Nutrition and Cancer, 62(5), 567–573
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ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635580903532408

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Freeze-Dried Ham Promotes Azoxymethane-Induced Mucin-Depleted Foci and Aberrant Crypt Foci in Rat Colon

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Processed and red meat consumption is associated with the risk of colorectal cancer. Meta-analyses have suggested that the risk associated with processed meat is higher. Most processed meats are cured and cooked, which leads to formation of free nitrosyl heme. We speculated that free nitrosyl heme is more toxic than native myoglobin. The promoting effect of a freeze-dried, cooked, cured ham diet was looked for in a 100-day study. Colon carcinogenesis endpoints were aberrant crypt foci and mucin depleted foci (MDF). A second study (14 days) was designed 1) to compare the effect of ham, hemoglobin, and hemin; and 2) to test the effect of sodium chloride, nitrite, and phosphate in diet on early biomarkers associated with heme-induced promotion. In the 100-day study, control and ham-fed rats had 3.5 and 8.5 MDF/colon, respectively ($P < 0.0001$). Promotion was associated with cytotoxicity and lipid peroxidation. In the short-term study, cytotoxicity and lipid peroxidation of fecal water, and the urinary marker of lipid peroxidation, increased dramatically in ham- and hemin-fed rat. In contrast, the hemoglobin diet, sodium chloride, nitrite, phosphate diet had no effect. Freeze-dried cooked ham can promote colon carcinogenesis in a rodent model. Hemin, but not hemoglobin, mimicked ham effect on early biochemical markers associated with carcinogenesis.

INTRODUCTION

Colorectal cancer is one of the main causes of death in affluent countries. Environmental factors are involved in this cancer, particularly diet. Modifications in dietary habits could reduce this cancer burden up to 70% (1). In its 2007 report, the World Cancer Research Fund panel judges that "the evidence that red meat and processed meat are a cause of colorectal cancer is convincing." The panel thus recommends one to "limit intake of red meat and avoid processed meat." Three recent meta-analyses have shown that consumption of red or processed meat is associated with a modest but significant risk of colorectal cancer (2–4). We have estimated, from these meta-analyses, that 1 g of processed meat increases the risk of colorectal cancer, respectively, 11 times, 6 times, or 2 times more than 1 g of fresh red meat (5). Thus, processed meat seems more closely associated with the risk of colorectal cancer than fresh red meat. This causes a challenge for the meat processing industry to 1) understand the mechanisms involved in the relationship between colorectal cancer and processed meat and 2) develop research to solve the problem (6).

Several mechanisms have been proposed to explain the relationship between the risk of colorectal cancer and red meat intake. Red meat enhances the formation of putative carcinogenic N-nitroso compounds (NOC) in human feces (7–9). But NOC in rat feces from a bacon-based diet do not initiate or promote preneoplastic lesions in rat colon (10). Meat cooked at a high temperature contains mutagenic heterocyclic amines (HCA) that induce colon, mammary, and prostate tumors in rodents and monkeys (11). However, HCA might not play an important role in colorectal cancer incidence, since 1) chicken intake is a major contributor of HCA intake, but it is not associated with the risk (12); and 2) doses of HCA that induce cancer in animals are 1,000 to 100,000 times higher than doses ingested by humans (13). Red meat also contains heme, the iron-bearing prosthetic group of myoglobin. Dietary hemin (free heme stabilized by freely exchangeable axial chloride group) in rat diet increases colonic epithelial proliferation and induces cytotoxicity

Submitted 26 March 2009; accepted in final form 21 September 2009.

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of fecal water in rats (14). Dietary hemin, hemoglobin, and heme in meat promote, dose-dependently, the formation of preneoplastic lesions in the colon, aberrant crypt foci (ACF), and mucin depleted foci (MDF). Dietary hemin (free heme) is more detrimental than hemoglobin (heme bound inside a hemoprotein) (15–17). Usual processes to make processed meat include salting, adding nitrites, smoking, and cooking (5). The addition of nitrite can nitrosylate heme iron, and cooking can release nitrosyl heme from myoglobin. We speculated that free nitrosyl heme in processed meat is more toxic than native heme in fresh meat, which would explain why processed meat intake is more closely associated with the risk of colorectal cancer than fresh red meat intake. This hypothesis fits with the fact that hemin is more toxic than hemoglobin (16). An alternative hypothesis would be that processed meat nitrite enhances the endogenous formation of carcinogenic NOC, but we did not test this hypothesis in this study.

Few experimental studies had been conducted on the impact of processed meat on colorectal carcinogenesis (10,15,18). Here, the promoting effect of cooked ham was looked for in a 100-day study. Carcinogenesis endpoints were dimethylhydrazine (DMH)-induced preneoplastic lesions (ACF and MDF) in rats. To test the hypothesis that free heme might be more toxic than globin-bound heme, a 14-day study was designed to compare the effect of cooked ham, hemoglobin, and hemin on early biomarkers previously associated with heme-induced promotion (17). This study shows for the first time that a cured meat, cooked ham, can promote colon carcinogenesis in a rodent model. Hemin, but not hemoglobin, mimicked the ham effect on early biochemical markers associated with carcinogenesis.

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MATERIALS AND METHODS

Animals and Diets

Fifty-five Fischer 344 female rats were purchased at 4 wk of age from Charles River (St. Germain l'Arbresle, France). Animal care was in accordance with the guidelines of the European Council on animals used in experimental studies. The animal colony and staff got official agreement No. 31–121 for animal studies by the French government.

Short-term study. Twenty-five rats were housed individually in metabolic cages. The room was kept at a temperature of 22°C on a 12-h light-dark cycle. After 2 days of acclimatization to the AIN76 powder, rats were randomly allocated to 5 dietary groups (5 rats/group) and fed experimental diets for 2 wk. Body weights and food intake were monitored at the beginning, at the middle, and at the end of the experiment. Feces were collected for the last 5 days and were frozen at –20°C. Urine was collected 1 day before the end of the experiment. Animals were killed 14 days after the start of the experimental diets.

Long-term study. Twenty rats were housed by pairs in stainless steel, wire bottomed cages. The room was kept at a temperature of 22°C on a 12-h light–dark cycle. Rats were allowed 7 days of acclimatization to the room and to the control diet before being injected intraperitoneally with the carcinogen 1,2 dimethylhydrazine (Sigma Chemical, St. Quentin, France; 190 mg/kg body weight) in NaCl (9 g/l). Usually, several injections are given to rats. We reasoned that the first shot initiates carcinogenesis, and the following shots promote it, blurring diet-induced promotion. We thus chose a single-shot protocol following Karkare et al. (19). Seven days later, rats were randomly allocated to two groups of ten and allowed free access to a control diet or a ham-based diet for 100 days. We chose to initiate all rats with the carcinogen, since the study was designed to show dietary promotion and because a 2.5% hemoglobin diet does not initiate ACF in rats (Pierre & Corpet, unpublished results).

Diets. Seven experimental diets shown in Table 1 were based on a modified standard AIN-76 diet (20), prepared and formulated in a powdered form by UPAE (INRA, Jouy-en-Josas, France). Diets were made every 14 days and maintained at –20°C. Calcium level is critical for heme promotion (17); calcium was thus excluded from the mineral mix, but dibasic calcium phosphate was included in all diets at a low concentration of 2.7 g/kg. For each study, one batch of cured cooked ham (*Jambon de Paris*) was obtained from Récapé S. A. (Lanta, France) and freeze-dried without irradiation by Lyophal (Salon-de-Provence, France). Ham diets contained 55% cured cooked ham (freeze dried, 11.5% fat) wt/wt. This amount corresponds to our previous meat and cancer studies (15–17). All diets were balanced for protein (50%), fat (21%), calcium (0.8 g/kg), and iron (0.14 g/kg) by the addition of casein, lard, calcium phosphate, and ferric citrate, respectively. Ham diets provide 0.25 μmol/g heme (short-term study) and 0.036 μmol/g heme (long-term study). This surprising discrepancy between heme concentrations may be due to our inability to measure properly the oxidized forms of heme. As discussed below, freeze-drying enhances polyunsaturated fat peroxidation. Hemin and hemoglobin (Sigma Chemical, St. Quentin, France) diets were formulated to provide the same amount of heme (0.25 μmol/g) and of sodium phosphate, nitrite, and chloride as the ham-based diet. All short-term study diets matched ham diet levels of sodium chloride, nitrite, and phosphate, but a low-salt control diet contained no added sodium nitrite and phosphate and little sodium chloride (Table 1).

ACF Assay

Rats were killed by CO_2 asphyxiation in a random order at Day 100 of the long-term study. Colons were excised from rats immediately post mortem, flushed with cold Krebs solution (Sigma chemical, St. Quentin, France), opened longitudinally, and fixed flat between two sheets of filter paper in 10% formalin (Sigma Chemical, St. Quentin, France) marked with a two-digit blinding code. Colons picked up in random order were stained for 6 min in a 0.05% filtered solution of methylene blue (21). Number of ACF per colon, and number of crypts in each ACF, were counted under light microscope at $\times$40 magnification in duplicate by 2 readers, blinded for the origin of the colon.

TABLE 1
Composition of diets (g/kg)

	Long-Term Study		Short-Term Study				
	Control	Ham	Control Low-Salt (LoSaCo)	Hemin High-Salt (Hemin)	Hemoglobin High-Salt (Hemog)	Control High-Salt (HiSaCo)	Ham High-Salt (Ham2)
Ham	0	550	0	0	0	0	550
Beef	0	0	0	0	0	0	0
Hemin	0	0	0	0.2	0	0	0
Hemoglobin	0	0	0	0	6.6	0	0
Lard	160	97	180	180	180	180	117
Safflower oil	50	50	50	50	50	50	50
Casein[a]	500	127	450	450	443	450	77
Corn starch	60	60	60	60	60	60	60
Sucrose	127	14	157	111	111	111	44
Cellulose	50	50	50	50	50	50	50
Methionine	3	3	3	3	3	3	3
Mineral mix[b]	35	35	35	35	35	35	35
Vitamin mix[b]	10	10	10	10	10	10	10
Choline bitartrate	2	2	2	2	2	2	2
$CaHPO_4 2H_2O$	2.7	2.35	2.7	2.7	2.7	2.7	2.35
Ferric citrate	0.13	0	0.13	0.02	0.02	0.08	0
Sodium phosphate	0	0	0	13.7	13.7	13.7	0
Sodium nitrite	0	0	0	0.19	0.19	0.19	0
Sodium chloride	0	0	0.54	33	33	33	0

[a]Low-calcium casein.
[b]AIN76 mix, but 500 g/kg of dibasic calcium phosphate replaced by sucrose in mineral mix.

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MDF Assay

MDF may predict colon carcinogenesis better than ACF, since Apc mutations are present in MDF with a frequency similar to that of tumors (22). Colons, after being scored for ACF, were stained with high iron diamine (HID) Alcian blue (AB) procedure to evaluate mucin production (23). Briefly, colons were rinsed in distilled water and left overnight in freshly prepared HID solution (50 ml of distilled water with 120 mg N-N′-dimethyl-m-phenylene diamine, 20 mg N-N′-dimethyl-p-phenylene diamine, and 1.4 ml of 60% ferric chloride). After rinsing, colons were counterstained in 1% AB solution for 30 min. MDF number, and number of crypts per MDF, were scored blindly under light microscope at ×40 magnification by a single reader.

Preparation of Fecal Water

Fecal pellets were collected under each cage of two rats for 24 h, thus leading to five samples per group. Freeze-dried feces were used to calculate dry fecal mass and to prepare fecal water by adding 1 ml of sterilized water to 0.3 g of feces. Samples were then incubated at 37°C for 1 h, stirring thoroughly every 20 min, followed by centrifugation at 20,000 *g* for 10 min. The aqueous phase was recentrifuged at the same speed and duration and the subsequent supernatant (fecal water) collected and conserved at –20°C until use.

Thiobarbituric Acid Reactive Substances (TBARS) and Heme Assay

TBARS were measured in fecal water according to Ohkawa et al. (24) exactly as previously described (15). Heme contents of freeze-dried feces and of fecal water were measured by fluorescence according to Van den Berg et al. (25) and Sesink et al. (14), respectively, as already described (15).

Cytotoxicity Assay of Fecal Water

Cytotoxicity of fecal water was quantified on a cell line according to Bonneson et al. (26) and as previously described (15). Briefly, cancerous mouse colonic epithelial cell line, CMT93 (ECAC), was seeded in 96-well microtiter plates (1.6×10^4 cells/well in 200 μl of medium) and treated for 24 h with fecal water sample diluted at 10% (vol/vol) in the culture medium. Cytotoxicity of each fecal water was quantified by the 3-(4,5-dimethyldiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) test.

Urinary 1,4-Dihydroxynonane Mercapturic Acid (DHN-MA) Assay

Each rat was placed alone in a metabolic cage for two days during the fifth wk of the experimental diet of the long-term study and at the end of the first wk of the short-term study. The 24-h urine was collected under each cage of one rat, thus leading to 10 and 5 samples per group for, respectively, long- and short-term study. DHN-MA assay was done by competitive enzyme immunoassay as previously described (27) using DHN-MA-linked acetylcholinesterase enzyme. Each urine sample was assayed in duplicate.

Statistical Analysis

Results were analyzed using Systat version 10 software for Windows and reported as mean ± SD. Biochemical long-term study data were analyzed by Student's *t*-test or Welsh's test when variances were not similar. ACF data were analyzed by 2-way analysis of variance (ANOVA; dietary group and reader). Short-term study data were considered first using 1-way ANOVA. If a significant difference was found between groups ($P < 0.05$), then pairwise comparisons were made with Tukey multiple comparison test (all other data). The Pearson correlation coefficient was used to determine the relations between ACF, MDF, and fecal or urinary values; and *P* values were calculated with Bonferroni correction for multiple comparisons.

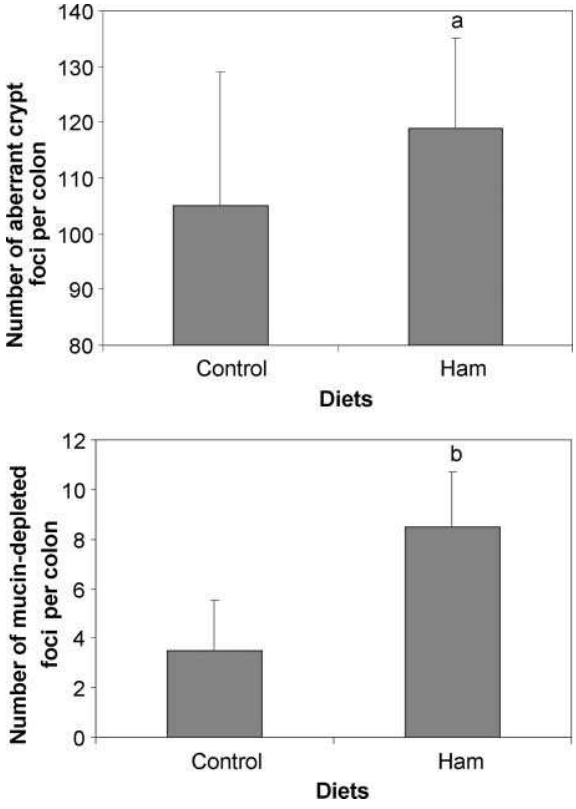

FIG. 1. Effect of a ham-based diet on putative precancerous lesions per rat colon 100 days after the injection of dimethylhydrazine. Values are means ± SD; $N = 10$. Top panel: number of aberrant crypt foci; a, significantly different from control group by 2-way ANOVA ($P < 0.05$). Bottom panel**:** number of mucin-depleted foci; b, significantly different from control group by Welsh's test ($P < 0.0001$).

RESULTS

Weight and Food Intake

Final body weight of rats was 200 ± 2 g after 100 days on experimental diets in the long-term study, without significant differences between both groups. Food intake was the same (data not shown), but ham-fed rats drank more water than control rats (23.8 ± 0.5 and 16.4 ± 0.3 ml/day, respectively; $P < 0.0001$).

Final body weight of rats was 132 ± 4 g in the short-term study, without significant differences between groups. Food intake was the same in all groups of rats, but rats given a low-salt diet drank less water than the other rats (data not shown).

ACF and MDF Data

Ham-based diet strikingly increased the number of MDF per colon (Student's *t*-test, $P < 0.0001$; Table 2 and Fig. 1). Ham also increased the number of ACF per colon (ANOVA $P = 0.048$; no difference between readers). No difference was observed between groups in the ACF and MDF size (Table 2).

TBARS and Cytotoxicity of Fecal Water

Heme intake values matched the study design: Ham, hemin, and hemoglobin groups had similar heme intake (Table 3). Heme can induce the formation of peroxyl radicals in fats, which may be cytotoxic and cleave DNA in vivo. Lipid peroxidation and cytotoxicity were thus measured in fecal water, respectively, by TBARS assay and MTT assay. Lipid peroxidation and cytotoxicity of fecal water of long-term ham-fed rats were 3 times higher than control values (Table 3, top panel; $P < 0.0001$; Welsh's test for unequal variances). Ham, hemin, and hemoglobin diets increased lipid peroxidation in fecal water in the short-term study ($P < 0.0001$), but the increase was smaller in hemoglobin-fed rats than in ham- and hemin-fed rats ($P < 0.0001$), although heme level was the same in the 3 experimental diets. Furthermore, as previously published, fecal water from hemin-fed rats was highly cytotoxic to CMT93 cells. Fecal water from ham-fed rats was also cytotoxic, but fecal water from hemoglobin-fed rats was not (Table 3).

Urinary DHN-MA Excretion

Table 3 shows that ham-based diet increased urinary DHN-MA excretion by approximately 200 compared with control diet in the long-term study ($P < 0.0001$). This striking change in DHN-MA excretion is an early event, since a 36-fold increase in DHN-MA was seen after 2 wk on ham-based diet in the short-term study. A striking increase in urinary DHN-MA level was also observed in hemin-fed rats but not in hemoglobin-fed rats (Table 3).

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TABLE 2
Effect of meat-based diets on aberrant crypt foci (ACF) and mucin-depleted foci (MDF) in the colon of rats 107 days after the injection of dimethylhydrazine[a]

		ACF				MDF			
	Heme Intake	ACF/Colon		Crypts/ACF		MDF/Colon		Crypts/MDF	
Diets[b]	μmol/Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0	105	24	2.3	0.2	3.5	2.0	4.6	1.7
Ham	0.45	119[c]	16	2.1	0.1	8.5[c]	2.2	4.3	1.2

[a]Values are means and SD; $N = 10$ rats/dietary group. A Student's test analysis of ACF data shown in Table 2 shows no significant difference ($P = 0.14$) because ACF counts from 2 readers have been grouped, which blurred the difference by increasing the variance. However, the two-way analysis of variance clearly showed the significant effect of diet on ACF number ($P = 0.048$).

[b]Diets were based on a low calcium formula, as shown in Table 1.

[c]Significantly different from control group by 2-way analysis of variance (ACF, $P < 0.05$) and by Welsh's test (MDF, $P < 0.0001$).

DISCUSSION

These data establish for the first time that processed meat in a low-calcium diet promotes colon carcinogenesis in rats. The intake of freeze-dried ham increased the number of ACF and MDF in the colon of carcinogen-induced rats. It also increased the mean number of large ACF and MDF per rat: 1.5 MDF containing more than 4 crypts were found in control rats vs. 3.5 in ham-fed rats (equivalent ACF data were 5.8 and 7.5, respectively). MDF promotion by ham diet correlated with fecal water cytotoxicity ($r = .78, n = 20, P < 0.001$) and TBARS ($r = .68, n = 20, P < 0.01$) and with urinary excretion of DHN-MA ($r = .51, n = 20, P < 0.05$) in line with our previous studies on red meat (15,17). Similar correlations were observed for ACF, but P values did not reach significance. Since MDF number would predict tumor outcome better than ACF number (23,28), the correlation between MDF and the preceding cited biomarkers suggest they may be useful as short-term surrogate endpoints.

Red meat promotion can be fully explained by its heme content, since the number of preneoplastic lesions is the same in the colon of beef meat and hemoglobin-fed rats (15). We speculated that ham promotion was related to heme intake and may be linked to the stimulation of peroxidation and cytotoxicity (15–17). However, in cured meat, the heme molecule can be modified by processing: The added nitrite nitrosylates heme iron, and cooking releases nitrosyl heme from myoglobin to form mononitrosylheme (29). Heme in cooked cured meat is thus different from heme in fresh red meat. In addition, ham is more salty than fresh meat.

In the short-term study, we first looked for the involvement of nitrite and phosphate salts that are usually added to ham.

TABLE 3
Effect of meat-based diets on fecal and urinary values in rats, notably, lipoperoxides and cytotoxicity[a]

			Heme Intake		TBARS MDF Equivalent		Cytotoxicity on CMT93 Cells		Urinary DHN-MA	
Study Length Days	Diets[b]	No. of Rats	μmol/Day	SD	μM	SD	% Lysed	SD	ng/Day	SD
100	Control	10	0	0	33	9	35	8	58	7
100	Ham	10	0.45[c]	0.1	113[c]	24	93[c]	2	11,448[c]	963
14	LoSaCo	5	0	0	40	30	0	0	139	7
14	HiSaCo	5	0	0	29	14	0	0	197	12
14	Ham2	5	2.75[d]	0.1	278[d]	23	68[d]	2	5,107[d]	19
14	Hemin	5	2.87[d]	0.3	284[d]	30	98[d]	2	9,707[d]	37
14	Hemog	5	3[d]	0	110[d,e]	22	0	0	71[e]	4

[a]Abbreviations are as follows: TBARS, thiobarbituric acid reactive substances; MDF, mucin-depleted foci; DHN-MA, 1,4-dihydroxynonane mercapturic acid. Values are means and SD.

[b]Diets were based on a low-calcium formula. See Table 1 for precise composition.

[c]Significantly different from control group by Student's t-test ($P < 0.05$).

[d]Significantly different from control group by Tukey multiple comparison test ($P < 0.05$).

[e]Significantly different from ham and hemin fed groups by Tukey multiple comparison test ($P < 0.05$).

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We previously suggested that fecal cytotoxicity and TBARS, and urinary DHN-MA, can be used as short-term biomarkers to screen meat induced promotion of colon cancer (17). Correlation between MDF promotion by ham and short-term biomarkers modulation in this study supports this use. Here, no difference in these biomarkers was seen between high-salt diet and low-salt diet-fed rats (Table 3). We thus suggest that nitrite and phosphate salts alone were not responsible for ham-induced modulation of the short-term biomarkers, and we can therefore extrapolate that they were not responsible for ham-induced promotion.

We then looked for the effect of heme in the form of free nitrosyl heme brought by cooked ham or hemin or globin-bound heme. Three diets were formulated with similar heme content in the form of 55% freeze-dried cooked cured ham, 0.2% hemin (free heme with freely exchangeable axial chloride group), and 6.6% hemoglobin (hemoprotein). Sodium chloride, nitrite, and phosphate were added to hemin and hemoglobin diets to match ham levels. The magnitude of effect of the ham diet on the three short-term biomarkers was closely similar to the effect of the hemin diet. By contrast, hemoglobin diet induced lower TBARs in fecal water, no cytotoxicity on CMT93 cells, and no urinary excretion of DHN-MA (Table 3). We have previously measured fecal water TBARs and cytotoxicity from beef-diet-fed rats (60% DM wt/wt) in a short-term study (data not shown) and a long-term study (17). The observed values were much lower than those measured here in feces from Hemin or Ham2 diet-fed rats. A similar difference had been seen between rats given hemin and hemoglobin diets with similar heme intake of 1.5 μmol/g of diet (16). Hemin and nitrosyl heme from ham were thus clearly more toxic than heme in hemoglobin and beef meat. We also made an attempt to synthesize pure mononitrosylheme by Pegg and Sahidi (29) and Sahidi and Pegg's (30) protocol and to give it by daily gavages to rats. However, no effect was seen on toxicity biomarkers in rat feces and urine, and we suppose that pure nitrosyl heme was not stable enough to resist stomach gavages. We speculated that free nitrosyl heme in ham is more toxic than native myoglobin in beef meat: It could explain why cancer risk associated with the processed meat intake is stronger than risk associated with fresh red meat intake (5). Indeed, magnitude of MDF promotion has been similar in ham-fed rats (this study) and beef-fed rats (our previous studies), although beef diets supplied 10 times more heme than ham diets (0.4 and 0.036 μmol/g diet, respectively). Furthermore, at equivalent molar dietary concentrations, hemin is more potent than hemoglobin to promote colon carcinogenesis (16); and hemoglobin and beef meat have the same promoting potency in rats (15). Here, hemin and ham diet induced the same effects on early biomarkers. As MDF promotion and modulation of early biomarkers were correlated in the long-term study, we propose that 1) processes leading to release of heme from globin could participate to the promoting effect of ham and that 2) hemin may be used as a model agent to study the effect of processed meat on colon carcinogenesis and hemoglobin a model agent for fresh red meat studies.

The mechanism of ham promotion is not known but, as stated previously, it can be linked to peroxidation and cytotoxicity. The urinary excretion of DHN-MA increases with heme intake in rats and humans, and it shows the formation of 4-hydroxynonenal (HNE) in foods or in the body (31). We have recently explored the effect of fecal water rich in HNE on normal (Apc +/+) and premalignant colonic cells (Apc Min/+) (32). Apc mutated cells survive heme-induced fecal lipoperoxides, notably HNE, that is toxic to normal cells. Selection of mutated cells by cytotoxic lipoperoxides may explain promotion of colon carcinogenesis by diet inducing large amount of HNE. However, in this study, we can suspect an overproduction of HNE in ham diets due to freeze drying. Indeed, formation of HNE, facilitated by concomitant presence of heme iron and omega 6 fatty acids, is enhanced by freeze drying (33). It would therefore be preferable in future studies to work with a meat that is not freeze dried.

An alternative hypothesis to explain processed meat promotion tells that nitrite in cured meat enhances the formation of carcinogenic NOC, in food and endogenously (7,9,34,35). This hypothesis was not tested in this study. Parnaud et al. (10,18) have shown that fried bacon-fed rats excrete 10 to 20 times more NOCs in feces than controls. Mirvish et al. (36) showed that hot dog contains 10 times more NOCs than fresh red meat. This high level of NOCs could explain why nitrite-treated processed meat is associated more strongly than fresh red meat with colorectal cancer risk (5). However, fecal NOCs from bacon do not initiate or promote preneoplastic lesions in the colon of rats (10,18); and we thus think this hypothesis is not supported by experimental studies in rodents, although it receives much support from human volunteers' studies (7,9,34,35).

In conclusion, this study shows for the first time that a cured meat, freeze-dried cooked ham, can promote colon carcinogenesis in a rodent model. It also suggests that hemin, but not hemoglobin, may be used as a model agent for processed meat studies.

ACKNOWLEDGMENTS

We thank Xavier Blanc (UPAE) for the preparation of experimental diets, Raymond Gazel and Florence Blas Y Estrada for the care of the animals, and Michael Charton for the participation in the short-term study. The studies were supported in part by the INRA, by the DGER, and by a grant of the French region Midi-Pyrénées.

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