[CANCER RESEARCH 37, 3533-3536, October 1977]

Effect of High-Risk Diets for Colon Carcinogenesis on Intestinal Mucosal and Bacterial β-Glucuronidase Activity in F344 Rats[1]

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SUMMARY

The effect of high-protein (beef or soybean protein) and high-fat (beef fat, corn oil, or lard) diets on large intestinal bacterial and intestinal mucosal β-glucuronidase was studied in female F344 rats maintained on these diets for two generations. Animals fed a 20% corn oil or 20% lard and 20% casein diet had a higher β-glucuronidase activity in the contents of cecum and colon than did rats fed a 5% corn oil or lard and 20% casein diet. The cecal bacterial β-glucuronidase activity was higher in animals fed diets with high levels of beef protein (40%) and beef fat (23%) or with high levels of soybean protein (39%) and corn oil (24%) than it was in rats fed diets containing 18.5% beef protein and 6.5% beef fat or 19% soybean protein and 5.4% corn oil. Animals fed diets containing high levels of beef protein and fat or high levels of soybean protein and corn oil had a higher small intestinal mucosal β-glucuronidase activity than did the other groups. No significant difference was observed in the colonic mucosal β-glucuronidase activity among the animals fed beef and soybean diets. It is concluded that diets high in fat and high or normal in protein are associated with elevated levels of bacterial β-glucuronidase activity in the large intestine of rats.

INTRODUCTION

Epidemiological data on the distribution of large bowel cancer and studies of migrants to the United States and of dietary habits of various population groups with different risks indicate that dietary factors, particularly high dietary fat and beef, are of major importance in the etiology of large bowel cancer (1, 2, 3, 7, 24). Although the effect of a diet high in fat and beef in the etiology of large bowel cancer is unclear, the following hypothesis on the etiology of colon cancer has been suggested. (*a*) Dietary fat changes the concentration of bile acids and cholesterol metabolites and also the concentration and metabolic activity of the bacteria in the colon, which may produce tumorigenic compounds from bile acids and cholesterol metabolites. (*b*) Conversion of cholesterol and dehydrocholesterol, which are normally present in colonic contents and mucosa, to reactive metabolites that act as carcinogens may be an important step in colon carcinogenesis. (*c*) Diet also influences mixed-function oxidases that could play a role in modifying colon carcinogenesis (9, 20, 23, 25).

It has been apparent in recent years that studies on the effect of diet on the composition of fecal bacterial flora have produced contradictory results (4, 8, 9, 11, 17). However, the overall metabolic activity of the intestinal bacteria rather than the actual numbers or types of bacteria in the intestinal tract may be important in evaluating the relationship between diet and the intestinal flora (6, 15, 18). Since the intestinal bacteria contain many inducible enzymes, the metabolic activity of the bacteria can be modified appreciably by diet or other environmental factors. We have investigated the effects of a high-fat, high-meat, mixed Western diet and of a balanced non-meat, low-fat diet on the metabolic activity of intestinal bacteria by use of bacterial β-glucuronidase as an inducible enzyme, since this enzyme is not only associated with many components of the gut bacteria but is also necessary to release active metabolites from their glucuronide conjugates in the lower gut (6, 15, 18). Intestinal microflora of subjects on a high-fat, high-meat diet were more able to hydrolyze glucuronide conjugates than were those of individuals on a non-meat diet (15, 18).

In the animal models it was found that rats fed a high-fat diet and treated with 1,2-dimethylhydrazine or azoxymethane had a higher incidence of colon tumors than did rats fed a low-fat diet (12, 13). Similarly, rats fed diets containing high levels of beef protein and fat or high levels of soybean protein and corn oil had a greater incidence of colon tumors than did rats fed diets with normal levels of such components and treated similarly (14).

Since high intake of fat and meat, which are linked as causative factors in colon carcinogenesis in humans, has been shown to modify the fecal bacterial activity, we extended our studies to investigate the effect of high-protein (beef or soybean protein) and high-fat (beef fat or corn oil) diets on cecal bacterial and small and large intestinal mucosal β-glucuronidase activity in rats. The present study also describes the effect of a diet high in lard or corn oil on cecal and colonic bacterial β-glucuronidase activity in rats.

MATERIALS AND METHODS

Inbred weanling male and female F344 rats obtained from Charles River Breeding Laboratories, Wilmington, Mass.,

[1] Supported by USPHS Contract CP-33208 from the National Cancer Institute and the Grant CA-16382 from the National Cancer Institute through the Large Bowel Cancer Project.

Received April 11, 1977; accepted June 30, 1977.

were randomly divided into 4 groups and housed in plastic cages with filter tops in a temperature- and humidity-controlled room. They were fed *ad libitum* one of the diets containing corn oil or lard at 5 or 20% level (Table 1). In a 2nd series, weanling male and female F344 rats were randomly divided into 4 groups and fed *ad libitum* with one of the diets (D_1, D_2, D_3, and D_4) with varying amounts of protein and fat (Table 2). Beef and soybean protein, beef fat, and corn oil were the sources of dietary protein and fat. The diets were analyzed for protein and fat and contained on the average the following: D_1, 39% protein and 25% fat; D_2, 19% protein and 5.4% fat; D_3, 40% protein and 23% fat; D_4, 18.5% protein and 6.5% fat.

At puberty female rats were mated with males and reared on their respective experimental diets. The litter size from each mother was reduced to 8, and rats were weaned to the same experimental diets consumed by their mothers.

At 30 weeks of age, all 2nd-generation female rats were killed under ether anesthesia. The cecum or colon was excised immediately and, after the end was tied with a string, slit open under anerobic conditions. The contents were transferred quantitatively and frozen at −40° until analysis for bacterial β-glucuronidase activity. The small intestine was also excised immediately, freed of adhering material, and slit open with scissors. The small intestine and colon were washed with ice-cold 0.9% NaCl solution, blotted gently to remove mucus and other adhering intestinal contents, and used for the analysis of mucosal β-glucuronidase activity.

All preparative procedures for the enzyme determination were carried out in a cold room at 4°. All determinations were performed in duplicate from each sample.

For the determination of enzyme activity from the cecal and colonic contents, a portion of the well-mixed sample was diluted with phosphate buffer (pH 7.0) and centrifuged at 100 × *g* for 15 min at 4° to remove undigested food particles and other coarse materials. The supernatant, which contained bacteria, was sonicated for 1 min at 0° and centrifuged at 10,000 × *g* for 30 min at 4° in an ultracentrifuge. Bacterial β-glucuronidase activity in the supernatant fraction was assayed as described (5, 15, 18). For the methods described below, the rates of reaction were linear with respect to time, substrate used, and amount of supernatant protein in the reaction mixture. The reaction mixture containing 0.1 ml of supernatant fraction, 0.1 ml of 0.1 M phenolphthalein glucuronide (pH 7.0), and 0.8 ml of 0.1 M phosphate buffer (pH 7.0) was incubated for 4 hr at 38° in a constant-temperature water bath. The reaction was terminated by adding 2.5 ml of 0.1 M alkaline glycine solution, 1 ml of 5% TCA,[2] and 1.5 ml of distilled water. Alkaline glycine solution was made so that a mixture of 2.5 ml of this solution, 1 ml of 5% TCA, 1 ml of phosphate buffer, and 1.5 ml of distilled water had a pH between 10.2 and 10.4 (5). The phenolphthalein liberated was measured at 540 nm in a spectrophotometer.

For the determination of enzyme activity in the small intestine and colon, the tissues were homogenized separately in 1.15% ice-cold KCl and centrifuged at 10,000 × *g*

[2] The abbreviation used is: TCA, trichloroacetic acid.

Table 1
Percentage composition of diets

	% composition of diets			
	Corn oil		Lard	
Ingredients	5%	20%	5%	20%
Casein, vitamin-free	20	20	20	20
DL-Methionine	0.3	0.3	0.3	0.3
Sucrose	58.7	43.7	58.7	43.7
Corn oil	5	20		
Lard			5	20
Alphacel	5	5	5	5
Salt mixture (20)	7	7	7	7
Vitamin mixture[a]	4	4	4	4

[a] The vitamin diet fortification mixture was obtained commercially from ICN Pharmaceuticals Inc., Cleveland, Ohio.

Table 2
Percentage composition of diets

	% composition of diets			
Ingredients	D_1	D_2	D_3	D_4
Beef hamburger (freeze-dried)			60.3	25.0
Soybean protein	40.0	20.0		
Corn oil	25.0	5.0		
Beef tallow			5.0	
Lysine	0.3	0.3		
DL-Methionine	0.3	0.3		
Corn starch	22.9	62.9	23.5	63.5
Alphacel	5.0	5.0	5.0	5.0
Salt mixture (Ref. 20)	4.5	4.5	4.5	4.5
Vitamin mixture[a]	2.0	2.0	2.0	2.0

[a] See Table 1, Footnote *a*.

for 30 min at 4°. Mammalian β-glucuronidase activity in the supernatant fraction was measured as described above for the cecal contents except that the mammalian enzyme had a pH optimum of 4.5. The reaction mixture containing 0.1 ml of supernatant fraction, 0.8 ml of 0.1 M acetate buffer (pH 4.5), and 0.1 ml of 0.01 M phenolphthalein glucuronide (pH 4.5) was incubated for 1 hr at 38°. The reaction was stopped by adding 1 ml of 5% TCA solution, 2.5 ml of 0.1 M alkaline glycine solution, and 1.5 ml of distilled water.

The protein in the samples was determined by the method of Lowry *et al.* (10). Results of each experiment were analyzed statistically with the Student's *t* test.

RESULTS

Table 3 summarizes the activity of bacterial β-glucuronidase in the contents of cecum and colon of rats fed semipurified diets (Table 1) containing corn oil or lard at 5 or 20% levels. Animals fed a 20% corn oil or a 20% lard diet had a higher β-glucuronidase activity in the contents of cecum and colon than did rats fed a 5% corn oil or 5% lard diet. The type of fat (corn oil *versus* lard) had no major influence on the β-glucuronidase activity in the cecum and colon.

Table 4 summarizes the β-glucuronidase activity in the cecal contents as well as in the mucosa of small intestine and colon of rats fed semipurified diets (Table 2) containing high or normal levels of beef or soybean protein or high or normal levels of beef fat or corn oil. No difference was noted

Table 3

Bacterial β-glucuronidase activity[a] in the contents of cecum and colon of rats fed high- or low-fat diets

Diet[b]	Cecum		Colon	
	Activity/mg protein	Activity/mg dry sample	Activity/mg protein	Activity/mg dry sample
20% corn oil	1860 ± 304[c, d]	95 ± 10[d]	1680 ± 205[d]	75 ± 9[d]
5% corn oil	880 ± 140	55 ± 8	945 ± 180	40 ± 5
20% lard	1708 ± 145[e]	110 ± 11[e]	1465 ± 95[e]	88 ± 9[e]
5% lard	1040 ± 100	40 ± 5	825 ± 65	45 ± 5

[a] μg phenolphthalein liberated per 4 hr at 38°.
[b] Number of rats/diet, 20.
[c] Mean ± S.E.
[d] Significantly different from 5% corn oil group ($p \leq 0.01$).
[e] Significantly different from 5% lard group ($p \leq 0.001$).

Table 4

Cecal bacterial and intestinal mucosal β-glucuronidase activity in rats fed diets high in protein and fat of vegetable and animal origin

Diet	No. of rats	Mucosal activity[a]/mg protein		Cecal bacterial contents	
		Small intestine	Colon	Activity[b]/mg protein	Activity[b]/mg dry contents
D_1	20	86 ± 6.7[c, d]	69 ± 4.3	2260 ± 406[e]	91 ± 10.9[e]
D_2	20	66 ± 3.4	71 ± 3.3	1010 ± 177	48 ± 10.9
D_3	18	81 ± 4.6[d]	68 ± 5.0	2050 ± 250[f]	122 ± 17.7[f]
D_4	25	58 ± 4.8	62 ± 4.5	1179 ± 94	50 ± 6.4

[a] μg phenolphthalein liberated per 1 hr at 38°.
[b] μg phenolphthalein liberated per 4 hr at 38°.
[c] Mean ± S.E.
[d] Difference between D_1 and D_2 or D_3 and D_4 is significant ($p \leq 0.05$).
[e] Significantly different from D_2 ($p \leq 0.01$).
[f] Significantly different from D_4 ($p \leq 0.001$).

in the colonic mucosal β-glucuronidase activity among the animals fed different diets. However, animals fed diets containing high levels of beef protein and fat or high levels of soybean protein and corn oil had a significantly higher enzyme activity in the mucosa of small intestine than did the other groups. The cecal bacterial β-glucuronidase activity was significantly higher in animals fed diets with a high content of beef protein and fat or a high amount of soybean protein and corn oil than it was in rats fed normal amounts of such components.

DISCUSSION

The aim of this investigation was to delineate the effects of type and amount of dietary fat and protein on the metabolic activity of intestinal microflora to understand the relationship of colon cancer to the type and amount of dietary fat and protein and to diet-mediated changes in the intestinal bacteria. Our recent results (14) indicate that rats fed a 20% corn oil or a 20% lard diet or rats fed diets containing high levels of beef and fat or high levels of soybean protein and corn oil and treated with 1,2-dimethylhydrazine had a higher incidence of colon tumors than did rats fed diets that had normal levels of such components and were treated similarly. The type of fat had no major influence on the incidence of colon tumors. Biliary excretion of total bile acids as well as cholic acid, β-muricholic acid, ursodeoxycholic acid, deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid was higher in rats fed a high-fat diet than it was in rats fed a low-fat diet (16).

The results of this investigation indicate that dietary corn oil or lard at 20% level and high dietary beef protein (40%) and beef fat (20%) or high dietary soybean protein (40%) and corn oil (20%) markedly increased the activity of bacterial β-glucuronidase of the cecal and colonic contents in rats. This would suggest that high dietary fat or high protein and fat increases the metabolic activity of the large bowel microflora. Weinstein *et al.* (21) showed that a change from a grain diet to a beef diet produces alterations in the types and concentration of bacteria in the colon of rats. Goldin and Gorbach (6) reported that rats fed a meat diet had higher levels of fecal bacterial β-glucuronidase activity than did grain-fed rats. Their data also suggested that the increase in β-glucuronidase activity was not simply an induction of enzyme synthesis but was rather a change in the composition of the flora. The present investigation confirms our previous studies in humans (15, 18) as well as the studies of Goldin and Gorbach (6) and extends the observation that not only a meat diet but also a high-fat diet or high-protein, high-fat diet changes the bacterial β-glucuronidase activity in the large intestine, which is indicative of the metabolic activity of the large bowel flora.

Results of the present study also indicate that feeding of high-fat and high-protein diets to rats had an effect on the small intestinal mucosal β-glucuronidase activity; however, no effect was observed on colonic mucosal enzyme activity.

Additionally, one should also consider the effect of caloric intake on β-glucuronidase activity in the contents of large bowel and in the mucosa of small intestine. In our study the caloric density of Diets D_1 and D_3 was higher than that of Diets D_2 and D_4 (Table 2); also diets with 20% corn oil or lard had a higher caloric density than did diets with 5% corn oil or lard (Table 1). Consequently, the caloric consumption for those fed a low-fat diet was probably reduced in relation to those fed a high-fat diet. In the present study, although variations in caloric consumption of different groups of animals may not exert an influence on β-glucuronidase activity in the contents of large bowel, this factor cannot be discounted from the available data; however, more information is needed.

Glucuronide formation is a major detoxification mechanism in mammals. Many exogenous compounds that are excreted in the bile as glucuronide conjugates are deconjugated by bacterial β-glucuronidase and modified further by intestinal bacteria in the large bowel (22). Since the intestinal microflora are changed by diet, these changes might alter the biological activity, toxicity, excretion, and reabsorption of many of the endogenous and exogenous compounds such as carcinogen and/or cocarcinogen metabolites. Since the microflora have high metabolic potential in populations on a high-fat, high-meat, mixed Western diet, these reactions including the release of tumorigenic or other toxic components would more probably occur in the intestine of populations on a high-fat, high-meat diet.

ACKNOWLEDGMENTS

The expert technical assistance of Yves-Marie Louis and Kathy Highland is gratefully appreciated.

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Cancer Res 1977;37:3533-3536.