Water-Soluble Pentosans from Rye: II. Effects on Rate of Dialysis and on the Retention of Nutrients by the Chick

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ABSTRACT

The properties of a water-soluble, pentosan-rich fraction that was isolated from rye flour were tested in vitro using a dialysis system and in vivo with chicks. The in vitro studies demonstrated that both an extract of rye and the pentosan-rich fraction impeded the rate of dialysis of three different salts and glucose, as well as glucose that was being enzymatically hydrolyzed from starch. When either the rye extract or the pentosan solution was digested prior to dialysis with a crude extract of *Trichoderma viride*, the viscosity was completely reduced in both fractions to that of water and the effect on the rate of dialysis of sodium chloride was eliminated. Pancreatin, in contrast, had no effect on these extracts. Nutritional studies with young chicks demonstrated that nearly all of the antinutritive activity of rye, as assessed by fat retention, was associated with the pentosan-rich isolate. The results suggest that the principle antinutritive factor in rye grain is a highly viscous, water-soluble, pentosan-rich carbohydrate.

Viscous carbohydrates, when present in the diet, reduce energy utilization and nitrogen and fat retention in chickens (Kratzer et al 1967) and the rate of glucose uptake in humans (Jenkins et al 1978). The water-soluble, viscous (gel-forming) polysaccharides also affect the in vitro rate of glucose diffusion (Brown 1979) and protein digestion by pepsin (Vaughan et al 1962). Rye contains a water-soluble and highly viscous compound that decreases the retention of all nutrients in the chick, particularly the long-chain saturated fat (Marquardt et al 1979, Antoniou and Marquardt 1982, 1983, Antoniou et al 1980, Ward and Marquardt 1983). Antoniou et al (1981) and Antoniou and Marquardt (1983) provided evidence that implicated the water-soluble pentosans. They were, however, unable to demonstrate that the antinutritive factor was a water-soluble pentosan since most of its viscosity was lost during the isolation procedure. The objectives of this study were to determine what effect the water-soluble and highly viscous pentosans, previously isolated from rye (Fengler and Marquardt 1988), had on the viscosity of an aqueous extract, on the rate of diffusion of certain electrolytes and glucose in vitro, on fat retention in vivo, and on the proportion of the total antinutritive activity of rye associated with the viscous water-soluble pentosans.

MATERIALS AND METHODS

Source of Chemicals and Dietary Ingredients

The method of preparing the dried water-soluble pentosans and their relative purity have been outlined (Fengler and Marquardt 1988). Most chemicals were from Fisher Scientific Ltd., Winnipeg, Manitoba, and Sigma Chemical Co., St. Louis, MO. Pancreatin (Sigma grade VI) and α-amylase (EC 3.2.1.1, Sigma grade VIA) were from porcine pancreas. *Trichoderma viride* cellulase concentrate (*T.v.* cellulase) was a gift from Miles Laboratories Inc. Elkhart, IN. This enzyme is a multi-enzyme complex high in hemicellulose activity, including pentosanase activity (Miles Laboratory 1976). The sources of grains and other chemicals were as given in Fengler and Marquardt (1988).

In Vitro Dialysis of Different Compounds in the Presence of Pentosan Preparations

In vitro dialysis was carried out according to the procedure of Jenkins et al (1984) at 25°C using 45-mm diameter Spectrophor membrane tubing having a molecular weight cutoff of from 12,000 to 14,000 (Fisher Scientific, Winnipeg, Canada). The dialysis bags contained 25 ml of solution, which was dialyzed against 500 ml of the same solution, except for the compounds being tested. The rate of dialysis of the samples was independent of rate of mixing, provided the sample was rapidly mixed, and was linearly dependent on time for a period of at least 1 hr.

In the first trial, 0.55 and 2.19 g of dried pentosans were solubilized in 200 ml of distilled water containing 0.05% sodium azide as described by Fengler and Marquardt (1988). Powdered NaCl, $CaCl_2$, and $(NH_4)_2SO_4$ were added to the above solutions so that the final concentration was 0.4*N*. The resulting solutions were dialyzed against the same crude pentosans solution without the added salts, and the conductivity in the dialysate was measured using a model CDC-114 conductivity meter (Radiometer, Copenhagen) set on the 1.5 milli-mho scale. Pentosans were included in the dialysate to avoid the osmotic effect associated with the viscous carbohydrates. Readings, which remained linear throughout the test, were made at 5-min intervals over a 60-min period. The conductivity of the pentosan solution alone was negligible.

In the second trial, a comparison of the rate of dialysis of NaCl in a pentosan solution or an extract of rye flour when pre-incubated with either pancreatin (Sigma) or *T.v.* cellulase was followed. Water extracts of autoclaved rye and wheat flour were prepared by adding four volumes of distilled water (w/v) to the flour, mixing at 250 rpm in a rotary shaker at 25°C for 90 min, and centrifuging at 13,000 × *g* for 15 min. The procedure for preparing the water-soluble pentosans from crude pentosans was as described for trial 1. Pancreatin (0 and 46 mg/250 ml) was added to the crude pentosans or water extract of rye, and the sample was incubated at 40°C for 6 hr, placed in boiling water for 15 min, and then centrifuged at 13,000 × *g* for 15 min to remove denatured protein. *T.v.* cellulase (0 and 9.2 mg/250 ml) was also added to the pentosan or water-extract of rye, and the mixture was incubated as the above samples were. NaCl was added to each of the different preparations so that the final concentration was 0.4*N*, and the sample was dialyzed using the procedure described for trial 1. Viscosity analysis was also carried out on the undialyzed samples using procedures outlined by Fengler and Marquardt (1988). The enzyme reaction was stopped 5 min after initiation of the reaction by placing the sample in a boiling water bath.

In the third trial, the rate of glucose dialysis in water and a pentosan solution was tested. Dialysis bags contained 25 ml of glucose solution (25 g/25 ml) in water or in the pentosan solution (2.19 g pentosans in 200 ml of distilled water containing 0.05% sodium azide as described above), and were dialyzed against 500 ml of the same solution, except for glucose. Samples of the dialysate (2 ml) were withdrawn for glucose determination at 10-min intervals over a 1-hr period. Glucose was determined using the colorimetric method of Dubois et al (1956) as modified by Frohlich and Dzialoszynski (1973).

In the fourth trial, the rate of dialysis of glucose, following starch

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hydrolysis by α-amylase, was compared for samples in buffer alone or in a pentosan solution with buffer. Crude pentosans (2.19 g) were solubilized in 200 ml of 0.05*M* phosphate buffer (pH 6.9) as described above. Two grams of soluble potato starch (Sigma S-2630) was further solubilized by heating to 60°C for 30 min in 0.05*M* phosphate buffer or in the pentosan solution. α-Amylase, at a final concentration of 10 mg/ml, was added immediately before initiation of dialysis. Glucose was determined in the hydrolysate as described in trial 3.

Chick Management, Diets, and Analysis

Three experiments were carried out with White Leghorn cockerels obtained from a commercial hatchery. One-day-old chicks were placed in a Jamesway poultry battery for five days with constant light. Feed from a commercial source and water were provided ad libitum. Six-day-old chicks were then segregated into weight groups after 24 hr of starvation. Birds from each weight group were evenly distributed into the experimental groups in such a way that all groups had similar weights. In each experiment there were six replicates per treatment group, and each replicate contained three birds for a total of 18 birds per treatment. The composition of the diets is given in Tables I and II. All diets were formulated to meet requirements according to the National Research Council (1977). Puma rye, Carman triticale, and Canada western red spring no. 3 (CWRS) wheat were used in the feeding trials. The pentosans were prepared as a semisoluble, homogeneous suspension by adding 25 volumes of water, followed by shaking at 250 rpm at 60°C for 2 hr. The suspension was added as a fine spray to the diets when they were being mixed. Finely ground pentosans were also added directly to the diet in the form of a 0.5% premix.

Birds for each replicate were placed in an 18 × 24 × 18-cm cage with a 1-cm squared wire mesh bottom. Each cage was equipped with a minifeeder and waterer that were designed to minimize spillage. Room temperature was 30°C and light was provided continuously throughout the experiment. Feed and water were provided ad libitum during the 48-hr nutrient retention trial. Excreta were collected on Teflon-lined paper that was placed under the raised cages. They were air-dried for four days, ground, and stored in sealed polyethylene bags at −20°C until analyzed.

Fat in the diets and excreta was determined by the method of Marchello et al (1971). Chromic oxide was determined following the procedure of Williams et al (1962). Nutrient retention was determined as described by Marquardt et al (1979). Moisture and protein were determined according to AOAC methods (1975). Statistical analysis was based on analysis of variance as described by Snedecor and Cochran (1980). Tukey's test was used for multiple comparisons of means.

TABLE I
Composition of Diets (experiment 1)

Component	Diets (g/kg) 1	2	3	4	5
Ingredients					
Ground wheat	524.0	···	526.3	528.5	533.0
Ground rye	···	560.0	···	···	···
Crude pentosans[a]	···	···	15.2	30.5	61.0
Casein	···	34.0	···	···	···
Cornstarch	70.0	···	52.5	35.0	···
Other ingredients[b]	406.0	406.0	406.0	406.0	406.0
Calculated analyses					
Crude protein, N × 6.25	21.2	23.5	21.2	21.2	21.2
ME[c] (MJ/kg)	12.45	11.98	11.65	11.92	11.38

[a]The ethanol-precipitated, water-soluble pentosans contained 42% xylose plus arabinose (Fengler and Marquardt 1988).
[b]As g/kg of diet: soybean meal, 284; tallow, 80; methionine, 1; calcium carbonate, 10; dicalcium phosphate, 15; chromic oxide, 3; vitamin mix, 10; mineral mix, 5. The vitamin mix per kilogram of diet consisted of vitamin A, 8358 IU; vitamin D_3, 870 ICU; vitamin E, 5.5 IU; riboflavin, 5.5 mg; Ca-pantothenate, 11 mg; niacin, 16.5 mg; choline chloride, 275 mg; menadione, 1.1 mg; santoquin, 250 mg. The mineral mix (mg/kg of diet) was composed of: manganese oxide, 92.5; zinc oxide, 56.2; ferrous sulfate·H_2O, 259; copper sulfate·$5H_2O$, 16.2; sodium selenite, 0.5; iodized salt, 4,080.
[c]ME = Metabolizable energy.

RESULTS AND DISCUSSION

Influence of Pentosans on the Rate of Dialysis of Salt Solutions

Previous studies have suggested that one of the principal effects of viscous carbohydrates is to impair digestion by interfering with the rate of diffusion of digestive enzymes to the substrate and to impede the rate of uptake of nutrient by the gastrointestinal tract (Elsenhans et al 1980). An in vitro system developed by Jenkins et al (1984) has been shown to correlate well with in vivo results. The objectives of the current studies were to determine if the viscous crude pentosans that were isolated from rye (Fengler and Marquardt 1988) also reduced the rate of dialysis of different salt solutions, and to determine if these effects were abolished when the viscosity factor was destroyed by use of a fungal enzyme.

Crude pentosans reduced the rate of dialysis of sodium chloride, calcium chloride, and ammonium sulfate to a similar degree with the effects appearing to be dose related (Table III). The similarity of results between salts, containing mono- and divalent cations and salts containing mono- and divalent anions would indicate that the effect was nonspecific and, as discussed subsequently, is attributable to the viscosity of the sample.

In the second trial, the effects of predigestion of the crude pentosans or a rye extract on the rates of dialysis of NaCl were followed. The results (Table IV) demonstrate that, although the pattern of response for both the crude pentosans and the rye extract was the same, the pentosan fraction was somewhat more potent than the extract. The reason for the difference was not

TABLE II
Composition of Diets (experiments 2 and 3)

Component	Experiment 2 (g/kg) 1	2	3	4	5	Experiment 3 (g/kg) 1	2	3	4
Ingredients									
Ground wheat	575.0	···	···	575.0	575.0	563.5	563.5	563.5	···
Ground triticale	···	575.0	···	···	···	···	···	···	···
Ground rye	···	···	560.0	···	···	···	···	···	560.0
Cornstarch	19.0	19.0	···	···	···	30.5	···	···	···
Crude pentosans[a]	···	···	···	19.0	19.0	···	30.5	30.5	···
Casein	···	···	34.0	···	···	···	···	···	34.0
Other ingredients[b]	406.0	406.0	406.0	406.0	406.0	406.0	406.0	406.0	406.0
Calculated analyses									
Crude protein (N × 6.25)	21.9	22.8	23.5	21.9	21.9	21.7	21.7	21.7	23.5
ME[c] (MJ/kg)	12.28	13.15	11.98	11.95	11.95	12.33	11.80	11.80	11.98

[a]Then ethanol-precipitated, water-soluble pentosans contained 42% xylose plus arabinose (see Fengler and Marquardt 1988). Pentosans were added in dry form to diet 2 and as an aqueous suspension to diet 3 in experiment 3.
[b]See Table I.
[c]ME = Metabolizable energy.

established. In both cases pancreatin, an enzyme complex capable of hydrolyzing the (1→4) α-linkages in carbohydrates, had no effect on the rate of dialysis of NaCl, whereas the addition of a crude fungal enzyme (*T.v.* cellulase), which completely and rapidly reduced the viscosity of both preparations (Table IV footnotes), also greatly increased the rate of dialysis of NaCl. The reduction in viscosity by pancreatin would not be expected, as it is not able to hydrolyze pentosans (Fengler and Marquardt 1988). As indicated in the footnote to Table IV, the rate of dialysis of the *T.v.* cellulase-treated samples was similar to that of water alone or that of the wheat extract. Also predigestion of wheat extract with *T.v.* cellulase or α-amylase had no effect on the rate of dialysis of NaCl. These results suggest that the viscous pentosan-rich carbohydrates in rye greatly reduce the rate of dialysis of salt solutions, and that a mammalian enzyme, pancreatin, has no effect on the rate of NaCl dialysis, whereas *T.v.* cellulase, which is high in pentosanase activity, is capable of reducing the viscosity of the pentosans and its effect on the rate of dialysis of different salts. The relatively small effect of the water-soluble pentosans from wheat on the rate of dialyses of NaCl is consistent with the previous observation (Fengler and Marquardt 1988) that its viscosity and water-soluble pentosan contents are also considerably lower than those of rye and with the observation that it has low growth-inhibiting activity compared to rye (Misir and Marquardt 1978).

A similar pattern of response as observed in trial 1 was also obtained with glucose or gelatinized starch that was subjected to enzymatic digestion with the mammalian enzyme, α-amylase, in the presence and absence of crude pentosans (Table V). These results suggest that both the rate of enzymatic digestion and the rate of dialysis were affected by the pentosans, because the relative effects in trial 3, which only evaluated the effects of glucose, were much less than those of trial 4, which evaluated both effects. Overall it would appear that the water-soluble and highly viscous pentosans do not have a specific effect on the rate of dialysis of different compounds, rather they seem to exert a general effect on all nutrients.

TABLE III
Effect of a Pentosan-Rich Fraction on the Rate of Dialysis of Salt Solutions (trial 1)

Concentration of Pentosans[a] (mg/ml)	Rate of Dialysis (mmol/[100 ml/20 min]) of		
	Sodium Chloride	Calcium Chloride	Ammonium Sulfate
0	3.3 ± 0.2 b[b]	3.0 ± 0.2 b	3.0 ± 0.3 b
1	2.6 ± 0.2 bc	2.9 ± 0.2 b	2.6 ± 0.2 b
4	1.9 ± 0.2 c	1.6 ± 0.1 c	1.8 ± 0.2 c

[a]The pentosans were from the ethanol-precipitated, crude pentosan fraction of Fengler and Marquardt (1988), which contained a total of 42% arabinose plus xylose. Values represent calculated amounts of pentosans in the sample.
[b]Means ± standard error of four samples. Means within columns with different letters differ significantly ($P<0.05$).

TABLE IV
Effect of a Pentosan-Rich Fraction and a Rye Extract on the Rate of Dialysis of 0.4*N* Sodium Chloride (trial 2)[a]

	Rate of Dialysis (mmol/[100 ml/20 min]) of Viscous Carbohydrates Plus		
	No enzyme	Pancreatin	*T.v.* cellulase
Pentosans, 4 mg/ml[b]	1.8 ± 0.2 d[c]	2.0 ± 0.2 d	3.5 ± 0.2 c
Water extract of rye, 1:4, w/v	2.8 ± 0.2 d	2.6 ± 0.2 d	3.9 ± 0.3 c

[a]The rate of dialysis ± standard error (SE) of NaCl in water was 3.3 ± 0.4 mmol/100 ml in 20 min. This value was not different ($P>0.05$) from that obtained when pentosans and *T.v.* cellulase were added to the salt solution. The rate of dialysis ±SE of NaCl in an extract of wheat was 3.5 ± 0.3 mmol/100 ml in 20 min, which was not different ($P>0.05$) from that obtained with the rye extract and the pentosan solution plus *T.v.* cellulase, but was different ($P<0.05$) from values obtained with pancreatin and no enzyme. The relative viscosities of the pentosan solution and the water-extract of rye, when diluted to a final concentration as outlined in Fengler and Marquardt (1988), were 2.4 ± 0.1 and 2.2 ± 0.0 prior to enzyme addition and were 1.13 ± 0.1 and 1.05 ± 0.1 10 min after enzyme addition, respectively.
[b]The concentration and source of pentosans were the same as that in Table III.
[c]Means ±SE of four samples. Means within rows with different letters differ significantly ($P<0.05$).

Digestibilities of Fat and Dry Matter in Chicks as Affected by the Dietary Addition of Water-Soluble Pentosans

The objective of this research was to provide further evidence that the water-soluble pentosans were responsible for the antinutritive effects of rye. In these studies only fat retention was monitored, because the quantity of crude pentosans was insufficient for growth studies. Previous studies (McNab and Shannon 1975; Misir and Marquardt 1978; Marquardt et al 1979; Antoniou et al 1981; Ward and Marquardt 1983; Campbell et al 1983a,b) have clearly demonstrated that there is a high degree of association between the effects of the antinutritive factor in rye grain on growth and its effects on nutrient retention, and that fat is the most sensitive indicator of its effect. In general, the antinutritive factor in rye suppresses weight gains, efficiency of feed utilization, and the digestion and absorption of all nutrients including dry matter, energy, fat, proteins (amino acids), and a fat-soluble vitamin (vitamin D). In addition, it alters the type and concentration of intestinal microflora, alters bile salt excretion, increases volatile fatty acid concentration in the excreta, and increases excreta viscosity.

The result of the first experiment demonstrated that the addition of increasing concentrations of pentosans to a wheat-based diet caused progressive decreases in the retention of fat and dry matter in chicks, and that the effects obtained with the two higher concentrations of pentosans were intermediate to those obtained with rye alone (Table VI). The second and third experiments further demonstrated that there was a marked reduction in fat and dry matter retention in chicks when water-soluble pentosans were

TABLE V
Effect of a Pentosan-Rich Fraction on the Rate of Dialysis of Glucose and Glucose Produced Digestion of Starch with α-Amylase (trials 3 and 4)

Concentration of Pentosans (mg/ml)[a]	Rate of Dialysis (mg/[100 ml/20 min]) of	
	Glucose (trial 3)	Glucose Produced After Hydrolysis of Starch (trial 4)
0	106 ± 3 b[b]	43 ± 4 b
4	75 ± 5 c	19 ± 1 c

[a]The source of pentosans was the same as that in Table III.
[b]Means ± standard error of three samples. Means within columns with different letters differ significantly ($P<0.05$).

TABLE VI
Fat and Dry Matter Retention in Young Chicks as Affected by Type of Diet (experiment 1)

Diet	Fat Retention (%)	Dry Matter Retention (%)
Wheat	76 b[a]	63 b
Rye	56 cd	51 c
Wheat + 0.5 units of pentosans[b]	74 b	63 b
Wheat + 1.0 units of pentosans	60 c	58 b
Wheat + 2.0 units of pentosans	51 d	49 c
SE	2.0	1.2

[a]Means within columns with different letters differ significantly ($P<0.05$).
[b]One unit of pentosans per kilogram of diet contained 30.5 g of crude pentosans and had a xylose plus arabinose content of 42% (Fengler and Marquardt 1988), which equals 12.8 g/kg of diet. This is equivalent to 110% of the water-soluble pentosan content of that present in the rye diet ([12.8 g added pentosans/kg diet] ÷ [20.89 g water-soluble pentosans/kg rye × 56% rye in the diet] × 100).

added to a wheat-based diet at a concentration equal to 69 and 111% of that present in the rye diet (Table VII). The form (powdered or partially solubilized) in which pentosans were added to the diet, however, did not affect the results. Studies with the triticale diet indicate that it has an effect on fat retention intermediate to its parents (wheat and rye) and similar to that of a wheat-based diet that contained added crude pentosans at one-half the amount present in the rye diet. In the third experiment, the concentration of added pentosans in the wheat-based diet (as shown in footnotes to Table VII) was similar to that present in the rye diet, as was its effect on fat retention. This suggested that the isolated pentosan-rich fraction was responsible for nearly all of the antinutritive activity in rye, that the factor was very potent as 1.3% of added water-soluble pentosans (3.05% crude pentosans) caused the same effect as obtained with a diet containing 56% rye, and that the activity of the factor, unlike that obtained in a previous study (Antoniou et al 1981), retained most of its original activity.

Other highly viscous carbohydrates produce effects similar to those obtained in the current study with the water-soluble pentosans. Burnett (1966) originally demonstrated that the viscous β-glucans in barley were responsible for its antinutritive activity and that a fungal enzyme extract added to the diet not only reduced the viscosity of the β-glucans but also improved animal performance. White et al (1981) demonstrated that the addition of viscous β-glucans to a corn-based diet decreased chick growth and feed efficiency, and that this effect was alleviated by the addition to the diet of a filtrate from a culture of *T. viride*. In addition, there was a parallel reduction in the viscosity of the chicks' intestinal contents. Similar effects were demonstrated by Jenkins et al (1978) in a study with human volunteers. They demonstrated that viscous carbohydrates from guar gum, pectin, tragacanth, and other sources decreased glucose absorption and that this effect was reversed when hydrolyzed nonviscous saccharides were ingested. Guar gum, gum karaya, gum carob, pectin, and agar were also shown by Kratzer et al (1967) to interfere with nitrogen retention and fat absorption and to reduce the metabolizable energy of the diet.

Although the viscous carbohydrates, and specifically those from rye, may be considered undesirable in the diet of domestic livestock, they are considered to be of benefit when consumed by humans because they increase volatile fatty acid production, reduce rate of uptake of glucose and total absorption of fat including cholesterol (Vahouny and Kritchevsky 1982; Jenkins et al 1978, 1984). Consumption of these carbohydrates has been shown to be beneficial to diabetic patients and to reduce the incidence of cancer and atherosclerosis. They also affect the loaf quality of bread (McCleary et al 1986). Further studies should be carried out with regard to the possible beneficial effects that rye can exert on humans since it is a particularly good source of water-soluble pentosans.

TABLE VII
Effect of Cereal Grains and a Pentosan-Rich Fraction Added to a Wheat Diet on Fat and Dry Matter Retention in Young Chicks (experiments 2 and 3)

	Experiment 2		Experiment 3	
Diet	Fat Retention (%)	Dry Matter Retention (%)	Fat Retention (%)	Dry Matter Retention (%)
Wheat	75 b[a]	66 b	78 b	70 b
Triticale	57 c	56 c	...	...
Rye	48 d	54 c	57 c	59 d
Wheat plus pentosans[b]	65 cd	59 bc	55 c	61 cd
Wheat plus solubilized pentosans[b]	67 cd	60 bc	61 c	62 c
SE	2.8	1.2	1.7	0.3

[a] Means within columns with different letters differ significantly ($P<0.05$).
[b] The diets in experiments 2 and 3 contained 19 and 30.5 g/kg of crude pentosans, respectively. The corresponding contents of water-soluble pentosans (xylose plus arabinose) were 8.0 and 12.9 g/kg, which was equivalent to 69 and 111% of those present in the rye diet. Calculations given in Table VI footnote.

In summary, it may be concluded that the water-soluble and highly viscous pentosan-rich carbohydrates that are present in rye grain at a relatively low concentration greatly impede the rate of dialysis of certain compounds in vitro and greatly reduce fat retention in the young chick. A fungal enzyme, *T.v.* cellulase, but not a mammalian enzyme, pancreatin, is able to reduce the viscosity of the pentosan-rich carbohydrate to that of water and, at the same time, eliminate its effects in vitro and in vivo. The results of these studies would suggest that the principle antinutritive factor in rye grain is a water-soluble pentosan. The results of a previous study (Fengler and Marquardt 1988) demonstrated that the pentosan is composed of xylose and arabinose, is located in the flour or endosperm of the seed, and that special precautions are required in order to isolate a highly viscous pentosan.

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