Dietary Carbohydrates Modify Azoxymethane-Induced Intestinal Carcinogenesis in Rats[1,2]

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ABSTRACT The effect of different dietary carbohydrates (sucrose, cornstarch and high amylose cornstarch) on intestinal carcinogenesis was studied in male Sprague-Dawley rats treated subcutaneously with azoxymethane (AOM) at a weekly dose of 8 mg/kg body wt for 8 wk. The diets, high in fat and low in calcium and fiber, were fed during and after AOM treatment. The number of colonic adenomas per rat in the groups fed either starch was lower ($P < 0.05$) than the number in the sucrose-fed rats [1.06 ± 0.38, 0.30 ± 0.10 and 0.41 ± 0.22 (means ± SEM), in the sucrose-, cornstarch- and high amylose cornstarch-fed groups, respectively]. The incidence of total intestinal tumors (adenomas + adenocarcinomas) was not affected by dietary treatment. However, the incidence of tumors in the small intestine of the rats fed the two cornstarch diets tended to be slightly lower than for rats fed the sucrose diet ($P = 0.075$). Adenoma dysplasia and adenocarcinoma differentiation were similar among the rats fed the three diets. However, the adenocarcinomas in the rats fed the cornstarch diet were significantly smaller than those in the rats fed sucrose [0.99 ± 0.14 cm^2 ($n = 13$), 0.56 ± 0.14 cm^2 ($n = 13$) and 0.55 ± 0.17 cm^2 ($n = 9$) in rats fed the sucrose, cornstarch and high amylose starch diets, respectively]. Moreover, in the rats fed the cornstarch diet, the adenocarcinomas showed lower invasive potential than those in rats fed the sucrose diet. The results suggest an overall inhibition of AOM-induced carcinogenesis in rats fed the cornstarch diets. J. Nutr. 124: 517–523, 1994.

INDEXING KEY WORDS:

- *diet* • *sucrose* • *starch*
- *rats* • *colon carcinogenesis*

Dietary habits affect the risk of colon cancer in both humans and experimental animals (Bruce 1987, Reddy 1983, Willett 1989). Dietary fat and refined sugar consumption have been associated with increased risk (Bristol et al. 1985, Willett et al. 1990), whereas complex carbohydrates, such as fibers and starches, have been proposed as protective factors (Bingham 1990, Jenkins et al. 1986a, Willett 1989).

We demonstrated in previous investigations that rodents fed high starch diets, compared with animals fed high sucrose diets, have lower cell proliferation of colonocytes, as assessed by labeling index after [^{3}H]thymidine incorporation (Caderni et al. 1988, 1989 and 1991a). We also showed that high starch-fed rats have a higher lumenal concentration of butyrate and other short-chain fatty acids in their cecal contents and a lower concentration of fecal bile acids compared with rats fed a high sucrose diet (Bianchini et al. 1992, Caderni et al. 1993). Moreover, we found that high starch diets reduce the growth of carcinogen-induced aberrant crypts in the colon (Caderni et al. 1991b).

Proliferative activity in colonocytes has been positively correlated with the risk of cancer in the colon, whereas increased production of butyrate and a decreased concentration of fecal bile acids have been proposed as protective factors (Bianchini et al. 1992, Lipkin 1988, Reddy 1983).

On the basis of these results, we wanted to verify whether intestinal carcinogenesis in rats caused by azoxymethane (AOM) might be affected by changes in the dietary levels of starch or sucrose.

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Utilizing the model of AOM-induced intestinal carcinogenesis we studied the effects of three different diets. Carbohydrates were supplied in the first diet by sucrose, in the second diet by cornstarch, and in the third diet by cornstarch in part and by a variety of cornstarch with a high amylose content. In the animals fed the last type of starch, given its lower digestibility, we expected that a greater quantity of starch would reach the colon and be available for fermentation. It has been suggested in fact that some of the effects of starch on the colon might be modulated by the digestibility of starch in different sections of the intestinal tract, and some authors have hypothesized that a higher amount of starch reaching the colon could be a protective factor against the development of colon cancer (Asp et al. 1987, Bartram et al. 1991, Thornton et al. 1988).

MATERIALS AND METHODS

Animals. We purchased male Sprague-Dawley rats weighing ~100 g (Nossan, Correzzana, Milan, Italy). After their arrival from the supplier, animals were housed in plastic cages with wire tops and bottoms and quarantined for 2 wk, during which time they were fed a standard nonpurified diet (Morini Inc., Reggio Emilia, Italy). The animals were afterwards divided into three groups of 25 rats each and fed the three experimental diets described below. The rats were maintained at a constant environmental temperature of 22°C, with an approximate 12-h light:dark cycle, according to internationally accepted ethical guidelines for the treatment of experimental animals (European Community Regulations on the Care and Use of Laboratory Animals, 1986, Law 86/609/EC). The rats were weighed weekly during the course of the experiment.

Dietary and carcinogen treatment. Animals were given free access to one of three different diets. Carbohydrates were supplied in one diet (sucrose diet), by sucrose (460 g/kg diet), in the second diet (the cornstarch diet) by "Globzeta" cornstarch (460 g/kg diet), and in the third diet (the high amylose cornstarch diet) by "Globzeta" cornstarch (230 g/kg diet) and "Hylon 7" cornstarch (230 g/kg diet) (for details on the formulation of the other dietary components, see Bianchini et al. 1992). All three diets were modifications of the AIN-76 purified diet (AIN 1977), varied as previously described (Bianchini et al. 1992, Caderni et al. 1991b) in order to contain a high level of fat (231 g corn oil/kg diet) and a high level of casein, vitamins and minerals, to maintain these nutrients at densities similar to those in the original AIN-76 diet. These diets also contained a low level of calcium (1 g/kg), at a concentration similar to that encountered in some Western diets, and a low amount of fiber in the form of cellulose (20 g/kg). We wanted to reproduce dietary conditions similar to those of Western populations that consume high fat, low calcium, low cellulose diets and that have a high incidence of colon cancer. The "Globzeta" starch used in the cornstarch diet is a standard cornstarch containing 30% amylose and 70% amylopectin, whereas in the high amylose cornstarch diet 50% of the "Globzeta" starch was replaced with "Hylon 7" starch, a modified cornstarch containing 70% amylose and 30% amylopectin. Therefore, the final concentrations of amylopectin and amylose were 322 and 138 g/kg, respectively, in the cornstarch diet and 230 and 230 g/kg in the high amylose cornstarch diet. All diet components were purchased from Piccioni Inc. (Gessate, Milan, Italy), except for "Hylon 7" starch (purchased from National Starch, Como, Italy).

After 1 wk of consuming the experimental diets, rats were treated subcutaneously with AOM (Sigma Chimica, Milan, Italy) at a weekly dose of 8 mg/kg body wt for 8 wk, following the protocol described by Bull et al. (1979). Each dietary group contained 20 AOM-treated animals and 5 saline-treated controls.

All diets were fed during and after AOM treatment. Between the 22nd and the 23rd weeks after the first treatment with AOM, animals were lightly anesthetized with ether and killed by decapitation.

All organs including intestines were then macroscopically examined for the presence of tumors or other pathological lesions. Tissues showing a deviation from normal morphology were fixed in 10% buffered formalin and embedded in paraffin blocks. Blocks were then sectioned and stained with hematoxylin-eosin to confirm the presence and type of tumors by histopathological examination, performed by a pathologist (A.G.) unaware of the coding of the specimens. Before embedding in paraffin blocks, suspected macroscopic lesions were measured using calipers to ascertain their dimensions by multiplying the two main diameters of each lesion.

Histopathological evaluation of the tumors. Cancer histological types were evaluated on the basis of histotype, grading and pattern of growth (Jass et al. 1986, Morson et al. 1992). Adenomas were classified on the basis of their microscopic architecture as tubular, tubulovillous and villous (Morson and Sobin 1976). The features used for grading adenomatous dysplasia were tissue architecture, nuclear alterations and cytoplasmic differentiation according to the method of Konishi and Morson (1982).

Statistical evaluation of data. The effects of the different diets on the body weight of saline- and AOM-treated animals were assessed by two-way ANOVA, considering both the effects of both diet and AOM treatment. The effects of the diets on tumor dimensions were assessed by one-way ANOVA. The significance of the pair-wise differences between rats fed each starch diet and those fed the sucrose diet, taken as the reference group, was evaluated by the Duncan test for multiple comparisons (Duncan 1955).

All animals were classified by dietary group and presence or absence of tumor. The contingency tables obtained for all tumors, classified separately by site (colon or small intestine) and by histopathology (adenomas or adenocarcinomas), were analyzed with the Pearson chi-square and likelihood ratio test (Agresti 1990). However, we were also interested in comparing the total number of tumors observed in each dietary group. To account for the slight imbalance in the number of animals per group (caused by deaths during the experiment), we corrected the experimental data using weighting factors based on the average number of animals at risk. The weighted contingency table (dietary group separated by tumor site or morphology) was analyzed using a Poisson regression model (Frome et al. 1973). The differences between rats fed the cornstarch diet and rats fed the high amylose cornstarch diet compared with those fed the sucrose diet were evaluated by the likelihood ratio test and Wald test for single treatment comparison. The Wald test is an approximate likelihood ratio test for a single parameter value (McCullagh and Nelder 1989).

The degree of dysplasia of the adenomas, grade of differentiation and extent of invasion of adenocarcinomas were analyzed by independently comparing the proportion of tumors in rats fed the cornstarch or the high amylose cornstarch diet to that in rats fed the sucrose diet. An exact test for trend was performed using the Statxact statistical package (Cytel, Cambridge, MA) (Armitage 1955). Values in the text are means ± SEM.

RESULTS

General observations. Some rats died during the experiment (two rats fed the sucrose diet, one fed the cornstarch diet and four fed the high amylose cornstarch diet). These animals died of pneumonia, and no other pathological findings were found during autopsy. These rats were excluded from the effective number of each dietary group. All the other animals, although apparently not infected, received two preventive injections of diaminocilline (60,000 IU/kg, intramuscularly) ~2 mo after the beginning of AOM treatment. The rats were weighed weekly during the experiment. The body weight of the AOM-treated rats at the end of AOM treatment (8 wk after the first carcinogen treatment) was significantly lower ($P < 0.01$) than that of saline-treated animals in all three experimental groups (saline-treated rats weighed 436 ± 19, 414 ± 27 and 417 ± 17 g and AOM-treated animals weighed 377 ± 12, 374 ± 8 and 378 ± 8 g in the groups fed diets containing sucrose, cornstarch and high amylose cornstarch, respectively). However, at the end of the experiment (22 wk after the first AOM injection) the weights of saline- and AOM-treated animals fed all diets were comparable (saline-treated rats weighed 588 ± 29, 577 ± 27 and 575 ± 22 g and AOM-treated animals weighed 562 ± 18, 559 ± 12 and 534 ± 14 g in the dietary groups fed sucrose, cornstarch and high amylose cornstarch, respectively).

Animals were killed 22–23 wk after the first AOM injection, when a certain number of them showed rectal bleeding.

Tumor induction. All tumors were found in the colon and small intestine and were classified as cancers of adenomas according to the histopathological criteria described in Materials and Methods. Because most cancers observed in the three groups of rats were adenocarcinomas (33 in total), with only one mucinous adenocarcinoma and two undifferentiated carcinomas observed, all cancers were considered adenocarcinomas. All the adenomas observed were of tubular type.

The incidence of colonic tumors (both adenomas and adenocarcinomas) was similar among the three dietary groups (**Table 1**). In the small intestine, the incidence of total tumors (adenomas + adenocarcinomas) in the rats fed the two diets containing cornstarch was slightly lower than in the rats fed the diet containing sucrose, although this effect was not statistically significant (using the likelihood ratio test, this difference showed a borderline significance, $P = 0.078$).

The results relative to the number of tumors per rat in each dietary group (**Table 2**) indicated that the animals fed the cornstarch and the high amylose cornstarch diets both had fewer colonic and total intestinal adenomas (defined as the sum of colon and small intestine adenomas) than did the rats fed the sucrose diet ($P < 0.05$). Although we observed fewer adenocarcinomas in the small intestine of the rats fed the two cornstarch diets compared with the rats fed

TABLE 1

Incidence of intestinal tumors in azoxymethane-treated rats fed three different diets containing sucrose, cornstarch and high amylose cornstarch as carbohydrate sources[1]

	Colon			Small intestine		
Dietary group	Ad	K	Total	Ad	K	Total
	%					
Sucrose (18)	44	44	67	6	22	28
Cornstarch (20)	30	40	60	0	5	5*
High amylose cornstarch (16)	25	44	62	0	6	6*

[1]Values are expressed as the percentage of animals with tumors in each dietary group. Numbers in parentheses under the diets are the number of animals in each dietary group. Ad = adenomas, K = adenocarcinomas, total tumors = Ad + K. *Likelihood ratio test, chi-square (2 df) = 5.11; $P = 0.078$, as compared with the sucrose dietary group.

TABLE 2

Number of tumors per rat in azoxymethane-treated rats fed three different diets containing sucrose, cornstarch and high amylose cornstarch as carbohydrate sources[1]

	Colon			Small intestine			Colon + small intestine		
Dietary group	Ad	K	Total	Ad	K	Total	Ad	K	Total
Sucrose	1.06	0.50	1.56	0.06	0.22	0.28	1.11	0.72	1.83
	(0.38)	(0.15)	(0.43)	(0.06)	(0.18)	(0.18)	(0.38)	(0.18)	(0.45)
Cornstarch	0.30*	0.65	0.95	0	0.05	0.05	0.30*	0.70	1.00
	(0.10)	(0.23)	(0.25)		(0.05)	(0.05)	(0.10)	(0.24)	(0.25)
High amylose cornstarch	0.41*	0.50	0.94	0	0.06	0.06	0.44*	0.56	1.00
	(0.22)	(0.16)	(0.23)		(0.06)	(0.06)	(0.22)	(0.18)	(0.24)

[1]Values are means, with SE in parentheses. Ad = adenomas, K = adenocarcinomas, total tumors = Ad + K. *Significantly different from the sucrose dietary group by Wald test ($P < 0.05$).

the sucrose diet (Table 2), the number of adenocarcinomas was not significantly different among the three dietary groups ($P = 0.14$ for the difference between sucrose- and cornstarch-fed rats and $P = 0.22$ for the difference between the sucrose- and high amylose cornstarch-fed rats). Moreover, no adenomas were found in the small intestine of rats fed the cornstarch diets.

The data relative to the number of tumors per tumor-bearing rat in the colon and small intestine showed a similar trend, although the effect of the starch diets was less marked.

Tumor site within the colon (distal vs. proximal) was similar among the three dietary groups (data not shown).

The grade of adenoma dysplasia (**Table 3**) in the entire intestine was similar among the three dietary groups (test for trend, $P = 0.25$), although in the animals fed the cornstarch diet very few adenomas showed a severe dysplasia. The dimensions of the adenomas were also comparable in the three dietary groups (0.20 ± 0.05, 0.22 ± 0.12 and 0.27 ± 0.06 cm^2 in rats fed the sucrose, cornstarch and high amylose cornstarch diets, respectively).

We also graded intestinal adenocarcinomas, but the differences in grading among the three diet groups were not significant.

The results relative to the grade of invasion of adenocarcinomas (intramuccosal, submucosal and intramuscular) indicated that in rats fed the cornstarch diet, the adenocarcinomas showed an extent of invasion significantly lower than that found in the rats fed the sucrose diet (**Table 4**). In fact, we found fewer intramuscular adenocarcinomas in rats fed the cornstarch diet, and the trend for the extent of invasion (from intramucosal to intramuscular invasion) was significantly different in rats fed the cornstarch diet compared with rats fed the sucrose diet. We also found that the trend for the extent of invasion in rats fed the high amylose cornstarch diet was similar to that observed in rats fed the sucrose diet.

The results also indicated (Table 4) that the adenocarcinomas in rats fed the cornstarch diet were significantly smaller than those observed in rats fed the sucrose diet. Adenocarcinomas in the rats fed the high amylose cornstarch diet also tended to be smaller than those found in the rats fed the sucrose diet, but the difference, which was quantitatively similar to that found in the cornstarch-fed group, was not statistically significant, due to the slight imbalance in group size. In fact, we observed 13 adenocarcinomas in both the rats fed the sucrose diet and those fed the cornstarch diet but only nine adenocarcinomas in the rats fed the high amylose cornstarch diet.

DISCUSSION

We described how different dietary carbohydrates (sucrose, cornstarch and high amylose cornstarch) affect AOM intestinal carcinogenesis in rats. The number of colonic adenomas per rat in the two groups fed cornstarch was significantly lower than in rats fed

TABLE 3

Grade of adenoma dysplasia in azoxymethane-treated rats fed three different diets containing sucrose, cornstarch and high amylose cornstarch as carbohydrate sources[1]

	Dysplasia		
Dietary group	Mild	Moderate	Severe
Sucrose	7/20 (35%)	5/20 (25%)	8/20 (40%)
Cornstarch	3/6 (50%)	2/6 (33%)	1/6 (17%)
High amylose cornstarch	1/7 (14%)	2/7 (29%)	4/7 (57%)

[1]Values are the number of adenomas in each grade of dysplasia divided by the total adenomas; these values are expressed in parentheses as percentages.

TABLE 4

Extent of adenocarcinoma invasion and dimensions of adenocarcinomas in azoxymethane-treated rats fed three different diets containing sucrose, cornstarch or high amylose cornstarch as carbohydrate sources[1]

Dietary group	Invasion			Dimensions[2]
	Intramucosal	Submucosal	Intramuscular	
				cm^2
Sucrose	0	7/12 (58%)	5/12 (42%)	0.99 ± 0.14
Cornstarch†	3/14 (24%)	9/14 (64%)	2/14 (14%)	0.56 ± 0.14*
High amylose cornstarch	0	6/9 (67%)	3/9 (33%)	0.55 ± 0.17

[1]Values are the number of adenocarcinomas in each category of invasion divided by the total adenocarcinomas in that group; these values are expressed in parentheses as percentages. †Trend test, $P < 0.05$, compared with rats fed the sucrose diet.

[2]Values are means ± SEM. *Significantly different ($P < 0.05$) from values for rats fed the sucrose diet by one-way ANOVA and Duncan's test. Values for rats fed high amylose cornstarch diet were not significantly different from values for the sucrose-fed rats because the number of adenocarcinomas in this group was smaller ($n = 9$) than in the cornstarch-fed group ($n = 13$).

sucrose. On the contrary, the incidence of total intestinal tumors (adenomas + adenocarcinomas) was not affected by the dietary variations, even though the incidence of tumors in the small intestine of the rats fed the two cornstarch diets was slightly lower, although not statistically significant ($P = 0.078$), when compared with the incidence for animals fed the sucrose diet.

We also found that the adenocarcinomas in the rats fed the cornstarch diet, and to a lesser extent the adenocarcinomas in the animals fed the high amylose cornstarch diet, were smaller than those of the rats fed the sucrose diet. Moreover, in rats fed the cornstarch diet, the adenocarcinomas had a significantly lower invasive potential than in those fed the sucrose diet.

Colon carcinogenesis is regarded as a multi-stage process in which colonic adenomas and colorectal cancer represent successive steps characterized by increasing dysplasia and the loss of cellular and architectural differentiation as the tumor proceeds along the carcinogenesis process (Chang 1984, Day 1984).

The observation that rats fed the two cornstarch diets had fewer adenomas compared with rats fed the sucrose diet suggests that cornstarch might have an inhibiting effect at some point in the process leading to colon cancer. We also found that the adenocarcinomas in the rats fed a "standard" cornstarch were smaller and had a lower invasive potential compared with those observed in sucrose-fed rats.

Using the same cornstarch with a low amylose content utilized in the present paper ("Globzeta" starch containing 30% amylose), we previously demonstrated that rats fed high starch diets, compared with rats fed high sucrose diets, have a lower proliferation in the colonocytes, a higher lumenal concentration in the cecum of short-chain fatty acids, and a lower concentration of fecal bile acids (Bianchini et al. 1992, Caderni et al. 1989, 1991a and 1993). We also demonstrated that high starch diets inhibit the growth of carcinogen-induced foci of aberrant crypts in the colon, suggesting that this type of starch is a protective factor in the early steps of colon carcinogenesis (Caderni et al. 1991b).

Some of the results obtained in the present paper using rats fed a cornstarch diet confirm this previous hypothesis, suggesting a protective role of cornstarch against colon carcinogenesis.

In the present study we also studied a cornstarch with a high amylose content (Hylon 7 starch, containing 70% amylose). It has been suggested, in fact, that some effects of starch on colon physiology and carcinogenesis may depend on the digestibility of the starch (Asp et al. 1987, Bartram et al. 1991, Thornton et al. 1988). Starch digestibility is itself affected by several factors, including amylose content. A high amylose content decreases the breakdown of starch in the small bowel and hence increases the amount of starch reaching the colon (Asp et al. 1987). A higher amount of starch in the colon, available for fermentation by the intestinal microflora, has been suggested to be in itself a protective factor against colon cancer (Bartram et al. 1991, Bingham 1990, Jenkins et al. 1986b, Thornton et al. 1988).

Some data obtained at the time of the killing of the same rats of the present study show that the cecal pH in the animals fed high amylose cornstarch was significantly lower than that found in the rats fed the cornstarch diet; the cecal pH of rats fed the cornstarch diet was also significantly lower than that in the rats fed the sucrose diet (values of cecal pH were 6.20 ± 0.12, 7.09 ± 0.03 and 7.34 ± 0.04 in rats fed the high amylose cornstarch, cornstarch and sucrose diets, respectively). Moreover, the concentration of short-chain fatty acids in the cecal content of the rats fed the high amylose cornstarch diet was higher than for both the rats fed the sucrose diet and those fed the

cornstarch diet (data not shown), suggesting that more fermentation occurs in the cecum of rats fed high amylose cornstarch.

Although some epidemiological studies have suggested that a lower pH, indicative of higher fermentation in the colon, is a protective factor against colon cancer, other studies have denied this hypothesis, showing that lower colonic pH increases colon proliferation and colon carcinogenesis (for a review, see Newmark and Lupton 1990).

The importance of higher levels of short-chain fatty acids in the colon is also controversial. Some authors deny their protective effects against carcinogenesis (Freeman 1986, Newmark and Lupton 1990).

The results of the present study do not support the hypothesis that higher fermentation in the colon is always associated with a low risk of colon cancer. In fact, the high amylose cornstarch diet was not more effective than the low amylose cornstarch diet.

It should also be noted that the high cornstarch diets used in the present study were sucrose-free. It has been recently reported that mice treated with oral boluses of sucrose, but not glucose, have higher proliferative activity in the colonic mucosa and are more sensitive to the induction by AOM of aberrant crypt foci, thus suggesting that sucrose in itself might enhance some of the early steps of colon carcinogenesis (Stamp et al. 1993). Therefore, the results observed in the present study might be explained as being due to the deleterious effect of sucrose on intestinal carcinogenesis rather than the protective effect of starch.

In conclusion, our results indicate that the composition of dietary carbohydrates affects colon carcinogenesis. Although some aspects of carcinogenesis were not varied in the animals fed cornstarch, our data suggest that by substituting starch for sucrose an inhibitory effect on some of the steps of intestinal carcinogenesis in rats can be obtained.

Given the importance of starch and sucrose in human diets, the definition of the effects of these nutrients on intestinal carcinogenesis deserves further attention.

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