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Effect of Meat (Beef, Chicken, and Bacon) on Rat Colon Carcinogenesis

Géraldine Parnaud, Ginette Peiffer, Sylviane Taché, and Denis E. Corpet

Abstract: High intake of red meat or processed meat is associated with increased risk of colon cancer. In contrast, consumption of white meat (chicken) is not associated with risk and might even reduce the occurrence of colorectal cancer. We speculated that a diet containing beef or bacon would increase and a diet containing chicken would decrease colon carcinogenesis in rats. One hundred female Fischer 344 rats were given a single injection of azoxymethane (20 mg/kg ip), then randomized to 10 different AIN-76-based diets. Five diets were adjusted to 14% fat and 23% protein and five other diets to 28% fat and 40% protein. Fat and protein were supplied by 1) lard and casein, 2) olive oil and casein, 3) beef, 4) chicken with skin, and 5) bacon. Meat diets contained 30% or 60% freeze-dried fried meat. The diets were given ad libitum for 100 days, then colon tumor promotion was assessed by the multiplicity of aberrant crypt foci [number of crypts per aberrant crypt focus (ACF)]. The ACF multiplicity was nearly the same in all groups, except bacon-fed rats, with no effect of fat and protein level or source ($p = 0.7$ between 8 groups by analysis of variance). In contrast, compared with lard- and casein-fed controls, the ACF multiplicity was reduced by 12% in rats fed a diet with 30% bacon and by 20% in rats fed a diet with 60% bacon ($p < 0.001$). The water intake was higher in bacon-fed rats than in controls ($p < 0.0001$). The concentrations of iron and bile acids in fecal water and total fatty acids in feces changed with diet, but there was no correlation between these concentrations and the ACF multiplicity. Thus the hypothesis that colonic iron, bile acids, or total fatty acids can promote colon tumors is not supported by this study. The results suggest that, in rats, beef does not promote the growth of ACF and chicken does not protect against colon carcinogenesis. A bacon-based diet appears to protect against carcinogenesis, perhaps because bacon contains 5% NaCl and increased the rats' water intake.

Introduction

Colorectal cancer is the second most common cause of death from cancer in Western countries, exhibiting a >10-fold excess compared with rural populations in less affluent

countries (1). Diet is supposed to influence the colorectal cancer etiology, but the precise causative factors are unknown. International ecological studies show a strong correlation between meat consumption and the colorectal cancer incidence (2). Most case-control studies (22 of 29) show an increased risk for development of colorectal cancer for those eating high amounts of meat (reviewed in Reference 3). However, cohort studies of meat intake and colon cancer have been less consistent. For example, an increased risk of colon cancer with high meat intake was found in women and men cohorts (4,5). In contrast, using a similar questionnaire, Bostick and colleagues (6) did not find a significant association between meat intake and colon cancer in Iowa women. European prospective studies also show no significant association between meat intake and colon cancer (7-9). Otherwise, the high intake of processed meat is associated with colorectal cancer risk in two cohort studies (4,8). Two prospective studies also show that consumption of white meat or fish is not associated with risk and might even reduce the occurrence of colorectal cancer (4,5). Animal studies on meat have received little attention compared with epidemiological studies (3). Only seven experimental studies have been published on the effect of meat or meat fractions on the colon tumor incidence in rodents initiated with chemical carcinogens (10-16). Data from these studies do not support the belief that red meat has a specific effect on intestinal carcinogenesis, except when it contains high levels of heterocyclic aromatic amines (16,17). The effect of white and processed meat on experimental colon cancer in rodents has not been studied.

The present study was designed to investigate the promoting effect of a diet high in red meat or processed meat and the protecting effect of white meat in the context of a high-fat diet. We used the rat-azoxymethane (AOM) model of experimental colon carcinogenesis. The study end point was the multiplicity of aberrant crypt foci (ACF) as a measure of ACF growth. We stopped the study after a feeding period of 100 days, which is adequate to quantify the promotion of ACF growth (18,19). Some ACF are dysplastic lesions of colonic mucosa thought to represent the earliest

precursors of colon cancer (19). We also quantified in feces the levels of specific components such as iron, fatty acids, and bile acids, because they might be meat-borne risk factors for the development of colon cancer. The results suggest that beef or chicken does not modulate the growth of ACF in rats. A bacon-based diet might protect rats against colorectal carcinogenesis, perhaps because bacon contains 5% NaCl and increased the water intake of rats.

Materials and Methods

Animals

Female Fischer 344 (F344) rats were obtained from Iffa-Credo (Lyon, France) at four weeks of age. One hundred animals were housed two rats per stainless steel wire drop-bottom cage at 22°C on a 12:12-hour light-dark cycle and were allowed free access to standard laboratory diet (UAR, Villemoisson, France) and water. After one week of acclimatization, the rats were initiated between 9 and 11 AM with a single injection of AOM (Sigma Chemical, St. Quentin, France; 20 mg/kg ip) in NaCl (9 g/l). Seven days later, they were randomly allocated to 10 groups ($n = 10$ in each group) and fed the experimental diets. Body weights were monitored weekly throughout the study, and food and water intakes were measured at periodic intervals. The animals were sacrificed 105–107 days after the carcinogen injection, 98–100 days after the start of the experimental diets, by cervical dislocation between 8 and 11 AM. The abdominal fat was excised and weighed. The colons were removed and fixed in formalin for ACF scoring.

Diets

One hundred F344 rats were randomized to groups after initiation and fed dry powdered diets based on a modified AIN-76 formula (UAR). Five groups received a relatively “low-fat” diet containing 14% fat and 23.5% protein. Five other groups received a very-high-fat diet containing 28% fat and 40% protein. Fat represented 32% of calories in the “low-fat” diet and 51.5% in the high-fat diet. These values are below and above, respectively, the average human intake in affluent countries (40%). Fat and protein were provided by dry powdered cooked meat, making 30% or 60% of the diet, olive oil (Carrefour) and vitamin-free casein, or lard and vitamin-free casein (UAR) (Table 1). Two sources of fat were used to make two control diets: olive oil and lard. Olive oil was chosen because it is “neutral” in colon carcinogenesis studies, because it does not enhance or reduce tumor incidence. Lard was chosen because its fatty acid composition is the same as that of bacon and is between beef and chicken. Beef (hamburger, Carrefour), chicken (with skin, Gastronomie), and bacon (Herta) were obtained from a local supermarket (Carrefour Purpan). The three types of meat were cooked in an oven for 15 minutes at 180–185°C. Each dish contained 500 g of 1-cm-thick meat. These cooking conditions may generate 1–15 ng/g of heterocyclic amines in beef, 15–65 ng/g in bacon, and 40 ng/g in chicken (17). After the meats were cooled, they were minced, frozen for 24 hours at –20°C, then freeze-dried. After the analysis of fat and protein contents in each type of meat (Table 1), meat diets were supplemented with casein to reach the 23.5% or 40% protein targets. The percentage of fat was adjusted with lard for beef diets and with chicken fat for chicken diets (Table 1).

Table 1. Composition of Experimental Diets^a

| | Low-Meat Diet | | | | | High-Meat Diet | | | | |
|----------------------|---------------|-----------|------|---------|-------|----------------|-----------|------|---------|-------|
| | Lard | Olive oil | Beef | Chicken | Bacon | Lard | Olive oil | Beef | Chicken | Bacon |
| Beef ^b | | | 30 | | | | | 60 | | |
| Chicken ^c | | | | 30 | | | | | 60 | |
| Bacon ^d | | | | | 30 | | | | | 60 |
| Casein | 23.5 | 23.5 | 5 | 7.3 | 10.2 | 40 | 40 | 3 | 7.6 | 13.9 |
| Lard | 14 | | 3.8 | | | 28 | | 7.6 | | |
| Olive oil | | 14 | | | | | 28 | | | |
| Chicken fat | | | | 0.6 | | | | | 1.2 | |
| Sucrose | 31.5 | 31.5 | 31.5 | 31.5 | 31.5 | 6 | 6 | 6 | 6 | 6 |
| Cornstarch | 17 | 17 | 17 | 17 | 17 | 10.2 | 10.2 | 10.2 | 10.2 | 10.2 |
| Cellulose | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 6.7 | 6.7 | 6.7 | 6.7 | 6.7 |
| Choline bitartrate | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Methionine | 0.34 | 0.34 | 0.34 | 0.34 | 0.34 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Corn oil | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| AIN-76 mineral mix | 4 | 4 | 4 | 4 | 4 | 4.68 | 4.68 | 4.68 | 4.68 | 4.68 |
| Vitamin mix | 1.13 | 1.13 | 1.13 | 1.13 | 1.13 | 1.33 | 1.33 | 1.33 | 1.33 | 1.33 |

a: Values are g/100 g diet. “Low-meat” diets contained 16.5% fat and 23.8% protein; “high-meat” diets contained 30.5% fat and 40.4% protein (including corn oil and methionine).

b: Freeze-dried cooked beef contained 34% fat and 61.7% protein.

c: Freeze-dried cooked chicken (with skin) contained 44.6% fat and 51.1% protein.

d: Freeze-dried cooked bacon contained 47.1% fat and 44.4% protein.

Assay of Bile Acids and Iron in Fecal Water

Fecal water preparation: Fecal water was prepared by reconstituting freeze-dried feces by adding 0.35 ml of distilled water to 1 g of freeze-dried feces (20). After they were homogenized, the samples were incubated for one hour at 37°C, then centrifuged for 10 minutes at 40,000 g. The supernatant was removed and stored at -20°C until use.

Assay of iron: Total iron in fecal water was measured using a colorimetric method based on the procedure of Persijn (21) (kit 565-C, Sigma Chemical).

Assay of bile acids: Bile acid concentration was determined using a fluorometric enzymatic assay based on the technique of Lapré and associates (20). All reagents for bile acid assay were obtained from Sigma Chemical (St. Quentin, France). The reaction mixture was made of 10 µl of appropriate dilution of fecal water (substrate source), 1,240 µl of 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 9), and 250 µl of the "3- α -Flu" solution (67 mM KH_2PO_4 , 55 mM NaOH, 4.4 mM sucrose, 1.8 mM NAD, 10 mM $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$, 0.13% bovine serum albumin, 714 U/l diaphorase, and 0.05 mM resazurin). To this reaction mixture, 10 µl of 3 α -hydroxysteroid dehydrogenase (enzyme, 2.5 U/ml) was added. After 15 minutes, the fluorescence was measured at 580 nm under a 565-nm excitation. The intensity of fluorescence was proportional to the bile acid concentration.

Fatty Acid Analysis of Fat in Feces and Food

Total fatty acid concentrations and profiles in feces and in cooked meat were determined according to the one-step extraction-transesterification procedure of Pritam and Palmquist (22). Fatty acids were analyzed on a gas-liquid chromatograph (model 1000, Dani) fitted with a peak simple chromatography data system (SRI Instruments), a split-splitless injector, and a flame ionization detector. Conditions were as follows: SP2340 fused silica capillary column (Supelco-Aldrich), temperature programmed from 74 to 80°C at 1°C/min, from 80 to 160°C at 8°C/min, and for 8 minutes at 160°C, then from 160 to 180°C at 4°C/min and 1 minute

at 180°C. Fatty acids were identified by their retention time and quantified in comparison with an internal C_{19} standard. The profiles of fatty acids in food are shown in Table 2. Lard and bacon showed similar fatty acid profiles with a high content of oleic acid. Rat feces contained more saturated fatty acids and less unsaturated fatty acids than were found in food; e.g., lard contained 16% stearic and 38% oleic acid, whereas feces from lard-fed rats contained 51% stearic and 15% oleic acid (other data not shown).

ACF Assay

Promotion was assessed by the multiplicity of ACF (number of crypts/ACF). ACF were scored using the procedure of Bird (23). Immediately after the animals were sacrificed, the colons were removed and flushed with Krebs Ringer solution (Sigma Chemical), then opened longitudinally and fixed flat between coded filter papers in 10% buffered formalin (Sigma Chemical). The colons were stained with methylene blue (0.1%) for 10 minutes, then the mucosal side was observed at $\times 32$ magnification. ACF were distinguished by their slitlike opening, increased staining, size, and pericryptal zone. The multiplicity was recorded for each ACF in each colon. All colons were scored blindly by a single observer.

Statistical Analysis

Results were analyzed using Systat 5 software for Windows. Values are means \pm SD, and *P* values are two sided. Data were analyzed by two-way factorial analysis of variance (ANOVA: factor 1, type of meat; factor 2, level of meat and fat content) using a general linear model. When factorial ANOVA showed a significant difference between groups (*F* test *p* < 0.05), multiple comparisons were done by the Dunnett's test comparing each group with the lard control group. Two separated one-way ANOVAs were used when the interaction was significant. Pearson correlation matrix was computed at the level of group means (*n* = 10) and individual rats (*n* = 99), and *p* values were computed with Bonferroni correction.

Table 2. Composition of Fatty Acids in Fat and in Meat Fat Used in Experimental Diets^a

| Fatty Acids | Lard | Olive Oil | Beef | Chicken | Bacon |
|-----------------------|------|-----------|------|---------|-------|
| Myristic acid (14:0) | 2.2 | 1.2 | 3.5 | 1.1 | 1.7 |
| Palmitic acid (16:0) | 26.3 | 13.6 | 28.1 | 21.0 | 26.3 |
| Stearic acid (18:0) | 16.4 | 4.7 | 17.3 | 6.4 | 16.7 |
| Oleic acid (18:1) | 38.2 | 68.3 | 40.3 | 45.5 | 42.4 |
| Linoleic acid (18:2) | 10.5 | 7.0 | 2.3 | 18.7 | 10.8 |
| Linolenic acid (18:3) | 2.2 | 1.0 | 1.1 | 4.4 | 0.6 |
| Other fatty acids | 4.2 | 4.2 | 7.4 | 2.9 | 1.5 |

^a: Values are percentages. Duplicate assays led to quasi-identical values.

Results

Body Weights and Food and Water Intakes

Body weight gains were higher in rats fed high-meat and high-fat diets than in rats fed low-meat and "low-fat" diets ($p = 0.001$; Figure 1, Table 3). Food and water intakes were measured per cage twice for a period of three days each (Days 26–28 and 63–65). Consumption of low-fat diets was higher than consumption of high-fat diets (8.6 ± 0.7 and 7.4 ± 0.6 g/day/rat, respectively, $p < 0.0001$). The energy intake was calculated with 4 kcal/g used for carbohydrate and protein and 9 kcal/g for fat. Rats fed low- and high-fat diets consumed energy at similar levels (37.75 and 37.34 kcal/day, respectively), suggesting that they self-regulated their energy intake. During the whole experimental period, rats fed the olive oil diets gained less weight per day and ate less food than rats fed the animal fat diets (Figure 1, Table 3). Rats fed the diets containing beef or chicken were the heaviest but did not eat more food than rats in the other groups (Table 3). Rats fed low-meat and low-fat diets drank less water than rats fed high-fat diets (12.3 ± 1.7 and 14.6 ± 2.6 ml/day, $p < 0.001$; Table 3). Rats fed bacon-based diets consumed more water than the other rats. The water intake was 32% higher in rats fed 30% bacon than in controls and 42% higher in rats fed 60% bacon ($p < 0.0001$). This is likely due to the salt in the bacon diets (1.7% and 3.3% NaCl in the diets containing 30% and 60% bacon, respectively).

Fecal Weight and Moisture Content

Fecal weight was not significantly different between groups of rats (data not shown, $p = 0.15$), but it was correlated with water intake ($r = 0.7$, $p < 0.001$, $n = 99$). Feces of rats fed the bacon diets were more humid than feces from the other groups. Feces from rats fed a diet with 60% bacon contained twice as much water as feces from lard-fed controls ($28 \pm 6\%$ and $15 \pm 2\%$, $p < 0.0001$). The difference did not reach significance between rats fed a diet with 30% bacon and lard-fed controls ($19 \pm 5\%$ and $14 \pm 2\%$, $p = 0.1$). The percentages of water in feces were not different between the other groups.

Iron and Bile Acids in Fecal Water and Fatty Acids in Feces

The iron concentration in fecal water was higher in rats fed the high-meat and high-fat diets than in rats fed the low-fat diets (11.3 ± 3.5 and 9.04 ± 2.2 $\mu\text{g/g}$ dry feces, respectively, $p < 0.001$). The fecal iron concentration was also affected by the nature of the diet (Table 4). Fecal iron was high in rats fed the control diets with olive oil and low in chicken-fed rats. Rats fed high-fat diets had more bile acid in fecal water than rats fed "low-fat" diets (4.04 ± 2.26 and 2.73 ± 0.91 $\mu\text{mol/g}$ dry feces, $p < 0.001$). Bile acid concentrations differed significantly among groups, with elevated values for rats fed the chicken diets and rats fed

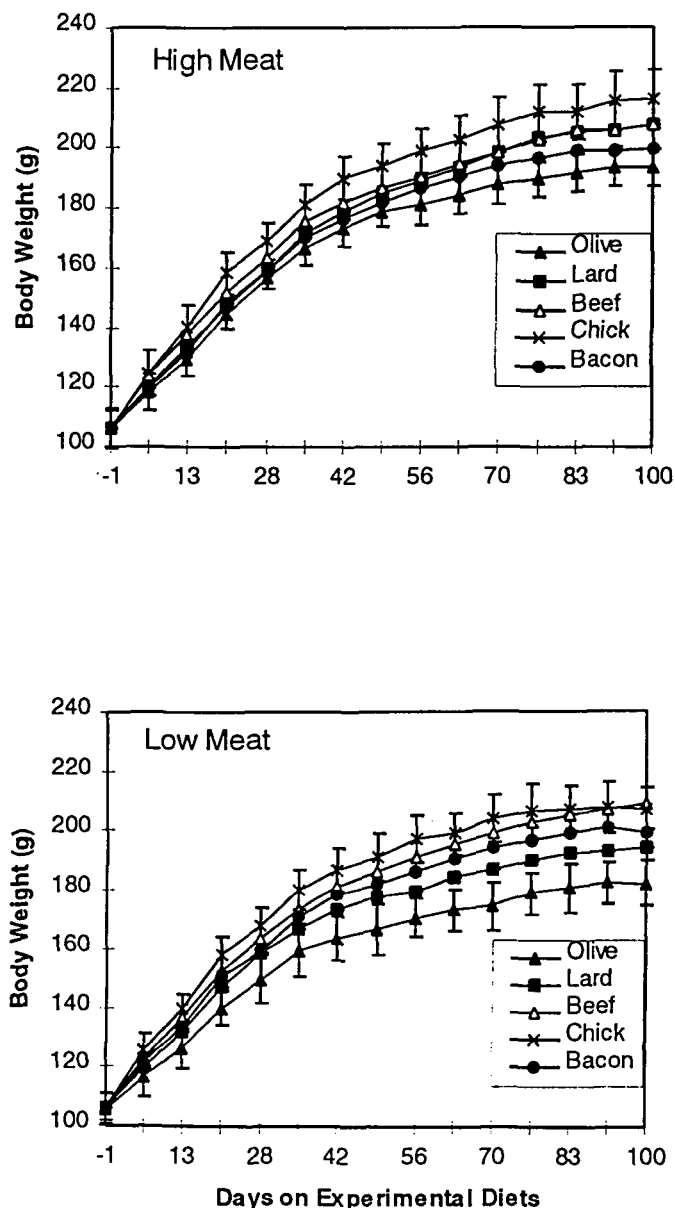


Figure 1. Effect of meat-based diets on body weight gain of female Fischer 344 rats. High meat: mean weight of 5 groups of 10 rats fed high-meat and high-fat diets containing 60% cooked freeze-dried beef, chicken, or bacon or 28% olive oil or lard. Low meat: weight of rats fed low-meat and low-fat diets containing 30% beef, chicken, or bacon or 14% olive oil or lard. SD bars are shown only for lightest and heaviest groups.

the olive oil diets (Table 4). Lard- and bacon-fed rats had similar fecal bile acid concentration ($p = 0.99$).

Fecal fatty acid concentrations were slightly affected by the fat content of diets, with higher concentrations in rats fed the high-meat and high-fat diets than in rats fed the low-fat diets ($p = 0.06$). The type of diet affected the fatty acid concentrations in feces (Table 4). With the same pattern in groups fed high-fat and low-fat diets, high concentrations of fatty acids were found in the feces of rats fed lard or beef diets and low levels were found in the feces of rats fed chicken or olive oil diets. Fatty acids were lower by a factor of 3 in feces of rats fed bacon than in feces of control rats

Table 3. Body Weight and Food and Water Intake of Female Fischer 344 Rats Fed Diets Containing 30% or 60% Meat^a

| | Body Wt ^b at Day 100, g/rat | | Food Intake, ^c g/rat | | Water Intake, ^c ml/rat | |
|----------------------------|--|-----------|---------------------------------|-----------|-----------------------------------|-------------|
| | Low meat | High meat | Low meat | High meat | Low meat | High meat |
| Lard | 194 ± 9 | 208 ± 7 | 8.9 ± 0.8 | 7.3 ± 0.6 | 11.5 ± 1.1 | 13.3 ± 0.8 |
| Olive oil | 182 ± 8 | 194 ± 6* | 7.9 ± 0.5 | 7.1 ± 0.5 | 11.0 ± 0.7 | 12.8 ± 0.4 |
| Beef | 209 ± 9 | 207 ± 7 | 8.9 ± 0.4 | 7.2 ± 0.3 | 12.2 ± 0.5 | 14.0 ± 0.7 |
| Chicken | 207 ± 7 | 216 ± 10* | 8.8 ± 0.2 | 7.5 ± 1.0 | 11.5 ± 0.3 | 13.4 ± 0.9 |
| Bacon | 199 ± 6 | 200 ± 6 | 8.3 ± 0.6 | 7.9 ± 0.3 | 15.4 ± 0.9 | 19.3 ± 0.9* |
| <i>P</i> Values (by ANOVA) | | | | | | |
| Meat level effect | 0.001 | | 0.000 | | 0.000 | |
| Meat type effect | 0.000 | | 0.077 | | 0.000 | |
| Level × type effect | NS | | NS | | NS | |

a: Diets are defined in Table 1. Statistical significance is as follows: *, $p < 0.001$ vs. lard-fed control by Dunnett's test. ANOVA, analysis of variance; NS, not significant.

b: Values are means ± SD for 10 rats/group, except high-meat beef group, where $n = 9$ (1 rat died on Day 75 because of aberrant teeth growth).

c: Values are means ± SD ($n = 5$ because rats were 2 per cage). Intake was measured between Days 63 and 65.

Table 4. Effect of Diets Containing 30% or 60% Meat on Concentrations of Iron and Bile Acids in Fecal Water and Total Fatty Acids in Feces^a

| | Iron, ^b µg/g dry feces | | Bile Acids, ^b µmol/g dry feces | | Total Fatty Acids, ^c mg/g | |
|----------------------------|-----------------------------------|-------------|---|--------------|--------------------------------------|--------------|
| | Low meat | High meat | Low meat | High meat | Low meat | High meat |
| Lard | 9.4 ± 1.2 | 10.3 ± 2.7 | 2.33 ± 0.64 | 2.29 ± 1.01 | 62.2 ± 13.4 | 84.5 ± 13.0 |
| Olive oil | 11.0 ± 0.7 | 15.5 ± 1.2† | 3.72 ± 0.98† | 5.55 ± 2.26† | 8.7 ± 3.5 | 12.7 ± 5.8† |
| Beef | 6.7 ± 1.2* | 11.9 ± 3.1 | 2.09 ± 0.57 | 3.39 ± 0.57 | 51.9 ± 14.7 | 72.6 ± 23.3 |
| Chicken | 7.3 ± 1.0 | 6.7 ± 0.7 | 3.44 ± 0.21* | 6.79 ± 2.08‡ | 7.1 ± 0.8 | 9.6 ± 2.6‡ |
| Bacon | 10.7 ± 2.3 | 12.0 ± 2.0 | 2.08 ± 0.43 | 2.44 ± 0.71 | 22.7 ± 0.1 | 26.9 ± 10.2‡ |
| <i>P</i> Values (by ANOVA) | | | | | | |
| Meat level effect | 0.000 | | 0.000 | | 0.058 | |
| Meat type effect | 0.000 | | 0.000 | | 0.000 | |
| Level × type effect | 0.003 | | 0.008 | | NS | |

a: Diets are defined in Table 1. Statistical significance is as follows: *, $p < 0.05$; †, $p < 0.01$; ‡, $p < 0.001$ vs. lard-fed control by Dunnett's test. Separated ANOVA and Dunnett's test were done for low-meat and high-meat subsets, for iron and bile acid values, because level × type interaction was significant.

b: Values are means ± SD ($n = 5$ cages).

c: Pool of feces from 10 rats in each group was assayed in duplicate for total fatty acids.

fed lard ($p < 0.001$, Table 4), although both diets contained 28% lard (Table 1).

Visceral Fat and Length of Colon

Visceral fat weight was higher in rats fed the high-meat and high-fat diets than in rats fed low-fat diets (14.2 ± 2.5 and 12.5 ± 2.8 g, $p = 0.01$; Table 5). Also, body weight of rats was correlated with visceral fat weight ($r = 0.73$, $p < 0.001$, $n = 72$). Among groups fed high-fat diets, rats fed the diet containing 60% bacon had the least visceral fat. Their colons were shorter than the colons of the other rats (compared with lard-fed controls, $p = 0.005$; Table 5).

Promotion of ACF

The number of ACF per colon, which is considered a marker for tumor initiation, but not for tumor promotion,

was not affected by the type of diet (Table 6). In contrast, the multiplicity of ACF (number of crypts/ACF), which is considered a marker for tumor promotion, was diet dependent ($p = 0.002$; Table 6). The multiplicity was nearly the same in all groups, except those fed bacon diets, with no effect of fat and protein source (Table 6; $p = 0.7$ between 8 groups, without bacon-fed rats, by ANOVA). Compared with lard-fed controls, olive oil, beef, and chicken diets did not change the growth of ACF. There was no effect of dietary fat level on ACF multiplicity: the number of crypts per ACF was 3.12 ± 0.41 in rats fed low-meat and low-fat diets and 3.03 ± 0.49 in rats fed the high-fat diet ($p = 0.34$). In contrast, compared with lard-fed controls, the ACF multiplicity was reduced by 12% in rats fed 30% bacon and by 20% in rats fed 60% bacon (significance of bacon effect $p < 0.001$). Moreover, the ACF growth reduction by bacon seems dose dependent, because Pearson correlation between multiplicity of ACF and bacon level in the diet was $r = -0.51$ ($p = 0.001$, $n = 40$ rats).

Table 5. Length of Colon and Visceral Fat of Female Fischer 344 Rats After 100 Days on Diets Containing 30% or 60% Meat^a

| | Colon Length, ^b cm | | Visceral Fat, ^c g/rat | |
|---------------------|-------------------------------|-------------|----------------------------------|-------------|
| | Low meat | High meat | Low meat | High meat |
| Lard | 14.3 ± 1.6 | 14.2 ± 1.0 | 11.3 ± 3.1 | 14.8 ± 2.7 |
| Olive oil | 13.1 ± 1.7 | 13.6 ± 1.4 | 10.9 ± 1.4 | 13.0 ± 1.7 |
| Beef | 13.1 ± 2.0 | 13.2 ± 0.9 | 15.6 ± 3.2* | 15.2 ± 1.3 |
| Chicken | 13.9 ± 0.7 | 13.8 ± 1.4 | 12.8 ± 2.7 | 17.1 ± 1.9 |
| Bacon | 13.1 ± 1.2 | 12.3 ± 2.0† | 12.9 ± 1.3 | 11.8 ± 1.3* |
| P Values (by ANOVA) | | | | |
| Meat level effect | NS | | 0.001 | |
| Meat type effect | 0.011 | | 0.000 | |
| Level × type effect | NS | | 0.003 | |

a: Diets are defined in Table 1. Statistical significance is as follows: *, $p < 0.05$; †, $p < 0.01$ vs. lard-fed control by Dunnett's test.

b: Values are means ± SD for 10 rats/group, except high-meat beef group, where $n = 9$ (see Footnote b in Table 3).

c: Values are means ± SD for 6–8 rats/group.

Table 6. Effect of Diets Containing 30% or 60% Meat on Number and Multiplicity of ACF in Colon of Fischer 344 Female Rats 100 Days After a Single Azoxymethane Injection^a

| | Multiplicity, ^b crypts/ACF | | No. of ACF/Rat ^b | |
|---------------------|---------------------------------------|--------------|-----------------------------|-----------|
| | Low meat | High meat | Low meat | High meat |
| Lard | 3.21 ± 0.47 | 3.27 ± 0.38 | 65 ± 34 | 75 ± 44 |
| Olive oil | 3.11 ± 0.28 | 2.94 ± 0.30 | 83 ± 30 | 61 ± 43 |
| Beef | 3.25 ± 0.44 | 3.15 ± 0.59 | 69 ± 23 | 71 ± 25 |
| Chicken | 3.16 ± 0.34 | 3.18 ± 0.32 | 76 ± 37 | 98 ± 30 |
| Bacon | 2.84 ± 0.45 | 2.62 ± 0.60* | 86 ± 47 | 72 ± 37 |
| P Values (by ANOVA) | | | | |
| Meat level effect | NS | | NS | |
| Meat type effect | 0.002 | | NS | |
| Level × type effect | NS | | NS | |

a: Diets are defined in Table 1. Statistical significance is as follows: *, $p < 0.001$ vs. lard-fed control by Dunnett's test. No other difference reached significance.

b: Values are means ± SD for 10 rats/group, except high-meat beef group, where $n = 9$ (see Footnote b in Table 3).

The multiplicity of ACF was not associated with iron ($r = -0.54$, $p = 0.11$, $n = 10$ groups), with bile acids ($r = 0.08$, $p = 0.83$, $n = 10$), or with fatty acid concentrations in feces ($r = 0.41$, $p = 0.23$, $n = 10$). Similar nonsignificant correlations were obtained at the rat level ($n = 99$).

Discussion

The present study yielded five major findings. 1) The intake of a diet containing 60% dry cooked bacon decreased the multiplicity of preneoplastic lesions in the colon of rats compared with control diets based on casein and lard. 2) Diets containing 30% or 60% dry cooked beef did not promote the growth of ACF in the colon of rats compared with casein-based control diets balanced for fat level. 3) Diets containing 30% or 60% dry cooked chicken did not reduce the ACF growth in rats compared with casein- or beef-based diets balanced for fat. 5) High-fat and high-meat diets in-

creased the rat body weight gains but did not promote the ACF growth compared with diets with less fat and less meat. 5) Fecal concentrations of iron, bile acids, and total fatty acids were not correlated with the promotion of ACF. These five points are discussed below.

The bacon-based diet appeared to protect rats against carcinogenesis in a dose-dependent manner. This finding was in contrast to our starting hypothesis that bacon diets would promote carcinogenesis. Our hypothesis was based on epidemiological studies showing that intake of processed meat (mainly pork) is associated with risk. We also thought that nitrite and *N*-nitroso compounds found in bacon might increase carcinogenesis. However, bacon-fed rats drank more water than controls (Table 3), probably because bacon is salty. We thus propose that a high water intake, and not the bacon intake, can protect the rats against carcinogenesis. The water intake is seldom measured in animal and human studies, although it plays a vital role in gut functions. Recently, two case-control studies have analyzed the connection between

the water intake and the risk of colorectal cancer (25,26). In Seattle, Shannon and colleagues (25) reported a reduced risk of colon cancer associated with a high intake of water. The adjusted odds ratio (OR) for women drinking more than five glasses of water per day vs. those drinking fewer than two glasses was 0.55, with a 95% confidence interval (95% CI) of 0.31–0.99. Lubin and colleagues (26) reported a similar OR (0.5) and 95% CI (0.3–0.9) for adenoma associated with highest vs. lowest tertiles of the mean daily water intake in Israel. Moreover, Stookey and colleagues (27) reported a strong negative association between water drinking and breast cancer in women (OR = 0.21, 95% CI = 0.07–0.62). In rats given dimethylhydrazine injections for 25 weeks, Ucheddu and colleagues (28) limited access to drinking water to only 1 h/day. The water-restricted group of rats had more colonic tumors and excreted fewer fecal pellets than control rats given water *ad libitum* (both $p = 0.02$). Although these results were poorly reported, they suggest that a low water intake may increase colon cancer risk, possibly by delaying the bowel transit time and by raising the concentration of toxic compounds in fecal water. Here, bacon-fed rats drank 45% more water (Table 3) and they had 90% more water in feces than rats fed the diets without bacon. On the basis of dry feces values, bacon-fed rats had low concentrations of bile acids and total fatty acids in feces (Table 4). Moreover, bile acids and fatty acids were more diluted in wet feces of bacon-fed rats than in less humid feces of rats in the other groups. Finally, bacon-fed rats had smaller ACF than rats fed the other diets (Table 6). Thus the protection afforded by the bacon-based diet might be explained by the dilution of promoting compounds in the gut content water phase.

Beef showed no promoting effect. This is not a new finding, and it is consistent with published studies where meat was given to rats during and after dimethylhydrazine injections (for review see Reference 3). Reddy and colleagues (10) reported that the incidence of colon tumors was the same in F344 rats fed a diet containing 60% beef and rats fed a diet similarly balanced with 40% soybean protein and 25% corn oil (tumor incidence beef-to-soy ratio = 1.1, $p = 0.79$). Clinton and colleagues (11) also showed that the incidence of adenocarcinoma was the same in Sprague-Dawley rats fed balanced diets containing 20% beef or 20% soybean protein (tumor incidence beef-to-soy ratio = 1.1, $p = 0.96$). Pence and colleagues (15) compared the effects of diets containing 50–60% beef with diets containing 17–21% casein on the promotion of colon carcinogenesis in Sprague-Dawley rats. The colon tumor incidence and burden were reduced in the groups of beef-fed rats compared with casein-fed controls (tumor incidence beef-to-casein ratio = 0.4, $p < 0.05$). Moreover, the study of beef effect in mice by Nutters and co-workers (12) and the study of kangaroo meat effect in rats by McIntosh and others (14) do not support promotion of colon cancer by that red meat in rodents. A recent study by Pence and colleagues (16) shows that well-cooked beef, containing a high heterocyclic amine content, can enhance colon carcinogenesis in rats only when fed

during dimethylhydrazine initiation and only in a low-fat diet context (tumor incidence ratio = 1.5, $p < 0.05$). The present study is the first in which meat was not given during carcinogen injections, but only after initiation. Its major limitation is that we stopped the experiment at the stage of ACF, which are putative precancerous lesions but not true cancers (19). These experimental data are in contrast to epidemiological data. In humans, most case-control studies and some prospective studies show an elevated risk of colorectal cancer associated with the red meat intake (3). It is possible that the rat model is not pertinent for human cancers, for instance, because the rodent diet is high in micronutrients or because detoxifying enzymes are more efficient in rats than in humans. Alternatively, it is possible that the epidemiological association between meat and colorectal cancer is entirely due to fat, and fat was fully balanced in this study, or to a compound that is sometimes, but not always, present in meat [e.g., heterocyclic amines (16,17)].

Chicken diets were not protective against carcinogenesis compared with beef or casein control diets. This is the first experimental study to examine the effects of a chicken-based diet on rat colon carcinogenesis. Two cohort studies have examined the association of chicken intake with the risk of colon cancer (4,5). Those who frequently eat chicken have a lower risk of developing colon cancer than those who consume no or very little chicken (relative risk = 0.47 and 0.82 in men and women, respectively). Similarly, in a recent case-control study, the intake of chicken without skin was negatively associated with risk in both genders (29). According to Giovannucci and co-workers (5), chicken would protect humans against colorectal cancer because it is high in methionine but low in (saturated) fat. In this study, the fatty chicken skin was included in the chicken diets to obtain identical fat levels in the five high-meat and high-fat diets and in the five “low-fat” diets. Thus the high fat content of chicken diets may have impaired the possible protective effect of lean chicken.

High-fat and high-meat diets did not promote the growth of ACF compared with the relatively “low-fat” diets. It is often assumed that fat promotes carcinogenesis. However, many rodent studies failed to show a colon cancer-promoting effect of saturated fat (16,30). In addition, the “low-fat” diets used in this study contained a high 32% of energy from fat. It seems that at a >30% threshold value, a rise in fat calories in the diet does not enhance the tumor yield in rats. Tang and colleagues (31) showed that the colon and mammary cancer incidence increased when fat was raised from 15% to 30% calories in the diet. There was no further increase of cancer risk at >30% of calories from fat. Similarly, Cohen and colleagues (32) showed no increase in mammary carcinogenesis when the fat content in the diet of rats was raised to >30% calories.

Bile acids in fecal water and fatty acids in colon content are toxic to the colonic mucosa (20). High concentrations of these surfactants may explain the promoting effect of dietary fat (33,34). Here, for each meat type, rats fed the high-fat diet

had more bile acids in fecal water than rats fed the "low-fat" diet ($p < 0.001$). Also, rats fed high levels of olive oil or chicken had high bile acid concentrations in fecal water (Table 4). The intake of unsaturated fatty acids, but not of saturated fat, may thus increase fecal bile acids (35–37). Lard- and beef-fed rats had high fecal fatty acid concentrations compared with olive oil- and chicken-fed rats. It is thus likely that a high intake of saturated fatty acids (palmitic and stearic) enhances fat and fatty acid excretion in feces (20). However, we did not find any significant correlation between any specific fatty acid in the diet, fecal fatty acids, bile acid concentration in fecal water, and the multiplicity of ACF. Because the variance in the ACF multiplicity was mainly due to the bacon diets and may come from differences in the water intake, we also analyzed data after removing the two bacon-fed groups. Again, we found no correlation between data. However, a limitation of this study is that we did not specifically assay the secondary bile acids or the free fatty acids, which may be more potent promoters of colon carcinogenesis than total bile acids and fatty acids (33).

Iron might be a risk factor derived from beef. In the present study, the fecal iron level in beef-fed rats was not higher than in controls fed the casein diets (Table 4). We did not measure, however, the serum iron levels. However, Lai and colleagues (38) observed that rats fed a beef diet appeared to use and absorb the iron better than control rats fed a casein diet, according to the serum iron levels. However, they did not show a promoting effect of increased dietary iron derived from cooked beef on the development of colon tumors. We did not show an association of the iron level in feces with the ACF multiplicity.

Dietary guidelines advise reduction in the intake of red meat and/or saturated fat to the benefit of "white" lean meat (39). The need to develop strong supporting data in animal models before conducting intervention trials has recently been stressed by De Luca and Ross (40) in a commentary on the failure of the recent α -tocopherol, β -carotene prevention studies. The present experimental study does not support the belief that, in a high-fat diet context, red meat consumption promotes, or that white meat protects against, colon carcinogenesis. This study nevertheless has two major limitations: 1) it was done in rodents, and we do not know whether AOM-initiated rats are good models for human colon cancers, and 2) the end point was the development of putative precancerous lesions. The multiplicity of ACF correlates with the adenocarcinoma incidence in most (41–43), but not all (44), rodent studies. In conclusion, this study has introduced a new and potentially important experimental finding concerning a possible beneficial effect of water intake on colon cancer prevention.

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