Steroid Metabolism, Transit Time, and Cecal Bacteria in Rats Fed Corn or Wheat Bran[1]

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ABSTRACT

The effects of corn and wheat bran on serum cholesterol concentration, bile acid excretion, bile acid degradation, bowel function, and anaerobic cecal bacteria were studied in rats. Neither wheat bran nor corn bran lowered serum cholesterol, but serum cholesterol was increased in one experiment when wheat bran was added to the diet. When rats were switched from bran containing diets to a bran free diet, the serum cholesterol concentration remained constant. In general, bran-fed rats excreted more bile acids than rats fed the low fiber control diet. Fat and fiber content of the diet affected both bile acid degradation and concentration in the large intestine of rats. Rats fed a 20% fat diet had increased bile acid degradation as the wheat bran content of the diet increased, but rats fed a 30% fat diet had extensive bile acid degradation regardless of wheat or corn bran content of the diet. Anaerobic bacterial concentrations in cecal contents were unaffected by dietary variables, but bacterial type or metabolism was altered when bran was added to the 20% fat diet. Bran feeding decreased bile acid concentration in intestinal contents and decreased the time that indigestible material remained in the intestine. Since bile acids are suspected to be carcinogenic or cocarcinogenic, bran may play a role in decreasing the incidence of colonic cancer by diluting bile acids in the large bowel.

In the past few years, much interest in the role of fiber in the diet has arisen. Some of the proposed beneficial effects of dietary fiber include its hypocholesteremic effect (Trowell 1972, Vijayagopal et al 1973), its role in regulating bowel habit (McConnell et al 1974, Williams and Olmsted 1936) and in alteration of gut microflora, and its protective effect against cancer of colon and rectum through a change in bile acid concentration and bile acid metabolism (Burkitt 1971, Cummings 1973, Pomare and Heaton 1973). Wheat bran is the most frequently used source of dietary fiber when the physiological effects of dietary fiber are studied. This study was undertaken to investigate the effect of corn bran and wheat bran on serum cholesterol concentration, bile acid excretion and degradation, intestinal transit time, and bacterial concentration in the cecum of rats. Also, the effects of diets with graded levels of wheat bran on these parameters were studied.

MATERIALS AND METHODS

Experiment I

Female Sprague-Dawley rats (140–350 g) were allotted into three groups of nine rats each so that the average weights for each group were equal. The rats were housed individually in stainless-steel cages with raised wire floors. The animal room was maintained at 20 ± 1°C with controlled humidity and a 12 hr light-dark cycle. The diet compositions are shown in Table I. Corn bran flour or wheat bran replaced corn starch and a portion of the casein in the control diet. All three diets contained approximately equal energy and protein per gram of diet. The diets were fed for six weeks. Food and water were supplied ad libitum. Throughout the feeding period, body weight and food intake were recorded.

Intestinal transit time was measured at the end of the fourth week. The rats were fasted from 10 p.m. to 9 a.m. the following morning and offered 5 g of food containing 100 mg of chromic oxide. Food refusals were recorded after 1.5 hr to determine the quantity of chromic oxide consumed. Fecal collections were made every 8 hr for the first day and then every 12 hr for the next two days. The feces were dried to a constant weight at 60°C in a forced air oven and stored for chromic oxide analysis. Chromic oxide was determined according to the method of Bolin et al (1952), and transit time was designated as the time required for 95% of the ingested chromic oxide to be excreted.

Collections for wet and dry fecal excretion and steroid excretion were conducted on four successive 24-hr collections on days 1–4 of the fifth week. The samples were dried as described above and pooled for steroid analysis.

Neutral steroids were extracted from aliquots of pooled feces as described by Miettinen et al (1965). 5-α-Cholestane was added to the dried steroids as an internal standard for gas-liquid chromatography (GLC). The dried steroids were silylated (Grundy et al 1965) and the trimethylsilyl (TMS) ethers were quantitated on a gas-liquid chromatograph equipped with flame ionization detectors. GLC conditions for separating TMS ethers of neutral steroids were: injector temperature, 280°C; detector temperature, 290°C; column temperature, 240°C; H_2 flow, 30 ml/min, and N_2 carrier gas flow, 35 ml/min. The column was 1.8 m long, 3.2 mm o.d., and packed with 3% SP2401 on Supelcoport 100/120. Cholesterol, coprostanol, and coprostanone standards were silylated and chromatographed with 5-α-cholestane as an internal standard. The area under the recorder tracing for each standard was cut out and weighed. Standard curves were prepared by plotting the ratio of neutral steroid to 5-α-cholestane vs. the quantity of neutral steroid injected. The quantity of neutral steroids in the feces was determined from the standard curves. Neutral steroid excretion is expressed as the sum of cholesterol, coprostanol, and coprostanone excretion.

Bile acids were extracted from aliquots of pooled feces according to Grundy et al (1965). Separation of bile acids from fatty acids was as described by Makita and Wells (1963). Methyl esters of bile acids were prepared with excess of etheral-diazomethane. The methylated bile acids were dried, silylated (Grundy et al 1965), and separated by GLC. GLC conditions were the same as for the neutral steroids except the column temperature was 210°C for 20 min after sample injection, 8°C/min to 270°C and held at 270°C until the last bile acid was eluted. Holding the temperature at 210°C for 20 min allowed remaining fatty acid methyl esters to be separated from the TMS ethers of bile acids. Cholic, lithocholic, deoxycholic, chenodeoxycholic, 12-ketodeoxycholic, and 3,12-diketodeoxycholic acid standards were prepared with 5-α-cholestane as an internal standard. Standard curves were prepared as for the neutral steroids and were used to quantitate bile acid excretion. An average standard curve was used to quantitate all bile acids, which are reported as the sum of all bile acids. Bile acids that did not chromatograph with cholic and chenodeoxycholic acids are designated as degraded bile acids.

Cecums from five animals with comparable body weights in each group were removed for anaerobic bacterial count and in vitro bile acid fermentation. A portion of cecal contents from each rat was serially diluted to 10^{-9} concentration and anaerobically incubated

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in roll tubes at 37°C for five days using M98-5 medium without hemin as described by Salanitro et al (1974). Colonies were counted after a five-day incubation period. This procedure was conducted as soon as the rats were killed and all steps were performed aseptically and anaerobically.

Cholic acid degradation was determined by incubating 1 ml of 10^{-1} dilution of cecal contents with 0.1 mmol of cholic acid, 10 ml of M98-5 medium without hemin or agar, and 1 g of diet that the animal had been eating. The fermentations were conducted anaerobically in 50 ml serum bottles at 37°C. Preliminary fermentations were conducted to determine appropriate assay conditions. Cholic acid degradation was linear between the 10th and the 40th hr when the logarithm of degraded cholic acid was plotted against time. Maximum extent of cholic acid degradation was 85–95% and the plateau in degradation occurred with 72–90 hr of incubation. In vitro cholic acid degradation reported in Results was determined with 24 hr incubations. Fermentation was stopped by injecting 2 ml of concentrated HCl into the serum bottles. Microbial degradation of cholic acid was designated as the quantity of cholic acid fermented to other bile acids minus blank fermentations. Bile acids were extracted from the fermentation reaction and analyzed as described for the fecal samples.

Serum cholesterol concentration was analyzed at the end of three and six weeks. Third week blood samples were obtained from the orbit of the eye (Sorg and Buckner 1964). Sixth week blood samples were collected by decapitation. Serum cholesterol was determined by the method of Searcy and Berquist (1960) with the modification described by Leveille et al (1962).

Experiment II

Male Sprague-Dawley rats weighing approximately 230 g were divided into 4 groups of 11 rats each. The control diet was similar to that of experiment I except it contained only 20% fat by weight (Table I). Three levels of wheat bran—9, 18, and 36 g—were isocalorically substituted for corn starch in the control diet. These three levels of wheat bran were chosen on the basis of neutral detergent residue (NDR) content, namely, 5, 10, and 20 g of NDR per 450 kcal of metabolizable energy. NDR of wheat bran was analyzed according to Van Soest and Wine (1967). These diets were fed for four weeks (period I) and then all animals were switched to the bran-free control diet for another 4 weeks (period II). Conditions of animal care were the same as in experiment I.

At the end of the fourth week of period I, blood was obtained from the orbit of the eye for serum cholesterol determination and three animals from each group were killed by decapitation. Their cecal contents were diluted and incubated for anaerobic bacterial count and in vitro cholic acid fermentation as described for experiment I.

In the beginning of the fourth week of period I, 100 mg of chromic oxide was administered intragastrically to six rats in each group to measure intestinal transit time. The feces were collected and analyzed for chromic oxide as in experiment I.

During the fourth week of experimental periods I and II, feces were collected for three successive 24-hr periods and analyzed as in experiment I. At the end of the fourth week of period II, all the rates were killed by decapitation. Serum cholesterol, anaerobic cecal bacterial counts, and in vitro cholic acid fermentation were done as above.

The data were tested for significance by Dunnett's procedure. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Experiment I

Table II summarizes the results of experiment I. Serum cholesterol concentrations were not different between the groups fed the control or the corn bran diets. However, wheat bran fed rats had higher serum cholesterol concentrations than either the control or corn bran fed rats for both the third and the sixth week experimental periods.

Corn bran and wheat bran fed rats excreted more wet and dry fecal mass than the control rats. Also, the intestinal transit time was significantly longer in the control group compared with either of the bran-fed groups.

Bile acid excretion was significantly higher for the corn bran fed group than the control or wheat bran fed groups (Table II). However, the concentration of bile acids in feces (milligrams of bile acid per gram of wet feces) was comparable for both the corn bran and the wheat bran fed rats. There was no difference in the extent of bile acid degradation among dietary treatments either in the feces or in the in vitro assay.

Neutral steroid excretion was greater and cholesterol absorption was less for the bran fed rats than for the control rats. The number of viable anaerobic bacteria per gram of cecal contents was similar for all groups, ie, did not differ by more than one log. The metabolizable energy intake was similar for the control and wheat bran fed groups, but the rats fed wheat bran grew significantly slower.

Experiment II

The results of experiment II are shown in Table III. The level of bran in the diet did not affect serum cholesterol concentration. Both wet and dry fecal mass increased as the concentration of bran in the

TABLE I
Composition of Diets

	Experiment I			Experiment II			
					Bran Supplements		
	Control	Corn Bran (g/100 g)	Wheat Bran	Control	+9 g	+18 g (g/450 kcal)	+36 g
Basal[a]	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Sodium-caseinate	25.0	20.0	20.0	21.0	19.0	19.0	19.0
Lard[b]	25.0	25.0	25.0	15.0	15.0	15.0	15.0
Corn oil[c]	4.0	4.0	4.0	6.0	5.0	5.0	4.0
Corn starch	39.5	...	...	52.0	49.0	45.0	38.0
Wheat bran[d]	...	...	44.5	...	9.0	18.0	36.0
Corn bran flour[e]	...	44.5	...	...	...	...	...

[a]Contained 1 g of vitamin mix, 4 g of mineral mix, 1 g of cholesterol, 0.3 g of L-methionine, and 0.2 g of choline • cl. The vitamin mix was composed of thiamine • HCl, 11 g; pyridoxine, 11 g; riboflavin, 11 g; calcium pantothenate, 33 g; *p*-aminobenzoic acid, 55 g; menadione, 25 g; inositol, 50 g; ascorbic acid, 100 g; niacin, 50 g; vitamin B_{12}, 15 mg; biotin, 0.3 g; folic acid, 2 g; retinol acetate, 1×10^7 IU; α-tocopherol, 50,000 IU; vitamin D_3, 110,000 IU; and cerelose to 5 kg. The mineral mix (salt mix 4164, Teklad, Inc., Madison, WI) contained, in g/100 g: calcium acetate • H_20, 6.293; calcium diphosphate • $2H_20$, 28.525; dipotassium phosphate, 28.443; ferric citrate • $5H_20$, 2.44; magnesium sulfate • $7H_20$, 10.053, potassium iodine, 0.65; sodium diphosphate • $12H_20$, 14.630; sodium chloride, 9.546.

[b]Farmer Peet's Shortenin' (pork and beef fat), Peet Packing Company, Chesaning, MI.

[c]Mazola oil.

[d]Soft red wheat bran from undetermined variety of wheat; 55% neutral detergent residue.

[e]Provided by Lauhoff Grain Company, Danville, IL. Corn bran was ground to pass through a 100-mesh screen and contained 64% neutral detergent residue and 24% acid detergent residue.

diets increased. When the rats were switched to the bran-free diet (period II), fecal mass decreased close to the control values of period I. The intestinal transit time was inversely proportional to the concentration of bran in the diet. No significant differences in total bile acid excretion were found between groups. However, the fecal concentration of bile acids was inversely proportional to the

TABLE II
Experiment I. Serum Cholesterol Concentration, Steroid Excretion, Intestinal Transit Time, Cecal Bacteria Concentration, Energy Intake, and Weight Gain in Rats Fed Corn Starch, Corn Bran, or Wheat Bran

	Diet[a]		
	Control	Corn Bran	Wheat Bran
Serum cholesterol, mg/dl			
Three weeks	135 ± 10.0	131 ± 5.7	180 ± 10.0*
Six weeks	149 ± 8.6	135 ± 12.1	197 ± 16.1*
Fecal excretion			
Wet mass, g/day/rat	0.6 ± 0.02	5.6 ± 0.20*	4.1 ± 0.20*
Dry mass, g/day/rat	0.5 ± 0.01	4.7 ± 0.20*	3.4 ± 0.10*
Transit time, hr	42.5 ± 3.8	24.0 ± 2.0*	19.0 ± 2.1*
Fecal bile acid			
Excretion, mg/day/rat	7.0 ± 1.0	10.8 ± 0.3*	6.9 ± 0.3
Concentration, mg/g wet feces	11.6 ± 1.7	2.0 ± 0.1*	1.7 ± 0.1*
Percent degradation[b]			
Feces	88 ± 1	89 ± 2	84 ± 2
In vitro	42 ± 3	42 ± 3	45 ± 2
Fecal neutral steroid			
Excretion, mg/day/rat	60 ± 8	143 ± 6*	108 ± 7*
Cholesterol absorption			
Apparent, mg/day/rat	63 ± 11	14 ± 9*	25 ± 18*
Bacterial count[c]	5.8	1.9	2.8
Energy consumption, kcal/day/rat	60 ± 1.9	70 ± 1.8	64 ± 1.3
Cholesterol consumption, mg/day/rat	122 ± 3	146 ± 4	134 ± 3
Weight gain, g/day/rat	1.9 ± 0.37	1.5 ± 0.27	1.1 ± 0.17*

[a]Means ± standard error for nine rats per dietary treatment.
[b]Percent degraded bile acids.
[c]Number of viable anaerobic bacteria per gram of cecal content × 10^{-10}

TABLE III
Experiment II. Bowel Function, Steroid Metabolism, and Cecal Bacteria in Rats Fed Graded Levels of Wheat Bran (WB)

	Control	+9 g WB	+18 g WB	+36 g WB
Energy consumption, kcal/day/rat				
Period I	86 ± 1.8	83 ± 1.4	84 ± 1.6	84 ± 2.0
Period II[a]	86 ± 2.0	86 ± 2.3	80 ± 2.5	72 ± 2.5
Weight gain, g/day/rat				
Period I	5.1 ± 0.2	4.9 ± 0.3	5.0 ± 0.2	5.3 ± 0.2
Period II	2.6 ± 0.1	2.3 ± 0.1	2.3 ± 0.2	2.3 ± 0.1
Cholesterol consumption, g/day/rat				
Period I	0.18	0.17	0.18	0.18
Period II	0.18	0.19	0.18	0.18
Serum cholesterol, mg/dl				
Period I	134 ± 4[b]	136 ± 4	134 ± 5	134 ± 2
Period II	130 ± 8	130 ± 5	120 ± 8	132 ± 5
Wet fecal mass excretion, g/day/rat				
Period I	1.0 ± 0.7	2.1 ± 0.14*	2.5 ± 0.12*	4.6 ± 0.25*
Period II	0.9 ± 0.12	1.5 ± 0.15*	1.1 ± 0.11	1.1 ± 0.10
Dry fecal mass excretion, g/day/rat				
Period I	0.6 ± 0.05	1.3 ± 0.05*	1.7 ± 0.09*	3.2 ± 0.13*
Period II	0.6 ± 0.05	0.8 ± 0.04	0.7 ± 0.04	0.7 ± 0.04
Transit time, hr	68.0 ± 5.0	62.7 ± 0.7	57.3 ± 1.9*	21.5 ± 1.9*
Fecal bile acids, period I				
Excretion, mg/day/rat	11.0 ± 2.6	15.1 ± 1.4	13.7 ± 1.6	17.4 ± 5.0
Concentration, mg/g wet feces	10.9 ± 2.8	8.0 ± 1.5	5.5 ± 1.6*	4.0 ± 1.2*
Percent degradation[c]				
Feces, period I	44 ± 2	57 ± 4	63 ± 2*	73 ± 2*
In vitro	28 ± 12	49 ± 11	37 ± 9	46 ± 8
Feces, period II	54 ± 2	52 ± 2	52 ± 2	52 ± 2
In vitro	31 ± 7	27 ± 7	26 ± 2	27 ± 10
Bacterial count[d]				
Period I	7.6	7.6	4.3	4.7
Period II	5.0	6.7	7.2	8.6
Cecal weight				
Period I	3.2 ± 0.2	4.1 ± 0.4	4.2 ± 0.2	3.7 ± 0.6
Period II	4.2 ± 0.2	3.6 ± 0.2	3.5 ± 0.4	4.8 ± 0.1

[a]All rats fed control diet in period II.
[b]Means ± Standard error for 8–11 rats per dietary treatment.
[c]Percent degraded bile acids.
[d]Number of viable bacteria per gram of cecal contents × 10^{-10}.

bran concentration in the diet. Fecal bile acid degradation paralleled the concentration of bran in the diet and was inversely related to bile acid concentration in the feces. When the rats were fed the control diet, the difference in bile acid degradation and excretion disappeared. There was also an increase in bile acid degradation in the in vitro fermentations from cecal contents of bran fed rats; however, the increase in bile acid degradation with increased level of bran in the diet was not seen in the in vitro fermentations. Bile acid degradation in the feces and the in vitro fermentation could not be related to the number of anaerobic bacteria in the cecal contents as the number of anaerobic bacteria per gram of cecal contents was relatively constant as the concentration of bran in the diet increased. Since there was no difference in cecal weights between groups, it is likely that the number of anaerobic bacteria per cecum also decreased as the concentration of bran in the diet increased.

DISCUSSION

Contrary to the hypothesis that dietary fiber may have a hypocholesteremic effect, our data show that the inclusion of either corn or wheat bran in the diet did not lower serum cholesterol, at least in rats. This finding is in agreement with other workers (Arbanitakis et al 1977, Jenkins et al 1975, Tsai et al 1976). Instead, when the diet contained 30% fat (experiment I), wheat bran fed rats had elevated serum cholesterol concentrations. Forsythe et al (1978) also reported increased serum cholesterol concentrations for rats fed wheat bran. When dietary fat was decreased to 20%, however (experiment II), wheat bran in the diet had no affect on serum cholesterol concentrations. The second experiment was conducted to verify that the addition of wheat bran would increase serum cholesterol in rats as seen in experiment I, although wheat bran fed at three levels did not alter serum cholesterol concentrations compared with the control rats.

Since the serum cholesterol concentrations of the wheat bran fed rats (experiment I) were higher than either the control or corn bran fed rats, cholesterol absorption could be expected to be greater or bile acid excretion to be less for the rats fed wheat bran. However, cholesterol absorption was greatest for the control rats and similar for both the corn and the wheat bran fed groups, whereas bile acid excretion was the greatest for the corn bran group and similar for the wheat bran and control groups. Thus, neither bile acid excretion nor cholesterol absorption can explain the elevated serum cholesterol in the wheat bran group. One notable difference for the wheat bran fed group compared with all the other groups in experiments I and II is the poor growth for the wheat bran group even though energy consumption was similar for the control and wheat bran fed groups in experiment I. If cholesterol synthesis rates were different between groups, this could possibly account for the differences noted in serum cholesterol.

Another difference between experiments I and II is that female rats were used in experiment I, whereas male rats were used in experiment II. The effects of sex and growth rate on the difference in serum cholesterol noted for wheat bran fed animals in experiments I and II cannot be ascertained at this time. The lower bile acid excretion in experiment I compared with experiment II probably reflects the lower cholesterol intake (Tables II and III) and a lower average body weight in experiment I than in experiment II.

The greater stool mass and shorter transit time in bran fed rats agree with the results of other investigators (Arbanitakis et al 1977, Jenkins et al 1975, Payler et al 1975). Many biological metabolites in the colon are thought to be cytotoxic to colonic cells (Hill 1974). These cell toxins could be important agents that cause colonic cancer. A larger stool mass and a shorter intestinal transit time might be important factors in preventing colonic cancer. Increased stool volume may dilute toxic substances and a shorter transit time may reduce the duration for which the tissue is exposed to toxic substances. The fecal bile acid concentrations were lower in bran fed animals in experiment I and fecal bile acid concentrations were inversely proportional to bran concentrations in the diets in experiment II. Jenkins et al (1975) also showed an increased bile acid excretion and reduced fecal bile acid concentration in human subjects fed diets containing wheat fiber. If bile acids are carcinogenic or cocarcinogenic, then the reduced bile acid concentration in colonic contents would decrease the quantity of bile acids that comes in contact with the colonic cell surface.

Inclusion of fiber in the diet has been thought to influence the type and number of microflora in the large bowel (Burkitt 1971). A modification in bowel microflora could lead to an alteration in the rate and in the extent of cholic acid and chenodeoxycholic acid degradation to secondary bile acids such as deoxycholic, lithocholic, and corresponding ketone derivatives. Our study shows that the absolute number of anaerobic bacteria in cecal contents decreased with the inclusion of bran in the diet, but it is doubtful that this decrease has any physiological importance. In this type of work, a difference of more than one log in bacterial count is considered physiologically important (Chung et al 1977).

When rats were fed a 30% fat diet, bile acids were extensively degraded regardless of bran content of the diet (Table II). When the fat content of the diet was decreased to 20%, however, bile acid degradation was less extensive with no bran in the diet (Table III). But bile acid degradation increased as the bran concentration in the diet increased when the lower fat diets were fed. The trend of bile acid degradation was the same for feces and for in vitro fermentation studies. Therefore, it appears that total number of anaerobic bacteria may not be directly related to degradation of bile acids. An alteration in type of anaerobic bacteria present in the cecum could change the extent of bile acid degradation as seen in experiment II. Since bacterial concentrations and cecal weights were similar in rats fed all levels of bran and there was a decrease in transit time for bran fed rats, the increased bile acid degradation could be due to a change in intestinal microflora. However, alteration in metabolic rates of the microflora due to a change in intestinal ecology cannot be overlooked. A change in metabolic rates would not require a change in type of microflora to produce differences in bile acid degradation.

Secondary bile acids generally are believed more active carcinogens or cocarcinogens than primary bile acids. Many investigators have postulated that fiber in the diet will decrease the degradation of primary bile acids to secondary bile acids in colon and, therefore, decrease the incidence of colonic cancer (Pomare and Heaton 1973). Our data are contradictory to this hypothesis, at least with respect to the effects of corn and wheat bran fiber in rats.

If bran is helpful in reducing colonic cancer, the major beneficial effects will be: 1) dilution of carcinogens or cocarcinogens in colonic contents and 2) a decreased time that colonic cells are exposed to carcinogenic or cocarcinogenic agents.

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