ethyl ether to facilitate drying. The holocellulose was first air-dried to remove ether so as to guard against explosion, and then dried in an air oven at 100 to 105° for eight hours, cooled in a desiccator

to room temperature and weighed.

(c) Wise, Murphy and D'Addieco.

Holocellulose was again prepared by Wise, Murphy and D'Addieco's method (98) Five g. of extractive-free fibers were used in the experiment. The sample was treated under a hood for one hour at 70° to 80° with 160 ml. of distilled water containing 1.5 grams of sodium chlorite and 10 drops of glacial acetic acid. The reaction was carried out in a three-neck round-bottom flask, having a stirrer in the middle and a thermometer in one of the other openings. Chlorine dioxide, among other vapours, was evolved slowly and reacted gradually with the lignin and extractives, the course of the reaction being followed readily by the colour changes in the fibers. At the end of the first hour, the residue was yellowish white and had retained the original fiber texture. The suspension was cooled in an ice-bath, filtered by suction through a Gooch crucible and then washed repeatedly with ice water and finally with acetone. The residue was then air-dried and finally oven-dried at 100 to 105° for eight hours, cooled in a desiccator to room temperature, and weighed.

Alpha-, Beta- and Gamma-Cellulose from Holocellulose

(a) Alpha-Cellulose

Alpha-cellulose was determined gravimetrically as per TAPPI Standard T. 429m-44 (116). A sample of lignin-free fibers was weighed into a 250 ml. Pyrex beaker. A 25 ml. volume of a

17.5 per cent (by weight) carbonate-free sodium hydroxide solution cooled to 20° was measured out in a small graduate. Ten ml. of this solution was added to the fibers in the beaker maintained at 20° in a water bath, and allowed to stand for five minutes. With a short glass rod, the end of which had been flattened out to form a small disc, the material was mascerated for ten minutes during which interval the remaining 15 ml. of sodium hydroxide solution of 20° was added in 5 ml. portions. The beaker was covered with a watch glass and allowed to stand for thirty minutes. Distilled water, 25 ml. at 20°, was added to the alkali-cellulose mixture accompanied by thorough stirring. The contents of the beaker were filtered immediately, by means of suction, to a previously weighed fine Gooch crucible. The residue in the crucible was washed with 100 ml. of distilled water at 20°. After releasing the suction, 15 ml. of 10 per cent acetic acid of 20° was added at 20° and allowed to react for five minutes, when the suction was again applied and the acid removed. The alpha-cellulose was washed with distilled water of 20° until free from acid as shown by testing with litmus paper. When free from acid, it was again washed with an additional 200 ml. of distilled water at 20°.

Excess water was wiped from the outside and bottom of the crucible with a cloth and the crucible, together with the weighing bottle, was dried in an oven at 100 to 105° for six hours, cooled in a desiccator to room temperature and weighed.

(b) Beta- and Gamma-Cellulose

Beta- and gamma-celluloses were determined by two different methods gravimetrically by O'Dwyer's method (102) and volumetrically by Becker's method (103). The filtrate from the alpha-cellulose, before adding the acetic acid, was collected and made neutral with sulfuric acid. The reaction flask was allowed to stand in the cold room for a few hours and the beta-cellulose that had settled at the bottom of the flask was recovered on a fine, previously weighed Gooch crucible. The beta-cellulose was washed first with acetone and then with ether, dried in an oven at 100 to 105° for six hours, cooled in a desiccator to room temperature, and weighed.

The filtrate from the beta-cellulose determination, before the addition of acetone, was diluted with 2 volumes of 95 per cent ethyl alcohol. The gamma-cellulose which precipitated, was washed first with acetone and then with ether, dried in an oven at 100 to 105° for six hours, cooled in a desiccator to room temperature, and weighed.

Beta- and gamma-celluloses were determined volumetrically by oxidizing an aliquot of the alkaline liquor containthem, before the addition of the acetic acid to the alpha-cellulose pad, with potassium dichromate solution. This alkaline filtrate was made up to 1 liter by the addition of distilled water. The reagents used in the oxidation were: potassium dichromate, 90.0000 g. per liter, ferrous ammonium sulfate 159.90 g. per liter (159.90 g. salt, 5 ml. concentrated sulfuric acid,

made up to 1 liter with distilled water), sulfuric acid, 50 per cent solution by weight, phosphoric acid, 85 per cent, sodium diphenylamine sulfonate, 0.32 g., barium diphenylamine sulfonate (E.K. No. 3104) dissolved in 100 ml. of distilled water (0.5 g. sodium sulfate added to precipitate the barium sulfate.)

Blank determination: Five ml. of the standard potassium dichromate solution was added to 100 ml. of a solution of the same strength sodium hydroxide as that in the test specimen. (75 ml. of 17.5 per cent sodium hydroxide diluted to 1000 ml.) Fifty ml. of cold concentrated sulfuric acid was added to the above and diluted with 150 ml. of distilled water and cooled to room temperature. Ten ml. of phosphoric acid and 0.5 ml. of sodium diphenylamine sulfonate indicator were added. Titration was carried out with the ferrous ammonium sulfate solution to the first green end-point.

Beta- plus gamma-cellulose determination: 100 ml. of the test specimen was pipetted into a 500 ml. Erlenmeyer flask containing five or six glass beads. Five ml. of the standard potassium dichromate solution was added by pipette, then 50 ml. of concentrated sulfuric acid. The mixture was boiled gently for ten minutes to oxidize the organic material present, care being taken to prevent bumping. The solution was then slightly cooled, diluted with 150 ml. of distilled water, and cooled to room temperature. Ten ml. of phosphoric acid and 0.5 ml. of the sodium diphenylamine sulfonate indicator were added, and titration carried out with the standard ferrous ammonium sulfate solu-

tion to the first green end-point. The indicator color change from red to blue to blue-green was the end-point.

Gamma-cellulose determination: One hundred ml. of the test solution was titrated with 50 per cent sulfuric acid solution to a phenolphthalein end-point, and the volume recorded.

Four hundred ml. of the test specimen was transferred into a 500 ml. Erlenmeyer flask. The volume of sulfuric acid required to neutralize the sodium hydroxide present was added, which was four times the ml. of acid required to titrate the 100 ml. test solution. The flask was equipped with an air condenser and placed in a hot water bath for 30 minutes to coagulate the beta-cellulose, cooled to room temperature, and filtered through a Gooch crucible (provided with an asbestos pad protected by a Wittman plate) into a dry filter flask. It was not necessary to filter more than 250 ml. If the filtrate was not clear, the asbestos pad had not retained all of the beta-cellulose, and the filtrate therefore was refiltered. Any moisture in the asbestos pad was removed by discarding the first few ml. of filtrate.

A 100 ml. aliquot plus the amount equivalent to the ml. of acid required to neutralize the sodium hydroxide solution in 100 ml. of the original test specimen, was pipetted from the filter flask into a 500 ml. Erlenmeyer flask containing glass beads. This total gave a sample that was equivalent to 100 ml. of the test specimen, less the beta-cellulose.

Into the above solution was pipetted 5 ml. of standard

potassium dichromate and 50 ml. of concentrated sulfuric acid. This mixture was boiled slightly, cooled, diluted with 150 ml. of distilled water, and cooled again. Ten ml. of phosphoric acid and 0.5 ml. of indicator solution were added and the mixture was titrated with ferrous ammonium sulfate to the first green end-point. The weight of cellulose in the aliquot was calculated from the fact that the consumption of 1 g. of potassium dichromate (after allowance for the blank) corresponded to 0.1375 g. of cellulose. The amounts of gamma-cellulose, and of the beta- plus gamma-cellulose, were found in parallel determinations and the beta fraction was taken as the difference between the two results.

Acid Hydrolysis

The hydrolysis of the fibers in acid under standard conditions was determined by Schorger's method (94). Approximately 2 g. of the fibers were weighed into a 250 ml. Erlenmeyer flask and 100 ml. of 2.5 per cent sulfuric acid was added. The flask was connected with a reflux condenser and the contents were boiled gently for three hours and then allowed to cool. The interior of the condenser was washed down with a little distilled water and the contents of the flask transferred to a 250 ml. volumetric flask. Distilled water, free from carbon dioxide was added to make up the volume, the mixture allowed to stand several hours with frequent shaking, and was then filtered.

The fibrous residue was recovered on a Gooch crucible, washed with cold distilled water, dried in an oven at 100 to 105°

and weighed in a weighing bottle.

Hemicellulose

Hemicelluloses were determined by the solubility method of O'Dwyer (102), which has been modified by Lovell (117) and Wise and Ratliff (118) with 1 and 5 per cent sodium hydroxide solution.

Delignified fibers, about 5 g., were weighed into a 150 ml. ground-stoppered Erlenmeyer flask and 50 ml. of 1 per cent carbonate-free sodium hydroxide solution was added. The fibers were stirred until they were completely disintegrated, then the air inside the flask was replaced with nitrogen, the flask was closed again and the extraction allowed to proceed overnight, at room temperature. The following day the contents were filtered on to a previously weighed Gooch crucible of coarse porosity. The fibers were washed with a small quantity of 1 per cent carbonate-free sodium hydroxide, and finally with cold distilled water. The crucible was dried at 100 to 105°, cooled in a desiccator to room temperature and weighed in a weighing bottle.

The solubility in 5 per cent sodium hydroxide was determined in the same way. In the larger scale preparation of hemicellulose, 5 g. of the delignified fibers were extracted overnight in a nitrogen atmosphere, with 100 ml. of carbonate-free 5 per cent caustic soda. The residue was re-extracted in the same way, was washed with acetic acid and water, dried and weighed. Yield, 44.44 per cent.

The soluble filtrates from the above extractions were combined and neutralized with sulfuric acid to precipitate hemicellulose fraction A. The filtrate from this operation was concentrated under reduced pressure on a steam bath and 2 volumes of 95 per cent ethyl alcohol were added to precipitate hemicellulose fraction B. The filtrate from the above was again concentrated under reduced pressure on a steam bath, and 2 volumes of commercial acetone were added. The precipitate was taken as hemicellulose fraction C, and the mother liquor, which was practically free of sodium sulfate was concentrated under reduced pressure to a syrup that was set aside for later study.

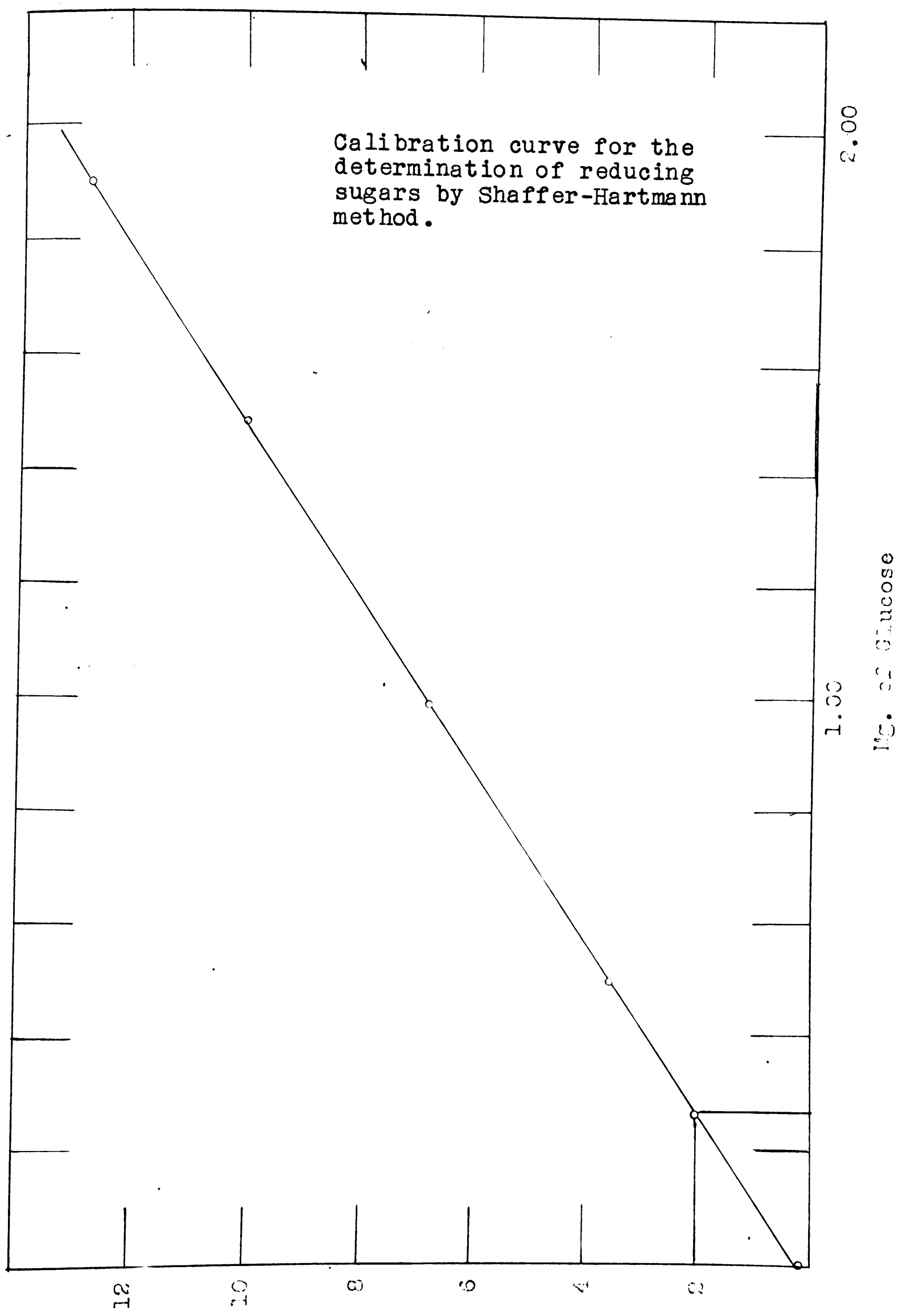
The fractions B and C were repeatedly dissolved in a small amount of water and precipitated with 95 per cent ethyl alcohol, but the separation of sodium sulfate, which had similar solubilities was incomplete. An attempt was made to remove the salt by dialysis through a cellophane membrane, but the operation caused a serious loss of hemicellulose.

To keep inorganic salt in solution during the precipitation of the hemicelluloses, the soluble liquor from the alkali extract was neutralized with hydrochloric acid instead of sulfuric acid (106). This change replaced sodium sulfate with sodium chloride, which was much more soluble in aqueous alcohol, and reprecipitations of the hemicellulose fractions rendered them free of inorganic materials.

Since it was necessary to dissolve the syrup in 50 per cent ethanol instead of water, the Shaffer-Hartmann reagent was calibrated against pure glucose dissolved in the aqueous alcohol.

The glucose calibration plot was determined as follows: 5 ml. of the reagent was measured into a large Pyrex test tube (250 x 25 mm.) and 5 ml. of the glucose solution added containing not less than 0.1 mg. of not more than 2.0 mg. of glucose. The two solutions were mixed by gentle shaking and the tube covered with an air condenser. The reaction test tube was then kept in a boiling water bath for fifteen minutes, cooled by placing in a shallow dish of water until the temperature was lowered to 35 to 40°. One ml. of 5 N sulfuric acid was added and all copper oxide precipitated was promptly dissolved. After 2 minutes the mixture was titrated with 0.005 N sodium thiosulfate solution. A blank titration on 5 ml. of the reagent and an equal volume of 50:50 ethyl alcohol and distilled water was also made.

The difference between the blank and the titration in presence of a known amount of glucose gave a copper reducing power expressed as ml. of 0.005 N thiosulfate. When ml. thiosulfate were plotted against mg. glucose, a straight line very nearly passing through the origin resulted (Graph 3.). Another straight line, probably differing in slope, is the form of the standard calibration plot against aqueous glucose. It was easy to determine the Shaffer-Hartmann reducing power of a known


Calibration curve for the determination of reducing sugars by Shaffer-Hartmann method.
2.00
1.00
Mg. of Glucose
12
10
8
6
4
2
Ml. of 0.005 N Sodium Thiosulfate

GRAPH 3

weight of another substance as ml. of thiosulfate, and to express the result as mg. of glucose by reference to the calibration plot.

The reducing power of the syrup was determined before and after hydrolyses with 1 N hydrochloric acid, for one- and three-hour periods, the pH before the addition of the acid and after neutralizing with 1 N sodium hydroxide solution being adjusted to the same value. Table XIV shows that the reduction was negligible both before and after acid hydrolysis for one hour, but increased to the equivalent of a 26.5 per cent glucose content after hydrolysis for three hours. Lack of material prevented confirmation of this percentage.

The methylation of the syrup was carried out by Challinor, Haworth and Hirst's method (119), employing 16.4 ml. of methyl sulfate and 37 ml. of 30 per cent sodium hydroxide for a 2 gm. sample. A three-neck reaction flask was used and the methyl sulfate and alkali were slowly added at a temperature of 35° with constant, vigorous stirring. After the additions were complete, the temperature was maintained at 70° for half an hour. Care was taken that during the entire process the reaction mixture was alkaline.

The reaction liquor was filtered and the filtrate neutralized with sulfuric acid. The filtration was then repeated to remove a little inorganic solid that separated and the filtrate was extracted with chloroform. Evaporation of the aqueous fraction under diminished pressure left a residue that was repeatedly extracted with methyl alcohol. The several methanol fractions

TABLE XIV

Determination of Shaffer-Hartmann Reducing Power

Substance[a] mg.	$Na_2S_2O_3$ ml.	Blank-Titer ml.	Reduction as Glucose mg.(b)
Calibration against glucose			
0.0	21.85(c)	21.85(c)	
0.5	18.3	3.55	
1.0	15.0	6.85	
1.5	11.8	10.05	
Reduction of syrup			
1.0	21.7	0.15	0.025(d)
1.0	21.7	0.15	0.025(e)
1.0	19.85	2.00	0.265(f)

(a) In 5 ml. of 50 per cent ethanol.
(b) From calibration plot (Graph 3).
(c) Shaffer-Hartmann blank.

(d) Prior to acid hydrolysis
(e) Hydrolyzed for one hour in boiling N hydrochloric acid.
(f) Hydrolyzed for 3 hours in boiling N hydrochloric acid.

were combined and concentrated under reduced pressure on a steam bath to a dark syrup with a methoxyl content of 15.36 per cent. The chloroform-soluble fraction was similarly concentrated under reduced pressure to a highly viscous dark syrup. The yield was less than a gram, an amount which was not enough, after determining the methoxyl content as 20.60 per cent, to carry out further methylations.

Oxidation of Refined Fibers with Sodium Paraperiodate

These oxidations were carried out according to Ritchie and Purves method (92) on one-gram lots of the fibers contained in a three-neck reaction flask immersed in a water bath at 20°. The reaction was carried out with constant stirring. Sodium paraperiodate, 200 ml. of 5 per cent strength, buffered to pH 4.1 with glacial acetic acid, was added and the amount of periodate remaining after various times was estimated volumetrically. Five ml. aliquots were transferred periodically to a 125 ml. Erlenmeyer flask, diluted to approximately 30 ml. and made alkaline with normal alkali to a phenolphthalein indicator. Solid dry ice was added to adjust the pH from 8 to 9 and then an excess of solid potassium iodide followed by 10 ml. of 0.1 N arsenious acid. After ten minutes' standing, the solution was titrated with 0.1 N iodine solution. The difference in iodine titration of the solution and a blank was taken as the amount of periodate consumed and the consumption plot drawn (Graph 1).

Calculation:

$$\text{Grams of Periodate consumed} = \frac{(\text{Titre} - \text{Blank}) \times 294 \times \text{N. of } I_2 \times T}{1000 \times 2 \times 10 \times 0.1000 \times V}$$

where T is the total volume of the solution

and V the volume of the aliquot.

When the curve had flattened out the oxidation was stopped and the oxidized residue was recovered by continuous washing, until the washings on acidification failed to liberate iodine from potassium iodide. The residue was boiled under reflux for three hours with water at a pH close to 7. After filtration and washing with water, the cycle of 9-hour oxidations, washing and hydrolyzing in water was repeated, care being taken that at no time the residue was permitted to dry in air, until the end of the sixth cycle when the consumption of periodate had become small, as per Graph 2, page 89. The residue, periodate lignin, amounted to 12.4 per cent of the original fiber and contained 8.4 per cent of methoxyl groups. Periodate lignin analyzed 46.1 per cent Klason lignin when submitted to the Klason estimation. The methoxyl content was determined on the lignins by the Vieböck and Schwappach method (120).

Examination of the Alcohol-Pyridine Extract from Typha Holocellulose

The combined alcohol-pyridine extracts obtained in preparing typha holocellulose by Ritter and Kurth's method was concentrated at reduced pressure on a steam bath and the last traces of solvent were evaporated in a vacuum oven at 55°. The

cooler sides of the flask appeared to be lined with white crystals. These crystals were taken up with a very small amount of 95 per cent ethyl alcohol which was distilled off at reduced pressure on a steam bath. When all the solvent had been distilled, a white mass of crystals had again sublimed to the neck of the flask. These crystals were recovered and purified by sublimation.

The sublimation was carried out in an oil bath which was heated by an electric mantle placed inside a desiccator (Fig. 7, page 90).

The crude crystals were placed on the watch glass, a perforated filter paper placed over it and then covered with a second watch glass. After closing the desiccator, the electric current was turned on to heat the oil bath, air in the desiccator was exhausted and then replaced with nitrogen gas. When the temperature had risen to 80° the crystals sublimed on to the upper watch glass. By thus subliming and resubliming the product was purified.

The above method of purifying the crystals proved to be most suitable as all attempts at recrystallization from different solvents failed. The crystals were highly hygroscopic, and appeared to decompose in air to a yellow liquid.

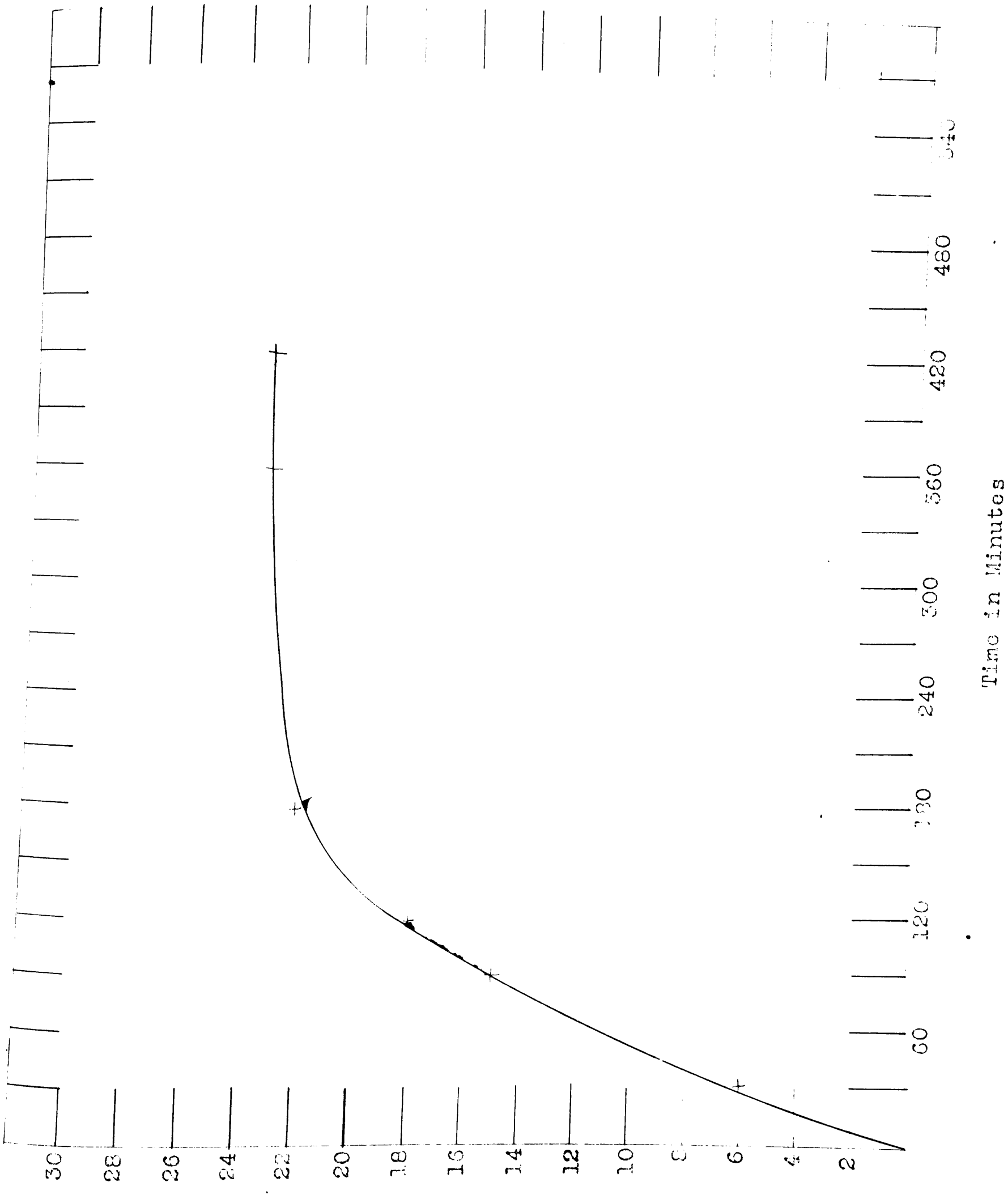
Examination showed that the white crystals had a somewhat soft melting point of 133° to 135°, were very soluble in cold water to give a solution neutral to litmus, and left no residue when ignited. A sodium fusion revealed the presence of chlorine

TABLE XV

Repeated Oxidation of One Gram of Refined Fibers by Sodium Periodate (a)

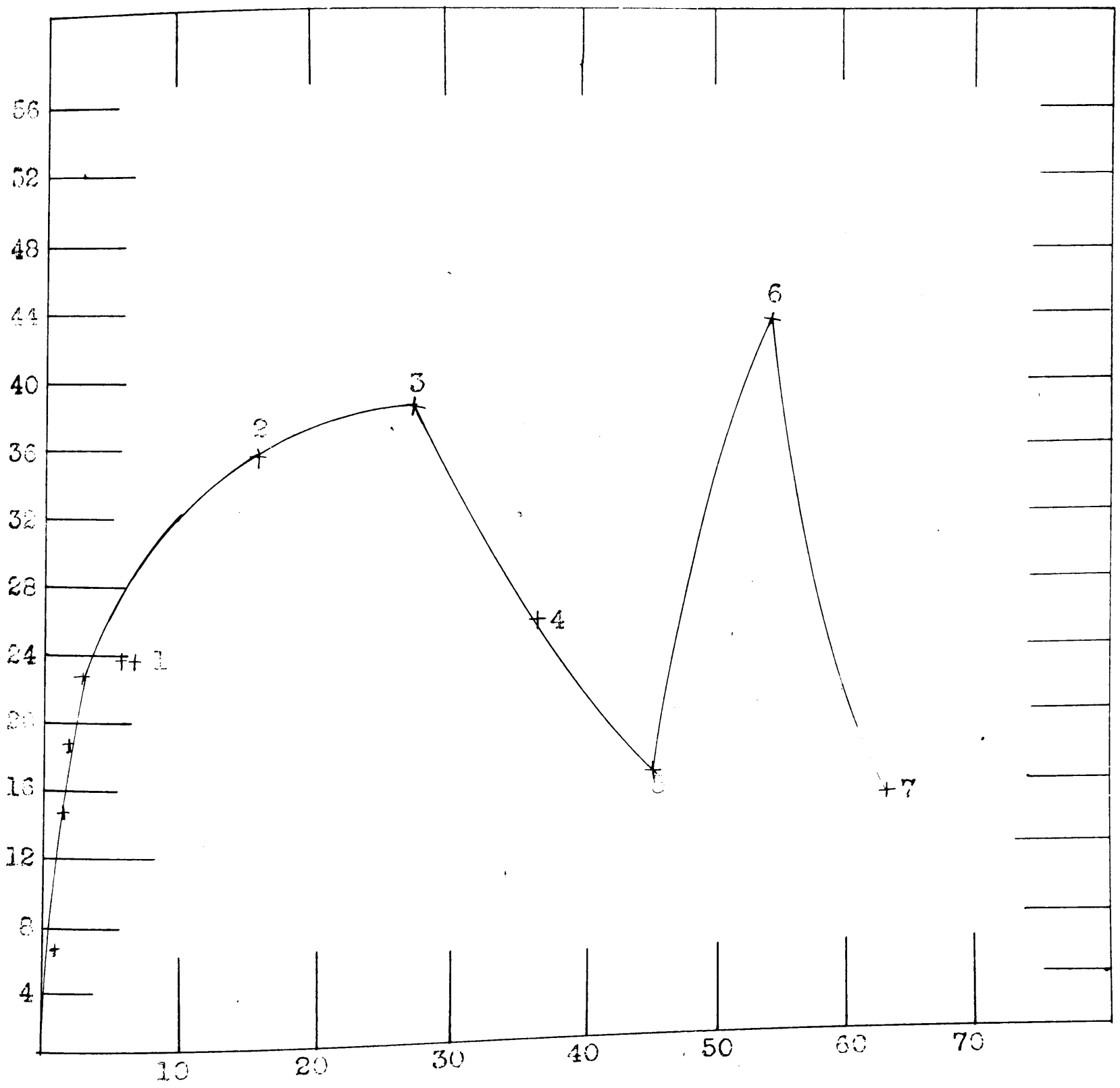
Time (hrs.)	Iodine Titer in ml. 0.1011 N	$Na_3H_2IO_6$ Consumed by Sample (gm.)	$Na_3H_2IO_6$ Consumed by Sample (mole)	$Na_3H_2IO_6$ Consumed per Gram Sample (gm.)	$Na_3H_2IO_6$ Consumed per Gram Sample (mole)
First Oxidation					
0.	1.00	0.	0.	0.	0. (b)
0.5	1.30	0.1693	0.0005759	0.1693	0.0005759
1.0	1.80				
1.5	1.80	0.4279	0.001455	0.4279	0.001455
2.0	2.00	0.5199	0.001768	0.5199	0.001768
2.5	2.00				
3.0	2.30	0.6372	0.002168	0.6372	0.002168
3.5	2.30				
4.0	2.30				
4.5	2.30				
5.0	2.30				
6.0	2.60	0.6655	0.002263	0.6655	0.002263
7.0	2.70	0.6817	0.002319	0.6817	0.002319
8.0	2.70				
9.0	2.70				
Second Oxidation					
9.0	3.70	1.091	0.003465	1.091	0.003465
Third Oxidation					
9.5	4.80	1.129	0.003823	1.129	0.003823
Fourth Oxidation					
9.0	4.30	0.7353	0.002501	0.7353	0.002501
Fifth Oxidation					
9.0	3.15	0.4790	0.001629	0.4790	0.001629
Sixth Oxidation					
9.0	5.60	1.247	0.004245	1.247	0.004245
Seventh Oxidation					
9.0	2.80	0.4105	0.001396	0.4105	0.001396

(a) Five per cent solution at 20° and pH 4.1
(b) Blank titration.



GRAPH 1

Course of first periodate oxidation on typha seed hairs.



GRAPH 2

Course of a series of seven periodate oxidations on typha seed hairs.

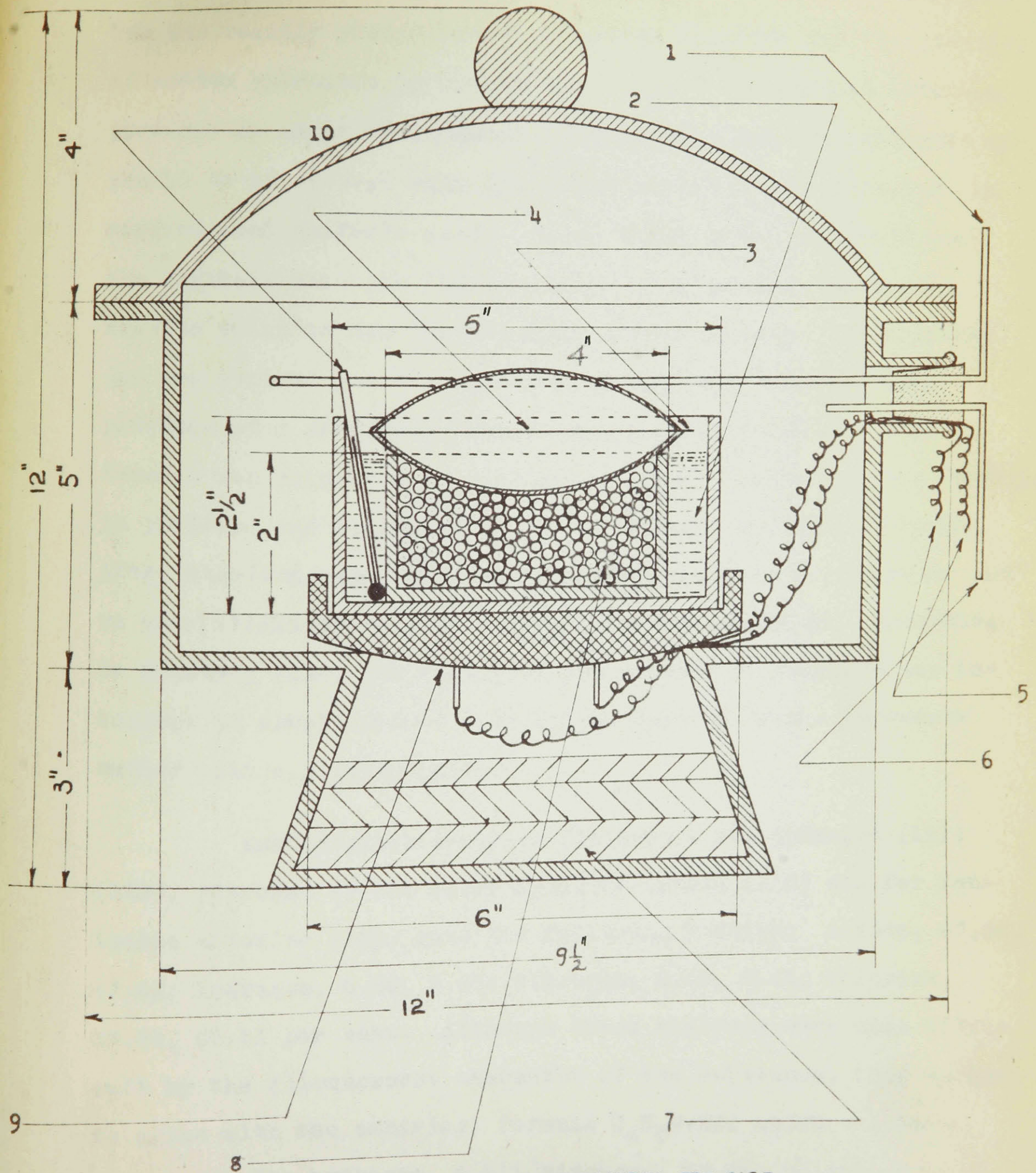


FIG.7. SPECIAL APPARATUS USED FOR SUBLIMING THE SALT

(1) Nitrogen inlet
(2) Oil bath
(3) Watch glass assembly
(4) Perforated filter paper
(5) Electrical leads to rheostat, to ammeter, to mains
(6) Exhaust to manometer, to suction pump
(7) Calcium chloride
(8) Glass beads
(9) Heating mantle
(10) Thermometer

and nitrogen but sulfuric and phosphorus were absent. The chlorine was readily precipitated as silver chloride and the addition of sodium hydroxide or sodium carbonate to an aqueous solution produced an odour reminiscent of ammonia. Hydrogen chloride appeared to be evolved when the solid crystals were dissolved in concentrated sulfuric acid. Since these tests suggested that the crystals were the hydrochloride of a volatile amine, an aqueous solution was treated with sodium nitrite. No nitrogen gas was evolved but the solution turned red, indicating the presence of a secondary rather than a primary amine. This inference was supported by dissolving a small amount of the crystals in pyridine and adding an excess of benzene sulfonyl chloride. After standing overnight, the solution was poured into water and an uncrystallized benzene sulfonamide was recovered. According to Hinsberg (120) the fact that the benzene sulfonamide was insoluble in alkali showed that it was derived from a secondary rather than a primary amine.

Quantitative analyses for carbon and hydrogen (121) (122), nitrogen by the micro-Kjeldahl method (123) and for ionizable chlorine (124) gave the following results: carbon, 47.29, 47.41; hydrogen, 5.66, 5.93; nitrogen, 8.45, 8.46; chlorine, 33.24, 33.51 per cent. Although these analyses were made difficult by the dilequescent character of the substance, they seemed to agree with the empirical formula $C_4H_5N \cdot HCl$ which requires carbon, 46.82; hydrogen, 4.91; nitrogen, 13.66; chlorine 34.56 per cent.

Dakin and Didley (125) report that Kjeldahl estimation on pyridine accounts for only about 70 per cent of nitrogen and hence the nitrogen percentage ascertained 8.45 may not be all the nitrogen present in the compound.

Lack of material prevented a more detailed investigation.

SUMMARY

A method suggested by Mr. R.W. Huddle, of Burgess-Manning Co., Libertyville, Ill., U.S.A., to separate the seeds from the seed hairs of Typha latifolia Linn. was successfully developed on a laboratory scale. Analyses of the crude and refined fibers for alcohol-benzene extractives, water and alkali solubles, ash, lignin, pentosan and nitrogen demonstrated a great difference in their composition. The low ash alkalinity observed for the latter suggested the absence of pectic materials.

The refined fibers were not entirely dissolved by the 72 per cent sulfuric acid used in the Klason lignin determination, and the apparent lignin content of 11.6 per cent accordingly required confirmation. Lignin prepared by the periodate method amounted to 12.8 per cent but only 46.1 per cent was recoverable as Klason lignin. The methoxy contents of the Klason and periodate lignins were 6.8 and 8.2 per cent respectively.

Chlorination and extraction with alcohol-pyridine carried out to prepare typha holocellulose by Ritter and Kurth's method failed to remove Klason lignin but eliminated 17 per cent of other substances. The alcohol-pyridine mother liquor yielded 14.6 per cent of white water-soluble crystals whose properties were provisionally thought to be those of a secondary amine hydrochloride of empirical formula $C_4H_5N \cdot HCL$. Although the large yield suggested these crystals were derived from the alcohol-

pyridine they could not be recovered in a blank experiment.

Chlorination and extraction with alcohol-monoethanolamine as in van Beckum and Ritter's holocellulose preparation, removed almost all the lignin and also about 25 per cent of other substances. The Wise, Murphy and D'Addieco treatment with sodium chlorite, however, resulted in a 90 per cent yield of nearly lignin-free typha holocellulose as creamy white fibers.

This chlorite holocellulose had alpha-, beta- and gamma-cellulose contents of 43.6, 1.86, and 4.65 per cent, respectively and about half of the material was not recovered. A study of the hemicelluloses obtained from the holocellulose by extraction with 5 per cent alkali showed that about 7 per cent was recoverable as a syrup, insoluble in water after isolation but soluble in ethanol and acetone. Although this syrup could not be thoroughly examined, it did not appear to be of a carbohydrate nature.

CLAIMS TO ORIGINAL RESEARCH

1. The typha latifolia Linn. seed hairs were successfully refined on a laboratory scale and published chemical analyses were repeated and greatly extended.

2. Direct lignin estimations were carried out on the refined seed hairs for the first time. The Klason and the Ross and Potter methods both employing 72 per cent sulfuric acid were obviously unreliable but the removal of all carbohydrate by oxidation with sodium periodate left a "periodate lignin" amounting to 12.8 per cent of the seed hairs and with a methoxyl content of 8.2 per cent. Only 46.1 per cent of this lignin was recoverable as Klason lignin. This low recovery, together with the low methoxyl contents of both lignins, showed that they were different from the lignins in woods.

3. Contrary to experience with woods, chlorinations followed by extractions with alcohol-pyridine failed to remove much lignin, whereas chlorinations followed by extractions with alcohol-monoethanolamine removed much carbohydrate as well as almost all of the lignin. Bleaching with aqueous sodium chlorite was the only satisfactory way of preparing nearly lignin-free holocellulose from the typha seed hairs. The yield was 91.8 per cent and the holocellulose, creamy white and in its native morphology.

4. The chloride holocellulose yielded 43.6 per cent alpha-, 0.2 per cent beta- and 8.1 per cent gamma-cellulose, and the balance, 48 per cent, was not accounted for. Extraction of the holocellulose with 5 per cent alkali left 44.4 per cent of cellulose undissolved and removed 55.6 per cent of hemicelluloses. Fractionation of the latter revealed the presence of 25 per cent of very soluble substances, not all of which appeared to be of a carbohydrate nature.

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