THE NATURAL OXYCELLULOSES.

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IN a previous short communication[1] we dealt with a controversial point which had arisen in regard to the constitution of the furfural-yielding constituents of plant-tissues. What there has been at issue between de Chalmot and ourselves in this matter, is at least clearly stated, and we have been engaged in accumulating experimental material as a further contribution to the solution of an important question in the chemistry of tissue-formation, which cannot usefully be further discussed *a priori*.

In this investigation we have been ably assisted by our friend Mr. Claude Smith, who has carried out the whole of the laboratory work, and Dr. Voelcker, the well known chemist of the Royal Agricultural Society, who has kindly cooperated with us in placing his laboratory at our disposal and supplying material for the investigation.

In the first instance we have traced the history of selected crops of barley, in relation to the elaboration of "furfuroids," to use a convenient short description of these characteristic furfural-yielding constituents.

The crops selected were grown upon two of the experimental plots of the Royal Agricultural Society's station at Woburn, (England):

Plot 1 representing a soil permanently unmanured and growing barley continuously.

Plot 6[2] on the other hand is manured with the maximum of fertilizers and gives continuously the maximum yield of straw and grain.

These plots represent therefore the extreme conditions of growth, unfavorable and favorable.

The plants were harvested at intervals of one month and investigated according to the subjoined scheme:

(1) The "total furfural," obtained by "distilling" the entire plants with hydrochloric acid, was determined.

(2) The plants were treated for the elimination of pentosans

1 This Journal, 17, 286.

2 Manured with potassium, sodium and magnesium sulphates, calcium superphosphate and sodium nitrate.

and generally, of constituents not belonging to "permanent tissue," as follows:

(*a*) Exhaustive treatment with boiling alcohol; (*b*) Digestion in alkaline solution of one and one-half per cent. sodium hydroxide for some hours, followed by washing, first cold, lastly at the boiling temperature; (*c*) Digestion in dilute one and one-half per cent. hydrochloric acid for some hours, followed as before, by cold and hot washings. The product of these treatments may be taken as fairly representing the cellular tissue of the plant *less* the cell-contents, and may be described as permanent tissue. This description we admit is somewhat arbitrary, but it will be conceded that the residue from the treatments above described is free from pentosans and all the more readily hydrolyzable constituents of the growing plant.

It is unnecessary to reproduce minutely the experimental details of the investigation. It is sufficient to state that the results were in all cases controlled by duplicate experiments.

Date.	Age of crop.	Plot.	Total dry weight in per cent.	Furfural per cent. of dry weight. (*a*)	Permanent tissue per cent. of total.	Furfural from permanent tissue. Per cent. tissue. (*b*)	original. (*c*)	ratio. *a* : *c*.
May 7	6 weeks	1	19.4	7.0	53.4	12.7	6.8	1.03 : 1
		6	14.7	7.0	55.9	12.3	5.8	1.20 : 1
June 4	10 weeks	1	17.6	7.7	52.9	11.6	6.1	1.26 : 1
		6	13.5	8.1	58.5	13.4	7.8	1.04 : 1
July 10	15 weeks	1	42.0	9.0	65.7	9.8	6.4	1.40 : 1
		6	32.9	10.6	65.7	12.5	8.2	1.30 : 1
August 21	21 weeks	1	64.0	11.9	70.0	14.5	10.1	1.17 : 1
		6	64.6	13.4	70.5	15.0	10.6	1.26 : 1
August 31[1]	22 weeks 3 days	1	84.0	12.6	75.0	16.5	12.4	1.02 : 1
		6	86.4	12.4	78.4	15.1	11.8	1.05 : 1

With regard to the distribution of the furfuroids in the plant the following determinations were made in specimens taken from plot 6, on July 18, the crop being sixteen weeks old:

	Whole plant (moist). Per cent.	Whole plant (dry). Per cent.
Stems	50.0	41.7
Leaves	15.3	19.6
Ears	34.6	38.6

[1] The samples taken on August 31st were *after* the crops were harvested.

	Dry matter. Per cent.	Ash, dry matter. Per cent.	Permanent tissue. Per cent.	Furfural gross.	Furfural in permanent tissue.
Stems	32.6	4.0	76.6	9.5	12.2
Leaves	50.1	7.0	49.7	12.1	16.0
Ears	44.2	7.0	79.0	7.5	9.6

It appears from these results that the leaves contain a large proportion of the easily hydrolyzable furfuroids, *e. g.*, pentosans; those of the stems and ears, on the other hand, are for the most part in the more resistant form of tissue-furfuroids. The higher proportion of furfuroids in the leaves may be taken as correlated with the special assimulating functions and more active oxidizing conditions obtaining in these organs, and as indicating that furfuroids of lower molecular weight may be assimulated or elaborated to permanent tissue. The probability of this must in fact be admitted. For although de Chalmot's experiments[1] contradict the hypothesis that the pentoses are so elaborated, the problem as regards oxidized hexose derivatives can hardly be considered as having been seriously attacked.

The investigations of these barley plots are being continued during the current year, with special attention to the more positive indications of the above results. The following are the results of the examination of specimens taken on May 15, the crop being seven weeks old; the figures represent percentages:

	Plot 1.	Plot 6.
Total dry matter	20.6	17.8
Ash of dry matter	13.7	18.0
Nitrogen of dry matter	3.65	3.87
Alcoholic extract	19.0	23.8
Nitrogen of alcoholic extract	2.5	2.8
Permanent tissue	53.9	56.7
Ash of permanent tissue	3.8	4.0
Nitrogen of permanent tissue	4.2	4.5

The furfural numbers calculated on the dry ash-free products are as follows:

		Furfural in permanent tissue.		
	Furfural gross. Per cent. (*a*)	Per cent. of tissue. (*b*)	Per cent. of original. (*c*)	Ratio. *a* : *c*
Plot 1	7.6	10.6	5.50	1.40 : 1
Plot 6	7.1	10.0	5.44	1.12 : 1

[1] This Journal, 16, 618.

These numbers confirm those of the 1894 crops in their general bearings. The season, however, has been so far very different, the week preceding the 15th being cloudless with high summer temperature, as against a cold wet week in the corresponding period of 1894. The higher ratio *a* : *c* accords with the higher rate of assimilation obtaining under such conditions.

The more extensive scale of the investigation has reference to a more strictly physiological scheme of observation to be made at the critical period of growth, *viz.*, flowering, fruiting, and ripening of the grain. These numbers lead to the following conclusions:

1. The "permanent tissue" (cellulose) of the cereals contains *ab initio*, a large proportion of oxidized groups, *i. e.*, oxycelluloses.

2. The furfuroids of the cereals (Haulm) are localized mainly in the cell-substance, in the earlier and later stages of growth, in fact, almost exclusively. Towards the period of most active growth the proportion of tissue furfuroids falls from ninety to seventy-five per cent. of the total.

3. Increasing again during the period of maturation indicates that the furfuroids of what may be termed the lower grade are assimilated to the more resistant, or cellulose, form.

4. Extreme variations of the soil-conditions, *i. e.*, supply of inorganic nutrient material—is without effect upon the permanent tissue in the earlier and later stages of growth, but determines some variations of the proportion of tissue furfuroids in the intermediate stages.

5. The results show that the composition of the permanent tissue is a constant of the plant, and largely independent of the particular conditions of cultivation. This latter result is confirmed by the result of a similar investigation of barley straws from the Rothamsted Experimental Station. Specimens of the fully matured straws grown upon plots selected as showing extreme variations of the conditions of cultivation, gave the following results:

	Plot.	Total furfural per cent.	Permanent tissue per cent.	Furfural in permanent tissue per cent. of tissue.	of straw.
Minimum fertilization.	1.*O*	14.5	72.4	15.8	11.4
	3.*O*	15.5	72.4	14.7	10.6
	6_1.	15.8	72.6	15.8	11.5
Maximum fertilization	4 *A.A.S.*	12.4	76.4	16.2	12.4
	4 *A.A.*	15.1	80.2	16.0	12.8
	7_2.	15.0	82.3	14.1	11.6

The following are the conditions of fertilization:

Plots 1.*O*. and 6_1., were unmanured continuously.

Plot 3.*O*., manured with sodium, potassium, and magnesium sulphates.

Plot 4 *A.A.S.*, manured with sodium nitrate, sodium silicate, calcium superphosphate and sodium, potassium, and magnesium sulphates.

Plot 7_2., fourteen tons farm-yard manure.

Plot 4 *A.A.*, potassium, sodium, and magnesium sulphate, calcium superphospate, and sodium nitrate.

These results are from an agricultural point of view of negative value. It was *a priori* possible that the composition of the permanent tissue would have varied with the prevailing conditions of assimilation. Such variations are not discoverable in the furfural-constants of the products, which express their most characteristic constitutional features. Physiologically, on the other hand, the results are of more positive bearing on the processes of assimilation, showing that these are in an important sense invariable.

It might be assumed in a superficial view that this conclusion was obvious *a priori*, and the experimental verification therefore gratuitous. On reflection, however, it will be conceded: (1) that we have not long been in possession of quantitative methods of diagnosing the constitutional features of the components of plant tissues—celluloses, oxycelluloses, lignocelluloses, etc.,—and (2) that, whatever the probability, the point is not specifically dealt with in works in plant-physiology,[1]

It is a point which we have dealt with before in regard to the typical lignocellulose, the jute fiber. In an investigation of the

[1] The current views on the subject of lignification and thickening of cell walls, imply that the permanent tissue in its earliest phases invariably consists of a pure (normal) cellulose. The chemist has to remember that the morphologist attaches no specialized significance to the term cellulose.

growth of the plant under the artificial conditions of "hot-house cultivation," Mr. A. Pears obtained the bast fiber, showing considerable divergence on ultimate analysis (C : H : O) from the normal Indian product, but with identical constitutional features. The low carbon percentage of the product was shown to be due to "dilution" by water of hydration; dehydration had not proceeded as far as obtained under the normal conditions of growth; but in all essential respects the quantitative chemical features of lignification were unaffected.[1] The point is therefore established in regard to two main types of tissue-formation.

Reverting now to the history of tissue-formation in barley plant.

In reference to the germination process, we commenced observations[2] upon the earliest stages of tissue-formation in regard to the formation and fixation of furfuroids, but, recognizing the prior claims of de Chalmot in this field of investigation, we are satisfied to leave the subject in such good hands, merely noting that we have joined issue with him in regard to the interpretation of the furfural-constants.

De Chalmot has especially investigated the influence of the two factors which might be expected to affect the formation and elaboration of these furfuroids, *viz.*, (1) light; (2) the supply of nitrogen (nitrate). Light was found to be without effect in these earlier stages of growth; and with a liberal supply of nitrates the amount of furfuroids in the young plants was found not to decrease. These results again point to the invariable habit of the cell in regard to the formation of tissue.

We have endeavored to produce a still more drastic variation of the conditions of assimilation, as follows: Young plants of oats seven weeks old were placed in the following solutions: (*a*) water; (*b*) dilute nitric acid of 0.25 per cent.; (*c*) of one-half per cent.; (*d*) of one per cent. The plants withered only gradually. After about ten days they were removed, dried and investigated. The results are given below:

Twenty plants before experiment weighed 25.8 grams and contained 16.7 per cent. dry matter.

Twenty plants in water (*a*) weighed 39.0 grams and contained 10.2 per cent. dry matter.

[1] *J. Chem. Coc.*, *1893*, 967; Cross & Bevan: "Cellulose," pages 111-113.

[2] *Ber. d. chem. Ges.*, **27**, 1061.

Twenty plants in dilute acid (*b*) weighed 18.0 grams and contained 21.3 per cent. dry matter.

Twenty plants in dilute acid (*c*) weighed 15.0 grams and contained 24.4 per cent. dry matter.

Twenty plants in dilute acid (*d*) weighed 13.0 grams and contained 31.0 per cent. dry matter.

From the dry matter the following percentages of furfural were obtained:

(*a*)	(*b*)	(*c*)	(*d*)
8.2	9.2	8.2	8.5

The uniformity of these numbers is curiously at variance with the extreme variations of the conditions of retrograde development, which are apparent from the statistics of the relative weights of these plants.

In a second series of experiments very different results were obtained. In this case the plants were taken at a more advanced stage of growth—thirteen weeks old. They were placed in water and dilute solutions of nitric acid, respectively, as before, remaining for ten days, when they showed signs of withering. They were removed, dried and analyzed for "total furfural," with the following results:

	Cultivations in water per cent.	Dilute nitric acid. per cent.		
		0.25	0.50	1.00
Furfural	4.6	4.9	5.9	7.8

These variations determined by artificial treatment may appear out of harmony with the invariability of the results obtained in the first series. It will be noted, however, that the oats show a very different initial proportion of furfuroids, and appear to contain carbohydrates susceptible of attack by nitric acid.

The more advanced period of growth (thirteen weeks) at which the plants were taken, has been previously shown to be one of maximum normal variation of the furfuroids, which is consistent with maximum variations under retrograde development, and we may in future experiments follow the indications of these observations, that the most active period of growth offers the most favorable conditions for the study of variations artificially determined.

The purpose of the present investigations being to accumulate experimental material, the observations were extended to products of widely different origin, life-history, or formation. Subjoined are the numbers obtained with (*a*) mangels, and (*b*) gooseberries:

(*a*) Long red mangels were taken at intervals from a selected field, and the furfural and other constants determined, as follows:

Date.	Average wt. per root	Dry matter per cent.	Ash of dry matter per cent.	Permanent tissue per cent.	Furfural whole root (dry) per cent.	Furfural Permanent tissue (dry) per cent.
June 30	1.5	12.7	11.0	25.3	4.1	12.6
August 6	300.0	11.5	12.0	28.8	4.3	11.4
October 11	1456.0	13.1	7.8	14.9	3.7	11.4

The furfural numbers, it will be remarked, are uniform over the whole period of growth. The proportion of furfuroids moreover is small, and further investigation of this group of products from our present point of view is therefore abandoned.

(*b*) The berries were examined at an interval of one month, with the following results:

Date.	Dry matter per cent.	Permanent tissue per cent.	Furfural whole fruit per cent.	Furfural permanent tissue per cent.
May 16	9.2	21.5	4.8	7.8
June 17	9.1	23.5	3.9	7.2

These numbers again are devoid of any characteristics such as invite further investigation. Other typical products were also examined with similar results. So far, therefore, the selection of the cereals as the typical case of the elaboration of cellulose-tissue with a maximum of furfuroid constituents is justified.

As we have before indicated it is not the purpose of the present communication to attempt any final conclusions as to the bearings of these results upon the general questions of assimilation and metabolism. We may perhaps again insist on the one prominent result of these investigations, which is to establish the uniform characteristics and uniform distribution of the furfuroids of the cereal straw throughout its substance:

(1) The entire straw in the matured state is characterized by yielding twelve to fifteen per cent. furfural; and (2) the "cellulose" isolated from the straw by the severe handling of the paper-maker; *viz.*, digestion with caustic lye (two to three per

cent. sodium hydroxide) at elevated temperatures (forty to sixty pounds steam pressure), is identically characterized, yielding twelve to fifteen per cent. furfural on boiling with condensing acids.

This uniformity of distribution and uniformity of resistance to alkaline hydrolytic treatment of the widest range, establishes the molecular homogeneity of the tissue-substance in regard to the relative proportion of furfuroids to normal hexose groups. The results of our investigations of the history of the formation of the tissue are strictly correlative, and the elaboration of products of such characteristics must be regarded as an essential and primary property of the unit cell, in the same sense that alcoholic fermentation is a property of the yeast cell. To put it perhaps more directly: the constitution of assimilated material in the plant is immediately determined by the molecular configuration of the assimilating material. This view has been expressly formulated by E. Fischer[1] in reference to the origin of the carbohydrates in the plant. The more evidence we have that the assimilating process remains invariable in its products, notwithstanding wide variations in the external conditions, the more necessary does it become to regard the essential *directive* factors of the process as material, *i. e.*, as residing in the material configuration of the cell rather than determined by external forces. The results of our investigations contribute to the establishment of this view.

PART II.

I. THE CONSTITUTION OF THE CEREAL CELLULOSES.

We have made some progress in the isolation of the "furfuroid" coutituents of celluloses. The more important differences which they exhibit from the normal celluloses we have dealt with in previous papers.[2] Although they are not resolved by alkaline hydrolysis we have found certain reagents which determine a satisfactorily sharp separation of their furfural-yielding component groups. Thus:

(*a*) Pure bleached straw-cellulose was treated with sulphuric acid at 1.62 specific gravity; the cellulose dissolves to a nearly

[1] *Ber. d. chem. Ges.*, 27, 3231.

[2] *Ber. d. chem. Ges.*, 27, 1061; C. Smith, *J. Chem. Soc.*, 65, 473.

colorless solution. After standing some time (three hours from the start) the solution was poured into water. The white gelatinous precipitate of cellulose (hydrate) was filtered off and exhaustively washed, dried, and weighed. It amounted to sixty-four per cent. of the original. The reprecipitated cellulose and the filtrate were both distilled for elimination of furfural; the proportions obtained, calculated on the original cellulose, were:

	Furfural. Per cent.
Reprecipitated cellulose	0.6
Products soluble on dilution	14.0

The "furfuroids" therefore are hydrolyzed to soluble derivatives by the process.

(*b*) A second specimen was dissolved by heating with a concentrated solution of zinc chloride, the viscous solution poured into water, and the precipitate filtered off. Similar determinations were made as in (*a*), with the following results:

	Furfural (calculated on original cellulose). Per cent.
Reprecipitated cellulose	0.9
Product soluble on dilution	10.4

It is to be noted that furfural is freely formed in the process of heating with the concentrated solution of zinc chloride, and some quantity is volatilized. The reprecipitated cellulose (hydrate) has the characteristic of the normal celluloses. On combustion of these products the subjoined numbers were obtained:

Carbon	43.00	43.07
Hydrogen	7.14	6.99

corresponding with the formula $3C_6H_{10}O_5.H_2O$.

The products in solution in the acid liquid would appear to be capable of isolation by simple means, *viz.*, by neutralizing with barium carbonate, filtering, and evaporating.

Being derivatives of unknown constitution it was deemed advisable to prepare them in large quantity, and we proceeded to carry out the above process of hydrolysis on the scale of 100 grams per operation, as preliminary to working on the still larger scale of kilograms.

Proceeding as described we have obtained, on evaporating the filtrate from the barium sulphate, a light-colored gum resembling dextrin.

The first preparation gave, on distillation with hydrochloric acid, only eight and two-tenth per cent. of its weight of furfural; whereas, had we succeeded in obtaining the furfuroids only, as in the experiments on the smaller scale, the products should have yielded forty to fifty per cent.[1]

On reverting to the small scale we obtained this result :

Two grams of the cellulose being dissolved and the solution diluted and filtered from the reprecipitated cellulose ; the filtrate was boiled with barium carbonate, filtered, and made up to 200 cc. Of this solution, eighty cc. on evaporation gave 0.144 gram organic solids ; a second, eighty cc., distilled for furfural, gave an amount corresponding to 46.6 per cent. of the weight of the organic solids.

On further investigation of the products obtained on the large scale we found a large proportion retained as an insoluble barium compound. On boiling the washed precipitate with ammonia it yielded an extract, which on drying and "distilling" with hydrochloric acid, gave 12.5 per cent. furfural. It appears, therefore, that the products are resolved into three groups :

1. Normal cellulose (hydrate) reprecipitated, on dilution.
2. Soluble in water, but combining with barium carbonate to form insoluble compounds.
3. Soluble in water, but neutral, in properties, and therefore forming no barium compounds.

It appears also that the discrepancies between the results on the small and on the large scale are due to the conditions affecting group 2. These conditions are being carefully studied with the view of controlling the operation on the large scale. We have further characterized the soluble products of the hydrolysis as obtained on the small scale under most favorable conditions, as follows :

(*a*) *Copper-oxide reduction*.—As first obtained in solution the reduction equivalent, referred to that of dextrose as 100, has been

[1] Unless we assume that the configuration of the products is changed by the process of solution in sulphuric acid, which is not an improbable hypothesis. See *J. Chem. Soc.*, 65, 477.

determined at 30.6. After further hydrolyzing by boiling in the solution diluted to contain two per cent. sulphuric acid, it reached a maximum at 68.3.

(*b*) *Osazone.*—After hydrolysis to this maximum the products were treated with phenylhydrazine under the usual conditions of formation of osazones. A characteristic product was obtained crystallizing well from solution in benzene. It was found to melt at 154°. On analysis it gave a quantity of ammonia corresponding to eight and four-tenths per cent. nitrogen. The parent substance is still, therefore, a product of relatively high molecular weight, approximately of the dimensions C_{16} to C_{18}, or, more strictly, containing one reactive CO group in the unit of these dimensions.

(*c*) *Decomposition by oxidants.*—On treatment with potassium permanganate in neutral solution it gives large yields of acetic acid. The products of oxidation by Fehling's solution also contain a large proportion of this acid.

(*d*) *Acetylation.*—The two products of hydrolysis not yet having been isolated on the large scale, the reaction with acetic anhydride has been studied with the parent substance. These celluloses contain reactive hydroxyl groups, forming an acetate on digestion with the anhydride at its boiling-point. The yield obtained in one experiment was 124 per cent. The product being insoluble in and unaffected by the usual solvents of the cellulose acetates, it was possible that a more complicated reaction had resulted, *e. g.*, condensation of the furfuroids to furfural and union with the anhydride to furfuracrylic acid. On distillation with hydrochloric acid the product yielded nine and a half per cent. furfural, which amounts to twelve per cent. of the original cellulose. The reaction will be further investigated with the isolated furfuroids.

(*e*) *Qualitative Reactions.*—The cereal-celluloses, as we have frequently stated, do not give the reactions characteristic of the pentosans, nor do the soluble products of acid hydrolysis obtained as described.

The celluloses on the other hand give a rose-red coloration on boiling with solutions of aniline salts, and the reaction is also obtained with the products of hydrolysis. From the behavior of the products with barium carbonate it appears we are dealing with two groups: a neutral group presumably aldoses, and a more acid group giving insoluble barium compounds, both characterized by the furfural reactions. The lines are obviously indicated upon which the separation and isolation of these two groups must proceed.

II. THE CONSTITUTION OF THE CEREAL STRAWS.

The straws are obviously complex structures and therefore not chemically homogeneous. They contain a considerable proportion of lignocellulose, the lignification being most marked in the groups of thickened polygonal cells situated in the hypodermal region, and evidently contributing chiefly to the rigidity of the stem.

The following constants[1] have been determined, certain of which may be taken as measuring the proportion of lignocellulose in the complex:

	Wheat straw.	Barley straw.
Cellulose, (chlorination method)	45.2	45.3
Chlorination, chlorine combining	2.5	2.5
" chlorine as hydrochloric acid	2.9	2.4
Methoxyl, $(O.CH_3)$	3.1	2.1
Furfural	14.15	14.15
Volatile acid, chiefly acetic, distilled with thirty-three per cent. sulphuric acid	6.4	6.3

The chlorination numbers correspond with a proportion of lignocelluloses amounting to thirty-three per cent. Of the cellulose isolated by the method of chlorination approximately one-half is obtained from this group, one-half being derived from the residual complex. The lignocelluloses giving seven to eight per cent. furfural, yield say two and six-tenths of the total fourteen and five-tenths of the straw.

The "residual" complex is therefore richer in furfuroids and has a correspondingly lower proportion of the resistant celluloses. It may be further resolved as follows:

		Yielding furfural of cellulose. per cent.	Yielding furfural of straw. per cent.
Resistant "cellulose"	25.0	12.0	3.0
Pentosans / Hemicelluloses	42.0		9.0

1 *Vide* the author's book: "Cellulose," pp. 111-113.

Assuming that of the latter group of easily hydrolyzable constituents the furfural-yielding bodies are exclusively pentosans, this would amount to one-half, and the entire straw (structural elements) might be expressed in terms of its proximate constituents, as follows :

Disposition in stem.			Containing cellulose.	Yielding furfural.
Hypodermal fibers and fibers of fibrovascular bundles.	Lignocelluloses ...	33.0	25.0	2.6
Vessels of fibrovascular bundles parenchyma and corbex.	Resistant cellulose.	25.0	2.50	2.6
	Hemicelluloses ...	21.0	...	..
	Pentosans	21.0	...	9.0
		100.00		14.6

It will be an object of our future investigations further to differentiate this complex.

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NOTES UPON THE DETERMINATION OF NITRITES IN POTABLE WATER.

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IN comparing the results obtained by Trommsdorff's iodo-zinc starch method and Griess' α-napthylamine test upon a large variety of waters, discrepancies were noticed which were very marked in the case of the peaty waters. These showed no blue by Trommsdorff's method, but in some cases as high as 0.0010 parts nitrogen as N_2O_3 per 100,000 by the Griess test. Upon decolorizing the waters the results agreed, showing that the peaty matter interferes with the formation of the iodide of starch, and unless nitrites are present in considerable quantity (above 0.0020 parts nitrogen as N_2O_3 per 100,000) this test is not capable of detecting them.

The decolorization was •affected in the cold, as heating increases the nitrites, by shaking up about 250 cc. of the water with three cc. of "milk of alumina,"[1] allowing to settle, and filtering through a filter which is washed free from nitrites. Even when using Griess' method it was found advantageous to decolorize the peaty waters, as their brown color modifies the pink tint, giving a slightly higher reading than would otherwise be obtained.

1 Prepared by precipitating a boiling solution of 125 grams potash alum per liter with ammonia, allowing the aluminum hydroxide to settle and washing by decantation.