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Hypocholesterolemic Activity of Mucilaginous Polysaccharides in White Leghorn Cockerels.* (31478)

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Previous studies in our laboratory with White Leghorn cockerels fed various semi-purified diets demonstrated that the response of plasma cholesterol levels to supplemental dietary cholesterol was markedly affected by the nature of the carbohydrate component of the diet(1). With sucrose as the sole source of carbohydrate, resultant plasma cholesterol levels were approximately double those obtained when glucose replaced sucrose. In the course of subsequent investigations, it was observed that certain mucilaginous polysaccharides elicited significant hypocholesterolemic activity when added to the basal cholesterol supplemented diet at levels as low as one per cent. Data are presented here on 16 mucilaginous polysaccharides which were found to have hypocholesterolemic activity in our test systems with the chick.

Methods. Three basal diets were employed. The composition of 2 of these diets has been previously reported(1); they were prepared from purified ingredients containing either 20% casein, 8% gelatin and 61.3% sucrose (Diet 1), or 25% purified soybean protein and 64% glucose (cerelose) (Diet 2). Another diet (Diet 3) was formulated from a variety of natural foodstuffs from vegetable and animal sources to simulate a commercial-type diet (Table I). In Diets 1 and 2, test substances (including 3 g cholesterol dissolved in 3 g corn oil/100 g diet) were added at the expense of carbohydrate. In Diet 3, all supplements were added directly to the complete diet. Day-old single-comb White Leghorn cockerels‡ were divided into groups of 12, and

TABLE I. Diet 3 (Commercial-Type Diet).

Ingredient	g/kg diet
Corn, yellow, fine ground[a]	614
Soybean meal (44%), fine ground[a]	200
Corn gluten meal (41%)[a]	50
Fish meal[a]	50
Alfalfa meal (20%)[a]	20
Distillers solubles (30%)[a]	25
Delamix[b]	1
Limestone, pulverized	20
Bone meal (steamed)[a]	12
Sodium chloride	5
Choline chloride (25%)	1
Fortafeed 2-49C[c]	1
Vitamins A + D[d]	1
Merck B_{12} (40)[e]	0.5

[a] Obtained from A. J. Mowerson and Co., Wyckoff, N. J.

[b] A trace mineral concentrate with calcium carbonate as carrier (obtained from Limestone Products Corp., Newton, N. J.); 1 g contains (ppm): Mn 60, I 1.2, Fe 20, Cu 2, Zn 0.1 and Co 0.2.

[c] A vitamin feed supplement (obtained from American Cyanamid Co., Wayne, N. J.); 1 g contains the following vitamins (mg): riboflavin 4.4, niacin 8.8, pantothenic acid 19.8, choline chloride 22 and folic acid 0.1.

[d] Approximately 8800 units vit A and 1770 units vit D_3 are added together to the diet by mixing appropriate quantities of Nopcay "10" and Super Nopdex "15" (obtained from Nopco Chemical Co., Harrison, N. J.) which contain 10,000 units vit A /g and 15,000 units vit D_3/g, respectively.

[e] A vit B_{12} concentrate (obtained from Merck & Co., Rahway, N. J.) containing 40 mg vit B_{12}/lb; 0.5 g ≡ 5 γ B_{12}.

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‡ Three different strains were imployed in the course of the studies: Mount Hope, Darby and Babcock, obtained from Kerr Chickeries, Frenchtown, N. J., Hall Brothers Hatcheries, Wallingford, Conn., and Spring Lake Farm, Wyckoff, N. J., respectively.

TABLE II. Hypocholesterolemic Activities in White Leghorn Cockerels of Various Mucilaginous Polysaccharides Administered at Levels of 10% to 0.25% in Diet 1 Supplemented with 3% Cholesterol (12 Chicks /Group).

Mucilaginous polysaccharide	% of diet	% Change in plasma cholesterol (treated-control)[a] at levels of mucilaginous polysaccharide equal to: 10	6	3	3	2	2	1.5	1.0	0.5	0.25
Carrageenin[b]											
Sea Kem #402				—67[1c]							
" " #9					—65[1]						
" " #1					—62[2]						
" " #201					—61[1]						
Viscarin					—59[4]	—50[12]			—22[4]		
Sea Kem #101					—54[1]						
Viscarin Ca salt					—52[6]		—39[6]		—12[6]		
Sea Kem #15			—76[1]		—51[9]		—28[10]		—11[12]	+3[9]	+6[6]
Gelcarin DG					—47[2]						
Viscarin K salt					—40[1]		—39[1]		—13[1]		
Kappa							—49[1]				
Lambda Mg salt							—48[2]				
Sea Kem #7							—32[2]				
Gelcarin LP							—32[2]				
Viscarin MCW							—14[2]				
Kappa, degraded (hydrolyzed)							—6[1]				
Sea Kem #22							—4[2]				
Lambda, degraded (hydrolyzed)							+7[2]				
Salep root											
#6-2026[d]				—63[2]							
#CP-4856[e]			—11[1]		0[7]	+7[8]			—6[6]		
?[d, f]									+2[3]		
Guar gum[d, e]				—60[51]		—52[12]			—28[49]	—13[13]	—2[8]
Karaya gum[d]											
#MCK 47				—58[2]					—4[1]		
#MCK 9					—46[1]						
#3803-P			—31[1]		—17[7]	—11[7]			+1[6]		
#G-126					—17[2]				+22[2]		
#G-127					+1[1]						
Locust bean gum											
Solubilized (1200 cps)[b]				—54[2]		—42[2]					
#2-13044[d]			—44[1]		—42[10]		—33[9]		—9[6]		
Solubilized (50 cps)[b]					—29[2]		—32[2]				
Carob flour[d]		—35[1]			—27[1]						
Ghatti gum[d]											
#269				—47[1]							
#3542-P			—8[1]		+8[7]	+6[6]			+4[6]		
Psyllium seed											
Husks #8-5091[d]				—44[2]							
Husks #8826-P[d]			—27[1]		—9[7]	—8[6]			+5[6]		
Whole #8-5082[d]					+73[1]						
Metamucil[g]								—29[1]			
Tragacanth gum[d]											
#3800-P			—43[1]	—42[11]		—37[8]			—16[7]		
#MCT 43									+6[2]		
Shiraz gum[d]											
?[f]				—32[1]							
#714			+2[1]		+8[7]	—7[7]			—2[6]		
Dextran CR[h]				—30[2]							
Pectin[i]											
N. F.			—38[1]	—29[20]		—14[6]			—4[15]		
Orange					—20[1]		+10[1]		+16[1]		
Agar[j]				—24[3]					+25[1]		
Alginic acid[k]				—20[2]							
Dialdehyde gum XO-200[l]				—18[1]		+1[1]			—2[1]		
Fucoidin[k]						—30[1]					
Polysaccharide Y-1401[m]						—51[2]					

TABLE II footnotes

[a] Average plasma cholesterol level and body weight of all control chicks at end of 27-day experimental period were 623 mg/100 ml and 240 g, respectively.
[b] Marine Colloids, Inc., New York.
[c] Superscript numbers refer to number of tests.
[d] Meer Corporation, New York.
[e] Stein, Hall & Co., Inc., New York.
[f] Lot number unknown.
[g] G. D. Searle & Co., Chicago, Ill.
[h] Pharmachem Corp., Bethlehem, Pa.
[i] Sunkist Growers, Ontario, Calif.
[j] Difco Laboratories, Detroit, Mich.
[k] Kelco Co., Los Angeles, Calif.
[l] General Mills, Minneapolis, Minn.
[m] Research Product, Northern Utilization Research & Development Division, Peoria, Ill.

each group was housed in individual cages with food and water offered *ad libitum*. Weekly body weight was determined for each group. At the end of 27 days on the experimental diets, final body weight and total food consumption were recorded for each group, then a 5 ml heparinized blood sample was taken *via* heart puncture from each chicken and assayed individually for plasma cholesterol either by the Trinder procedure (2) or by a modification of the Leffler procedure(3) adapted for use on a Technicon AutoAnalyzer.

Results and discussion. Data on the hypocholesterolemic activity of the 16 mucilaginous polysaccharides, expressed as per cent reduction from the control group and arranged in decreasing order of activity at the 3% dietary level, are summarized in Table II. All tests employed the casein-sucrose basal diet (Diet 1). The figures to the left of each column are the per cent changes obtained with the most active preparation of a particular polysaccharide. The superscript numbers associated with each of the values in the Table represent the total number of experiments included in the average value shown.

Only one agent, salep root (carob flour), was tested at the relatively high level of 10% of the diet: a 35% reduction of plasma cholesterol resulted ($p \leq 0.05$), but there was no significant effect on body weight or total food consumption.

Of the agents tested at 6% of the diet, carrageenin (Sea Kem #15) was the most active (—76% change from control); moderate activity (—44% to —27% change from control) was shown by locust bean gum (#2-13044), tragacanth gum (#3800P), pectin N. F., karaya gum (#3803P) and psyllium husks (#8826P), arranged in decreasing order of activity; inactive at this dose level were salep root (#CP-4856), ghatti gum (#3542P) and shiraz gum (#714).

The greatest number of experiments with the various mucilaginous agents were carried out at the 3% dietary level, and for this reason the relative listing in Table II according to descending order of hypocholesterolemic activity was derived from these data obtained with the most active preparation of each agent tested. It is readily apparent from inspection of Table II that not all preparations of the same polysaccharide have equal activity. In every instance, except in the case of guar gum, widely different activities were found depending upon the preparation tested. A striking example of this occurred with salep root, where preparation #6-2026 appeared to be one of the most active agents tested, and another preparation (#CP-4856) obtained from the same supplier was completely inactive. Similarly, one batch of karaya gum (#MCK47) had high activity, whereas other batches had lower activities, one of which was inactive (#G-127); however, in this case, the inactive product was obtained from a different supplier. Other examples of activity variation between different preparations of the same polysaccharide were observed with ghatti gum (#269 *vs* #3542P), psyllium seed husks (#8-5091 *vs* #8826P and whole psyllium seed, #8-5082) and shiraz gum (unknown batch number *vs* #714).

A number of carrageenin preparations other than the most active product (Sea Kem #402) were tested, and although variations in activity were also encountered here, they all had reasonable activity ranging from 40% to 65% reduction of plasma cholesterol.

The reasons for the observed differences in activity between the various preparations of the same polysaccharide are not apparent. However, the data obtained with locust bean gum indicated that viscosity and water solubility of the product influenced activity since the pregelatinized preparation with the higher viscosity (1200 cps) was more active than the 50 cps product or the native ungelatinized material (#2-13044). Furthermore, in experiments with certain carrageenin preparations at the 2% dietary level, it was found that reduction of the mucilaginous character of the polysaccharide by acid hydrolysis to yield products of lower molecular weight resulted in complete loss of activity; thus, the hydrolyzed kappa and lambda fractions were inactive, whereas the unhydrolyzed materials were approximately as active as one of the best corrageenin preparations, Viscarin.

In experiments at the 2% dietary level, fucoidan exhibited moderate activity (30% reduction) and polysaccharide Y-1401 high activity (51% reduction). Of the agents included in Table II, only guar gum and carrageenin (Viscarin) demonstrated significant activity at the 1% dietary level, and finally, guar gum was the only substance which was found to be active at 0.5% of the diet. The data shown for guar gum are a summation of a number of different commercial preparations, all of which were approximately equal in activity.

Some of the substances which were found to be inactive when tested at a level of 3% in the casein-sucroe basal diet (Diet 1) are: acacia gum, Alphacel (cellulose), alumina, carbowax (type 1500), cellulose triacetate, chondroitin sulfate, dextran H, ethocel, kaolin, Magnesol-celite (40/60), methyl cellulose (15 cps), Microcel (calcium silicate), polyvinylpyrrolidone and silicic acid. Of particular interest are the polysaccharides such as acacia gum, chondroitin sulfate, dextran H, and the various cellulose derivatives. The lack of activity of these agents again emphasizes the difficulty in relating chemical structure and activity.

In our previous publication(1), we pointed out the variation in response of plasma cholesterol of White Leghorn cockerels when dietary cholesterol was incorporated in different basal diets. Therefore, it was of interest to test several of the active polysaccharides in the 3% cholesterol supplemented soybean protein-glucose basal diet (Diet 2) and also in a similarly supplemented commercial-type diet (Diet 3). The results obtained with either 3% carrageenin (Sea Kem #15) or 3% pectin N. F. are summarized in Table III. Although the plasma cholesterol levels of the supplemented control groups were only about half those obtained with the usual casein-sucrose basal diet (Diet 1), statistically significant reductions were obtained in every instance except with pectin N. F. in Diet 2.

The effects of carrageenin and guar gum

TABLE III. Effects of 3% Carrageenin or Pectin Administered in Either Diet 2 or Diet 3 Supplemented with 3% Cholesterol on Plasma Cholesterol, Body Weight and Total Food Consumption of White Leghorn Cockerels (10 Chicks/Group).

Agent	Basal diet	Avg plasma cholesterol (mg/100 ml) Control	Treated	% Change plasma cholesterol (treated—control)	% Change body wt[a]	% Change total food consumption[a]
Carrageenin (Sea Kem #15)	2	286 ± 22[b]	166 ± 8.1[b]	—42[c] ± 5.3[b]	—10	—11
Pectin N. F.	2	286 ± 22	304 ± 40	+ 6 ±16.2	—16	— 4
Carrageenin (Sea Kem #15)	3	361 ± 35	272 ± 22	—25 ± 9.5[e]	— 1	+ 7
Pectin N. F.	3	361 ± 35	260 ± 18	—28 ± 8.5[d]	+ 1	+15

[a] Average body weight and total food consumption of the control group consuming Diet 2 were 260 g and 5.0 kg, respectively; corresponding values for the control group consuming Diet 3 were 278 g and 4.8 kg. Body weight and food consumption were determined for entire group and not calculated by averaging individual values for each chick.

[b] Standard error of the mean. [c] $p < .001$. [d] $p < .01$. [e] $p < .025$.

TABLE IV. Effects on Plasma Cholesterol, Body Weight and Total Food Consumption of White Leghorn Cockerels Fed Diet 1 Supplemented with Carrageenin or Guar Gum.

Agent	% of diet	Avg plasma cholesterol (mg/100 ml) Control	Treated	% Change plasma cholesterol (treated—control)	% Change body wt[a] (treated—control)	% Change total food consumption[a] (treated—control)
Carrageenin (Sea Kem #15)	3.0	$175^{12} \pm 8.4$[b][c]	$169^{12} \pm 10.2$[b][c]	-3 ± 10.7[c]	−5	−4
Guar gum	3.0	$199^{10} \pm 5.8$	$145^{10} \pm 6.9$	-27[d] ± 4.1	+2	−3
" "	2.0	$199^{12} \pm 13.3$	$148^{11} \pm 10.2$	-18[e] ± 7.1	+12	+8
" "	0.5	$199^{12} \pm 13.3$	$164^{10} \pm 16.3$	-18 ± 9.9	+3	+23

[a] Average body weights of control groups consuming the basal diet supplemented with 3% carrageenin, 3% guar gum, and either 2% or 0.5% guar gum were 247 g, 247 g and 200 g, respectively; corresponding total food consumptions for these same control groups were 4.9 kg, 3.5 kg and 3.9 kg, respectively.

[b] Superscript numbers indicate number of individual values employed in calculation of average.

[c] Standard error of the mean. [d] $p < .001$. [e] $p < .025$.

in Diet 1 unsupplemented with cholesterol are presented in Table IV. Carrageenin at the 3% dietary level did not affect plasma cholesterol levels, whereas both 3% and 2% guar gum gave statistically significant reductions. The results indicate that as little as 2% guar gum lowers the endogenous plasma cholesterol level of chickens under these experimental conditions.

In Table V are summarized the effects on plasma cholesterol, body weight and food consumption of carrageenin, guar gum and pectin N. F. at the 3% and 2% dietary levels in Diet 1 supplemented with 3% cholesterol. Guar gum appeared to be slightly more active than carrageenin, and both of these agents were significantly more active than pectin N. F. Because of the large number of tests included in the data presented in Table V, it was possible to demonstrate statistically significant reductions in body weight gain with all 3 polysaccharides at the 3% level, but the reduction observed with guar gum (3%) was minimal. At 2% of the diet, pectin still gave a significant reduction in body weight gain, whereas carrageenin and guar gum produced a slight increase. No apparent reduction in total food consumption was observed with any of the agents at the 2 dietary levels employed. Vohra and Kratzer(4) reported that feeding 2% carrageenin or guar gum in a stock diet similar to Diet 3 resulted in a 25% to 30% inhibition of growth of day-old chicks after 20 to 21 days on the experimental diets. Our data do not support this finding. It is possible that this dis-

TABLE V. Effects of Carrageenin, Guar Gum and Pectin N. F. Administered in Diet 1 Supplemented with 3% Cholesterol on Plasma Cholesterol, Body Weight and Total Food Consumption of White Leghorn Cockerels (12 Chicks/Group).

Agent	% of diet	No. of tests	Plasma-cholesterol (treated—control)[a] % Change	± S.E.	Body wt (treated—control)[a] % Change	± S.E.	% Change total food consumption (treated—control)[a,b]
Carrageenin[c]	3	28	−54[e]	2.7	−9[e]	1.7	+10
Guar gum[c]	3	51	−60[e]	2.0	−3[g]	1.2	+2
Pectin N. F.[c]	3	20	−29[e]	3.2	−8[e]	2.0	+1
Carrageenin[d]	2	36	−40[e]	2.6	+4[g]	1.6	+22
Guar gum[c]	2	42	−52[e]	2.2	+3[h]	1.4	+7
Pectin N.F.[c]	2	6	−14[g]	5.9	−10[f]	3.6	−5

[a] Average plasma cholesterol level, body weight and total food consumption of all control groups were 638 mg/100 ml, 241 g and 4.55 kg, respectively.

[b] Standard errors not given since statistical analysis revealed no significant differences.

[c] Summation of all experiments with agent tested at indicated dietary level (see Table II).

[d] Summation of experiments with carrageenin tested at 2% dietary level except those employing Viscarin MCW, kappa and lambda (degraded, hydrolyzed) and Sea Kem #22 (see Table II).

[e] $p < .001$. [f] $p < .01$. [g] $p < .025$. [h] $p < .05$.

crepancy may be due to the different strain of chicks, basal diets or cholesterol supplementation employed in most of our experiments. Ershoff and Wells(5) found no significant depression in the growth of rats which were fed 10% pectin, guar gum, locust beam gum or carrageenin for 28 days in a casein-sucrose basal diet similar to Diet 1 supplemented with 1% cholesterol. However, in our experiments, cholesterol supplementation would not appear to be a significant factor since chicks fed 3% corrageenin or guar gum in Diet 1 without supplemental cholesterol did not exhibit reduced body weight gain (Table IV).

Ershoff and Wells(5) stated that maximal activity is obtained only with citrus pectin samples assaying in the range of 10.7% methoxyl on a moisture-ash-free basis. The pectin N. F. (also obtained from a citrus source) which was employed in our experiments had a methoxyl content of 10.5%.

Our results do not agree with the claim of Ershoff and Wells(5) that pectin N. F. is the most active polysaccharide. Instead, guar gum and certain carrageenin preparations demonstrated the greatest activity. No satisfactory relationship between polysaccharide structure and hypocholesterolemic activity was established.

Summary. Sixteen mucilaginous polysaccharides fed to chickens for 28 days at various levels between 10% and 0.5% of a casein-sucrose basal diet supplemented with 3% cholesterol exhibited hypocholesterolemic activity. Relative decreasing order of activity of 14 of these agents tested at the 3% dietary level is: carrageenin, salep root, guar gum, karaya gum, locust bean gum, ghatti gum, psyllium seed, tragacanth gum, shiraz gum, dextran CR, pectin N. F., agar, alginic acid and dialdehyde gum XO-200. The two other active agents, fucoidin and polysaccharide Y-1401, were tested only at 2% of the diet. Carrageenin demonstrated activity at the 1% dietary level and guar gum as low as 0.5%. Carrageenin was active in a soybean protein-glucose synthetic diet and in a commercial-type diet supplemented with 3% cholesterol. Significant reductions in plasma cholesterol levels were obtained with 3% and 2% guar gum in the casein-sucrose basal diet unsupplemented with cholesterol, indicating that guar gum can lower the endogenous level of chickens consuming this experimental diet; 3% carrageenin was inactive under these conditions. Minimal reductions (<9%) in body weight gain were obtained with 3% carrageenin, guar gum and pectin N. F. in the casein-sucrose basal diet supplemented with 3% cholesterol; at the 2% dietary level, pectin N. F. still inhibited body weight gain, whereas carrageenin and guar gum gave a slight increase. No apparent reduction in food consumption was observed with carrageenin, guar gum and pectin N. F. at the 3% dietary level. A relationship between polysaccharide structure and hypocholesterolemic activity was not established, although there was a possible correlation between the activity of certain preparations of a particular polysaccharide and parameters such as water solubility, viscosity and molecular weight.

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