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Potential Carcinogenic Mechanisms by Red & Cured Meat

Denis E. Corpet

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ENVT - Ecole Nationale Vétérinaire Toulouse - France



D.E. Corpet – KSLA - Stockholm - 2012





Eating Meat Promotes Cancer

PETA



WARNING: HOT DOGS CAN STRIKE YOU OUT—FOR GOOD

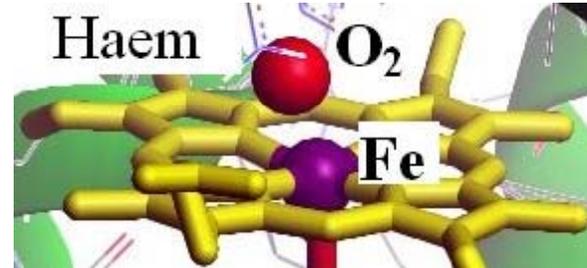
CancerProject

Exaggerated, but true. Why?

Five Hypotheses on Meat & Colorectal Cancer

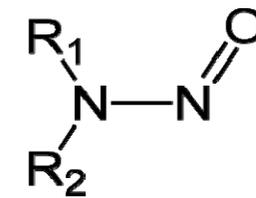
The relative importance of heme iron, nitrate & nitrite, and HCAs and PAH on CRC burden appears to be roughly equivalent, according to three recent cohort studies.

H1- Red meat contains myoglobin with **heme iron**

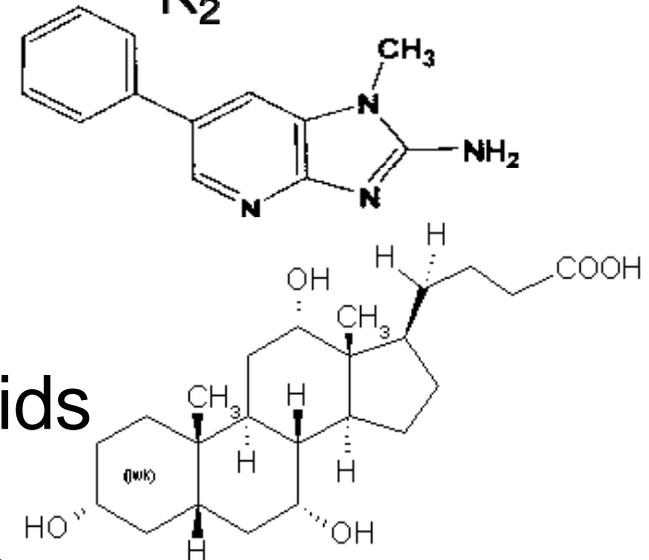


But epidemiology cannot provide true evidence. Experimental studies are needed to prove that a cause produces an effect, and to understand mechanisms

H2- Cured meat contains nitrate & nitrite => **N-nitrosated compounds (NOC)**



H3- Cooking at a high temperature => **Heterocyclic Amines (HCA)** and Polycyclic Aromatic Hydrocarbons

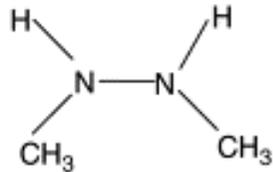


H4- Fat => excess calories & bile acids

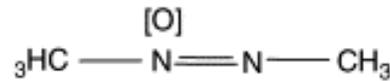
H5- Meat only lacks protecting agents (e.g., calcium, phytochemicals)

Method: Rats given a colon carcinogen

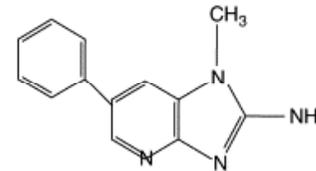
DMH, AOM, PhIP, MNU, MNNG...



Dimethylhydrazine - DMH

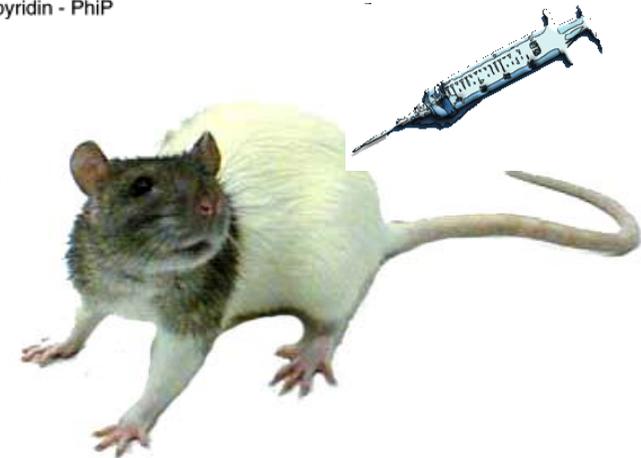


Azoxymethane - AOM

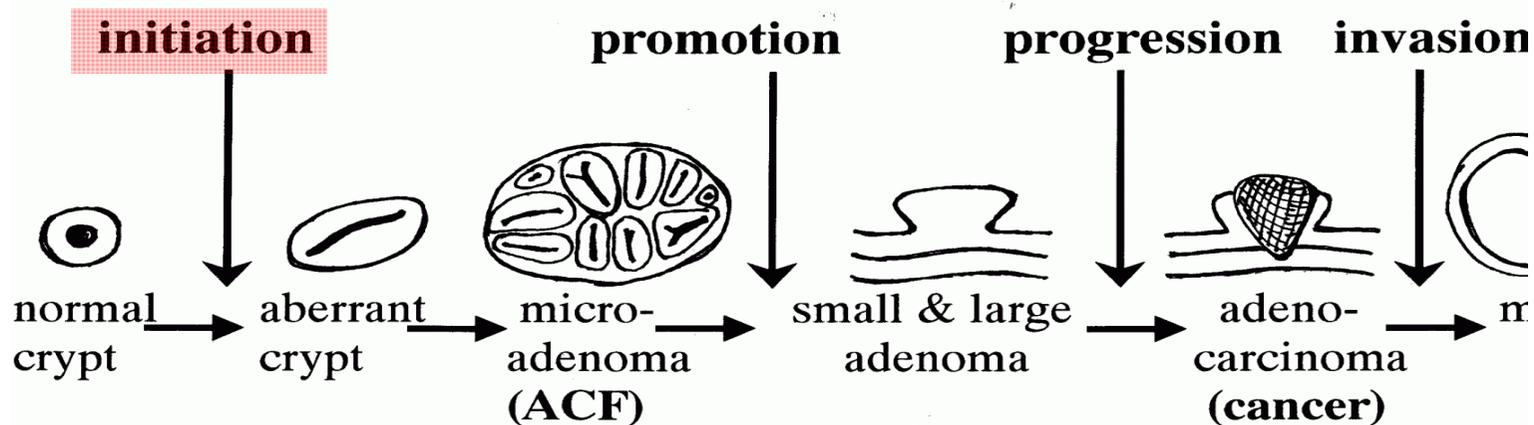


2-amino-1-methyl-6-phenylimidazol
[4,5-b]pyridin - PhIP

No spontaneous colon cancer in rats
but **easy to induce ACF, MDF & cancers**



Tumor development & pathology
are similar in rats and in humans





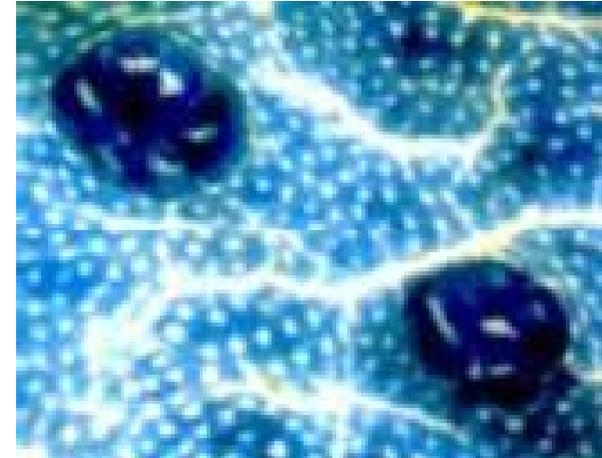
Rat model with precancer lesions

ACF, Aberrant Crypt Foci

Methylene Blue staining x40,
15d after carcinogen initiation

Correlation with cancer, not 100%
(Bird, *Cancer Let.* 1987)

Ki-ras mutation



MDF, Mucin Depleted Foci

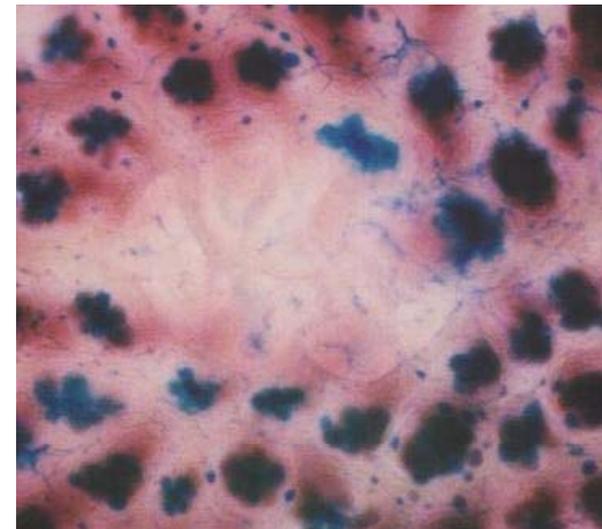
HIDAB staining x40 high iron diamine Alcian blue

100d after carcinogen initiation

MDF-cancer correlation >> ACF

(Caderni, *Cancer Res.* 2002)

Apc mutation (Femia, 2007)



Experimental testing of the 3 major hypotheses

- Carcinogen-initiated rats given meat-derived factors for 100d in a 2x2x2 design

H1- Hemoglobin 1% in diet. **Heme**

H2- Nitrite + Nitrate (0.17 + 0.23 g/l NaNO₂ + NaNO₃) in drinking water
0 = zero Nitrite / N = added Nitrite

H3- Heterocyclic amines

(PhIP + MeIQx) in diet. **HCA**

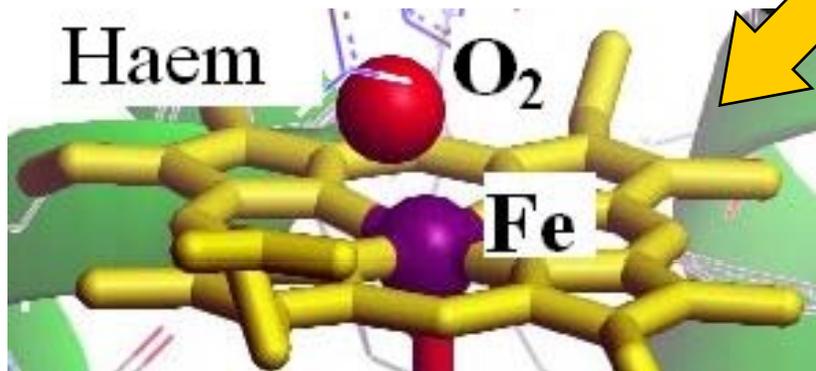
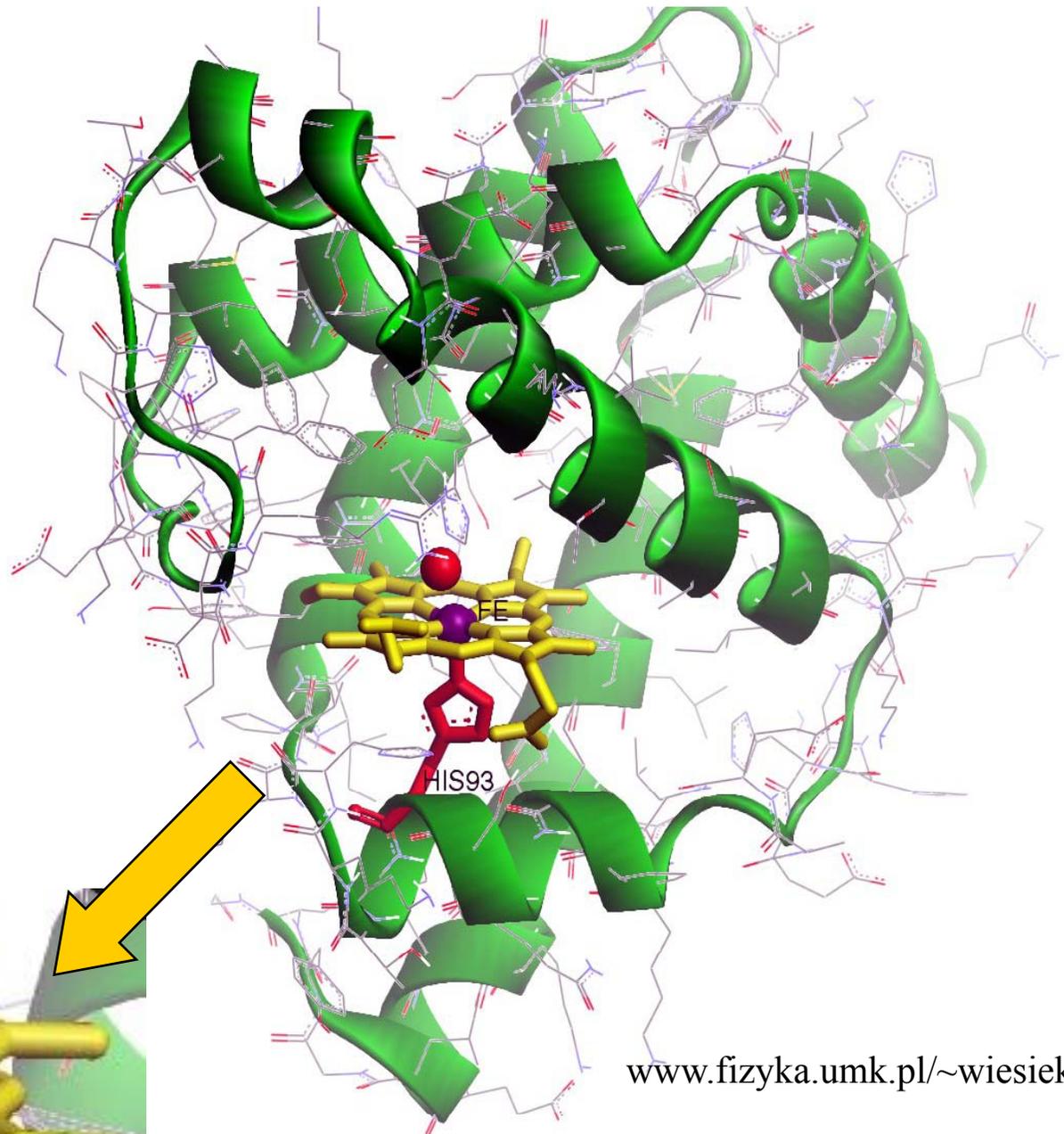
- Endpoint: Number of precancer lesions per rat. **MDF per colon**

MDF per colon

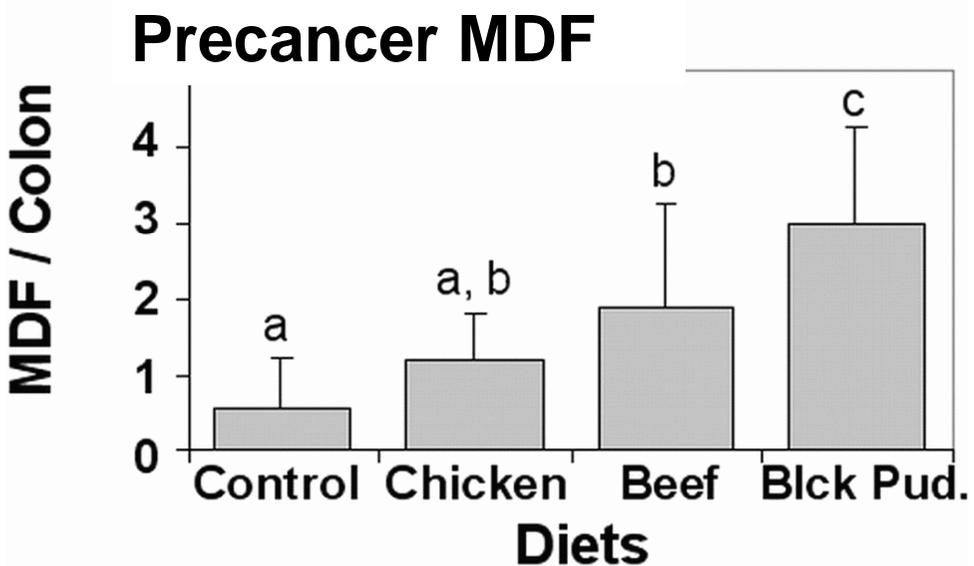
Data shown in Stockholm KSLA clearly show that heme only promoted MDF. Data removed because they are not yet published.

Hemoglobin diets significantly increased the number of MDF per colon ($p < 0.001$), independently of the two others factors

H1:
Red Meat
contains
Myoglobin
contains
Haem
contains
Iron

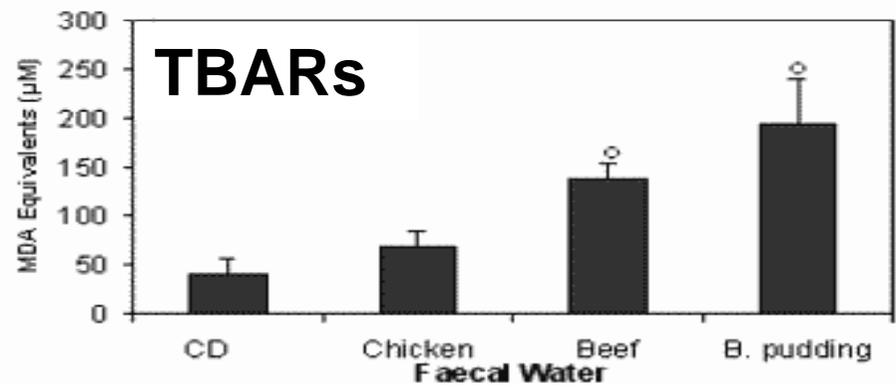


Rat Model → First evidence of red meat & heme promotion of colon carcinogenesis:



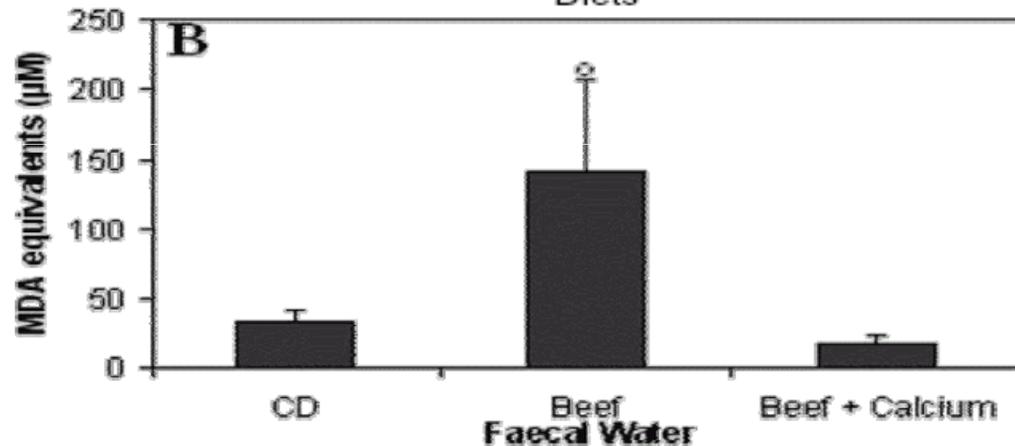
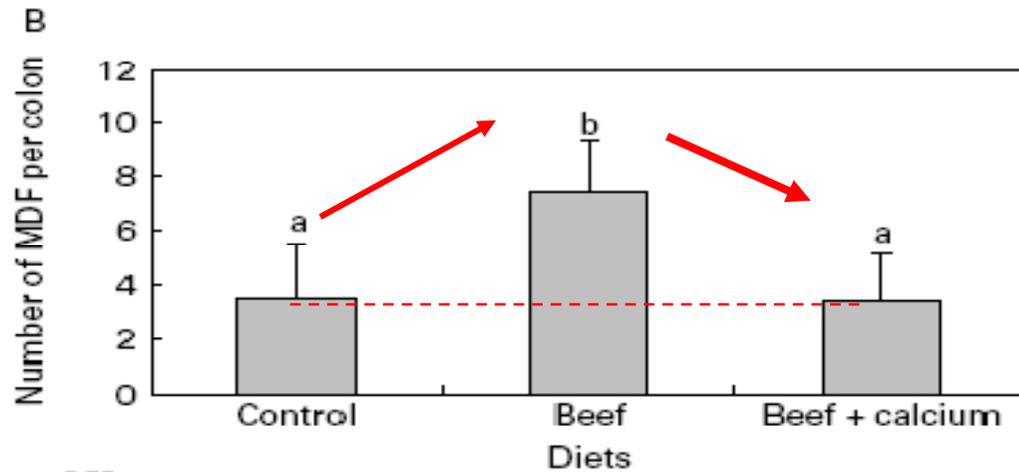
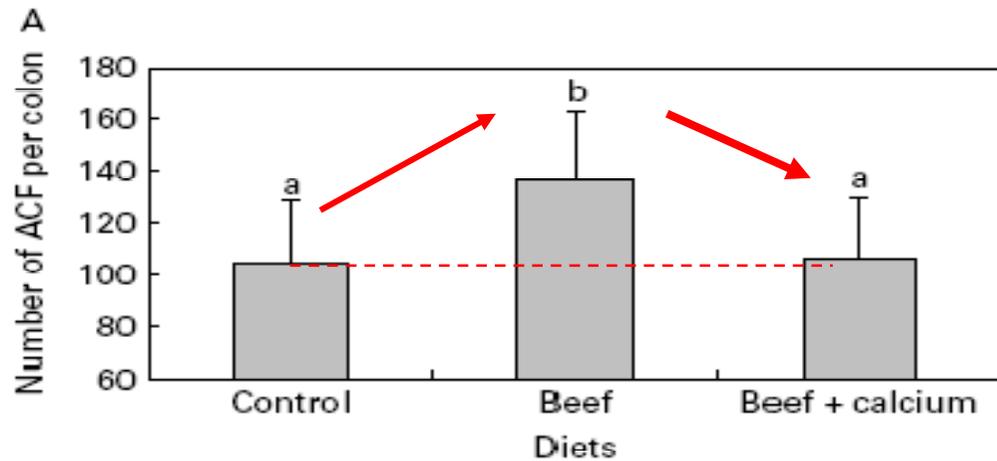
In a **calcium-depleted diet**, Beef meat & Black pudding (blood sausage) promote Mucin Depleted Foci (& ACF)
More heme = more MDF

Pierre et al., J.Nutr. 2004



Beef meat & Black pudding raise fecal & urinary markers of fat peroxidation: TBARs, DHN-MA, cytotoxicity
More heme = more peroxides

Dietary Calcium normalizes beef meat effects



=> Calcium fully suppressed beef-induced promotion

Fat peroxides: MDA genotoxic & cytotoxic

-> Calcium fully suppressed beef-induced lipoperoxidation

MECHANISMS

Apc mutation induces resistance of colonic cells to lipoperoxide-triggered apoptosis induced by faecal water from haem-fed rats

F.Pierre^{1,*}, S.Tache¹, F.Guéraud², A.L.Rerole¹,
M.-L.Jourdan³ and C.Petit¹

¹Ecole Nationale Vétérinaire Toulouse, UMR INRA-ENVT Xénobiotiques,

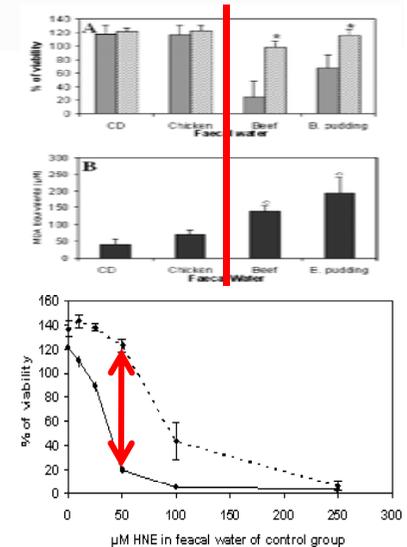
Published mechanistic studies suggest that heme-induced fat peroxides promote colon cancer by **selection of *apc* mutated cells** in the colonic mucosa

In all our previous rats studies, carcinogenesis promotion by fresh red meat or by heme was associated with fat peroxidations biomarkers:

- Fecal water TBARs
- Fecal water cytotoxicity
- Urinary DHN-MA (metabolite of 4-hydroxynonenal)

Pierre et al., 2003, 2004, 2006, 2007; Santarelli et al., 2008

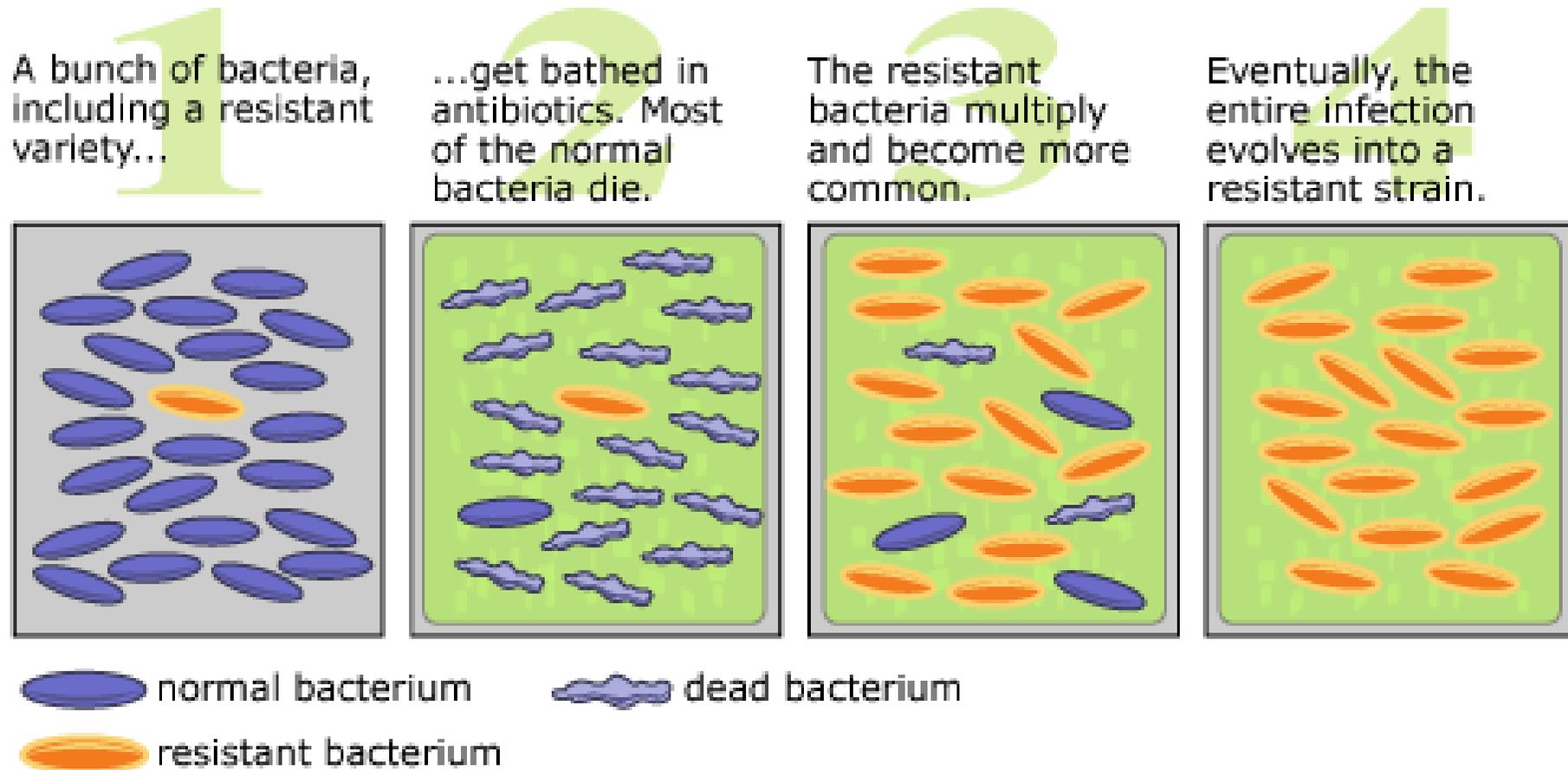
The agents that decrease the level of these biomarkers also suppress carcinogenesis in rats



How can haem & lipoperoxides promote cancer?

We guess it is by **selection** of cancer cells

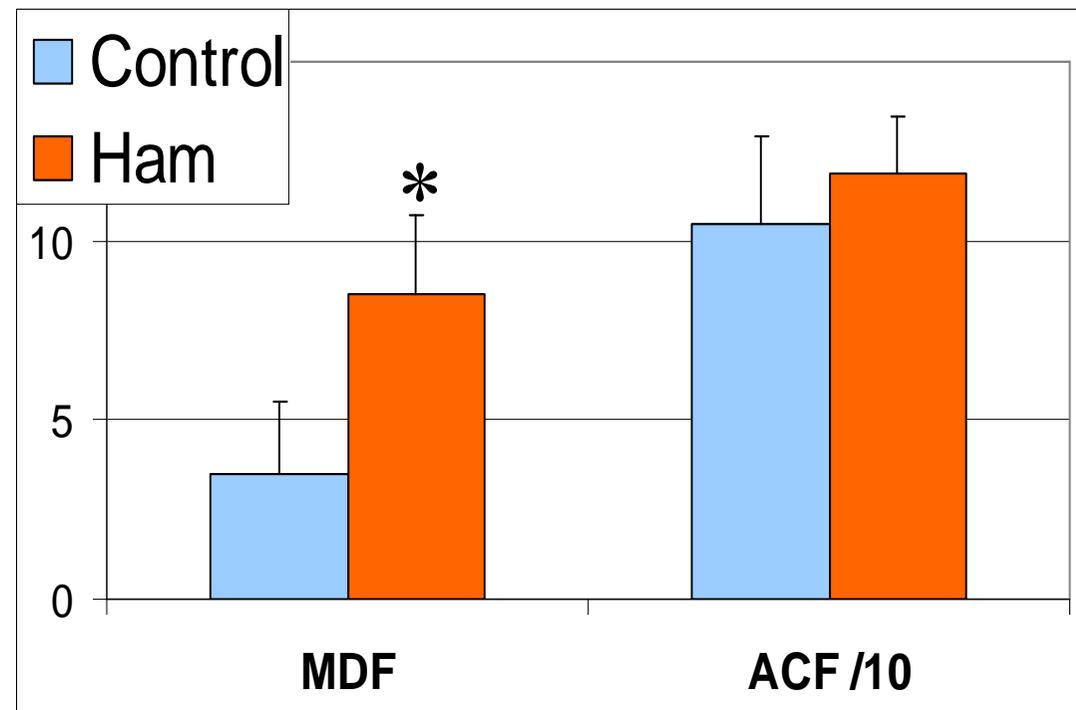
Similar to selection of drug resistant bacteria by antibiotics



Processed cured meat

Freeze-dried oxidized cooked ham

given to initiated rats promotes precancer MDF and increases markers of lipoperoxidation & cytotoxicity

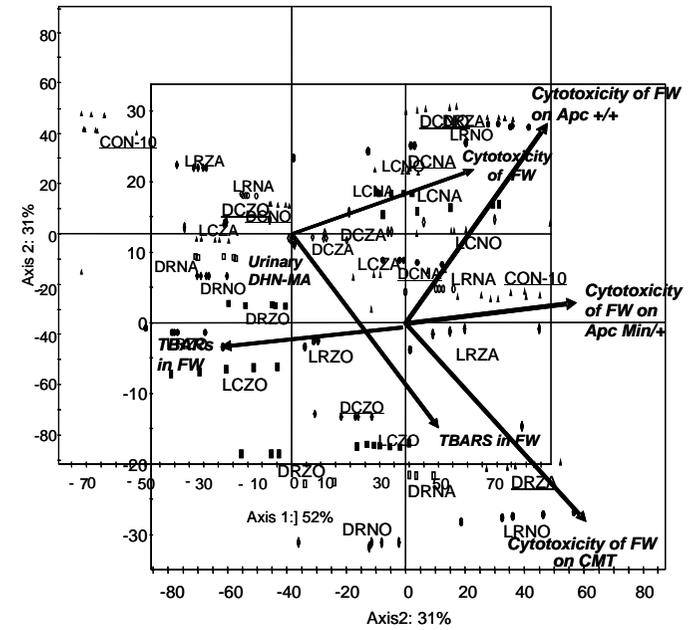


Pierre et al., Nutr. & Cancer, 2010

Design of a short-term study in rats given 16 models of cured meat



16 processes	Anaerobic packaging	Five day air exposure	Added Nitrite
Dark meat shoulder	DCZA DCNA	DCZO DCNO	DRZO DRNO
Light meat longiss. dorsi	LCZA LCNA	LCZO LCNO	LRZO LRNO
	Cooked 70°C	Raw 50°C	Cooked 70°C
			Raw 50°C



Principal component analysis of biomarker data, to choose 4 cured meat models → 100 d carcinogenesis study

Short-term study of 2x2x2x2 factors = 16 models of cured meat.
 End-points: Early fecal and urinary biomarkers used as screening tools



Dark Cooked Nitrited Oxidized = DCNO
 Dark Raw Zero-Nitrite Oxidized = DCZO
 Dark Raw Zero-Nitrite Anaerobic = DRZA
 Dark Cooked Nitrited Anaerobic = DCNA
Given for 100 days to carcinogen-initiated rats

Meat Processing and Colon Carcinogenesis: Cooked, Nitrite-Treated, and Oxidized High-Heme Cured Meat Promotes Mucin-Depleted Foci in Rats

Cancer
Prevention
Research

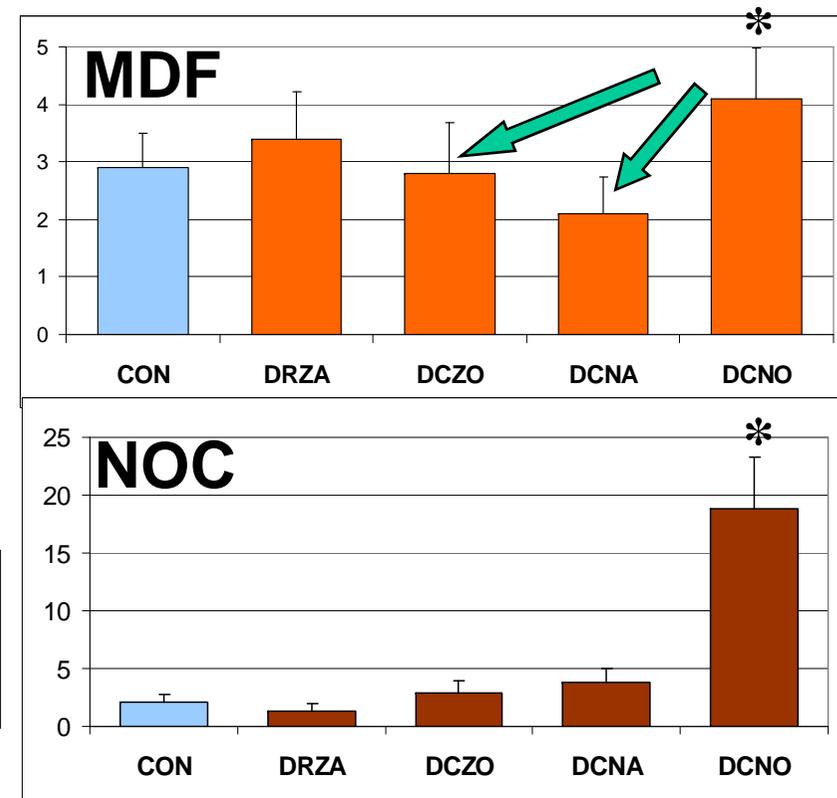


Raphaëlle L. Santarelli^{1,2}, Jean-Luc Vendevre², Nathalie Naud¹, Sylviane Taché¹, Françoise Guéraud¹, Michelle Viau³, Claude Genot³, Denis E. Corpet¹, and Fabrice H.F. Pierre¹

DCNO model cured meat (Dark, Cooked, Nitrite-treated and Oxidized high-heme meat) promotes colon carcinogenesis and increases fecal end products of lipid peroxidation in rats (Santarelli et al., *Cancer Prevention Research*, 2010).

But if No Oxygen or No Nitrite
DCNA & DCZO → No promotion

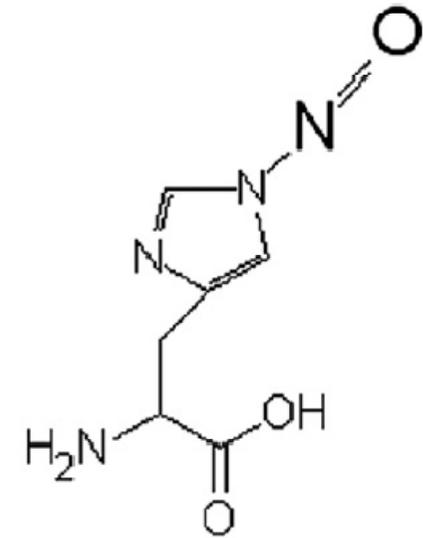
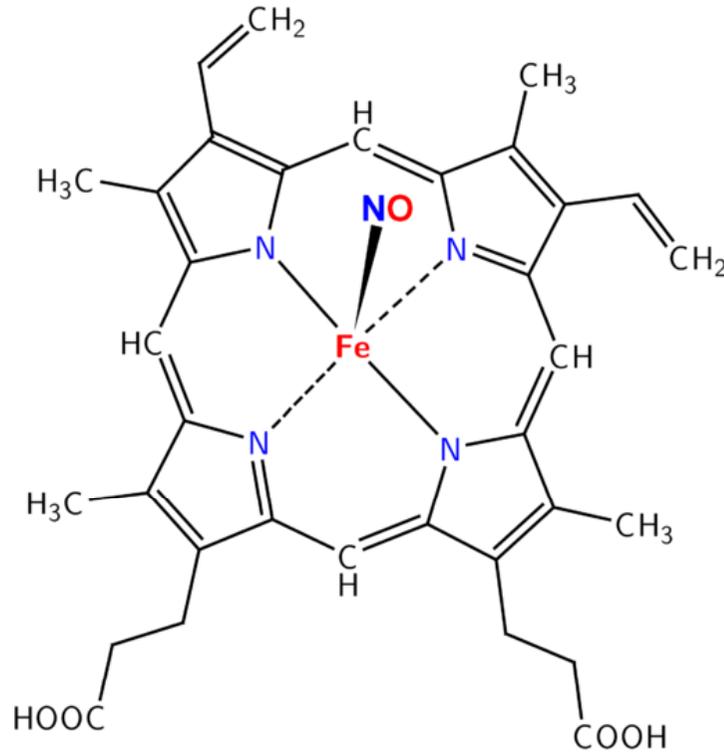
N-nitrosated compounds (NOCs):
Fecal **NOCs** associated with promotion



Effect of a cured meat diet on MDF and NOC formation in the colon of rats 106 days after carcinogen injection (values are means \pm SD, $n = 10$. * significantly different from control $P < 0.05$)

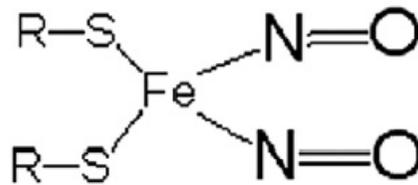
Nitrosyl
Heme

NOC structure

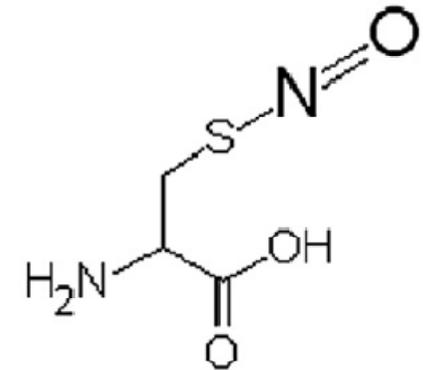


N-nitrosamine
(e.g. *N*-nitrosohistidine)

Carcinogenic
Nitrosamines
and many
other NOCs



Dinitrosyl iron complex



S-nitrosothiols
(e.g., *S*-nitrosocysteine)



H2: N-nitroso-compounds, NOC in vivo studies

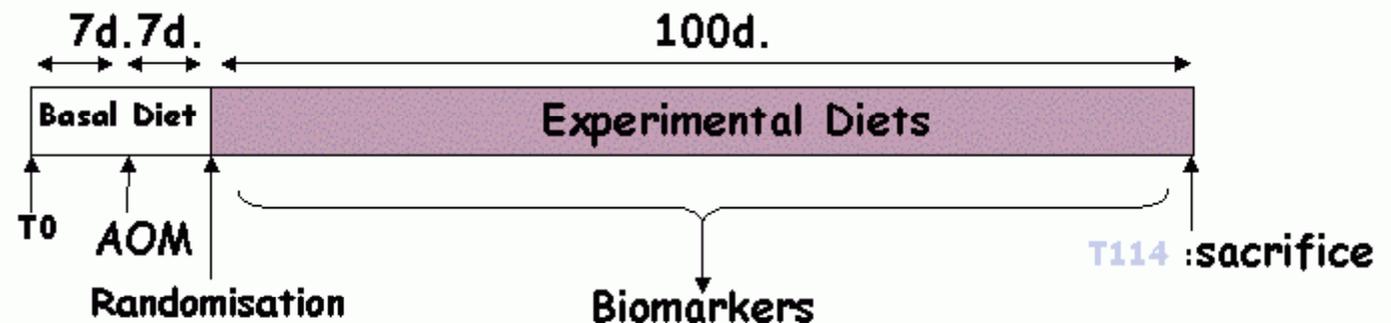
- Volunteers given red meat have more faecal NOC
Sheila Bingham 1996, Hugues 2001 : MRC Dunn, Cambridge, UK
- Haem is the NOC-inducing agent (*Cross & Bingham 2002*)
- Rodents given hot-dog : more faecal NOC (*Mirvish 2002*)
- Ileostomised study shows that NOCs are (*Kuhnle 2007*)
 - Nitrosothiols, formed in the acidic stomach
 - Nitrosyl Haem formed in the anaerobic colon
- None is carcinogenic (?), but they can "Nitrosate"
(transmit =NO to produce carcinogenic Nitrosamines)

Unpublished **Prevention Study**
in Rats and Humans



Aim: to **prevent** the promoting and pro-oxidant effects of cured meat with a heme iron binding-additive or an antioxidant-additive.

Calcium carbonate (150 μ mol/g) or **α -tocopherol** (0.05%) added to the model cured meat diet DCNO, and given for 100 days to rats pretreated with a carcinogen. Colons were scored for preneoplastic MDF.



Results 1: Prevention of Cured Meat MDF Promotion in Rats



In rats, cured meat DCNO increased the number of MDF/colon after feeding for 100d (p=0.01)

Calcium and α -tocopherol fully normalized the number of MDF/colon (p=0.01)

Data shown in Stockholm KSLA clearly show that DCNO ham promoted MDF, but adding calcium or alpha-tocopherol to the diet fully prevented promotion by DCNO. Data removed because they are not yet published.

Effect of cured meat diets on MDF formation in the colon of rats 99 days after the injection of 1,2-dimethylhydrazine, (*values are means \pm SD, n = 10*)
* *Significantly different from CON diet (P = 0.01)*
Significantly different from DCNO diet (P = 0.01)

Results 2: Prevention of Cured Meat-Induced Biochemical markers in Rats



In rats, cured meat increased fecal NOCs and TBARs (fat peroxides)

Calcium, but not tocopherol, reduced fecal TBARs, NOCs & cytotoxicity, and urinary DHN-MA, in cured meat-fed rats (only TBARs data are shown)

Data shown in Stockholm KSLA clearly show that DCNO ham increased fecal NOCs and TBARs, but adding calcium to the diet fully prevented these DCNO effects. Data removed because they are not yet published.

Effect of cured meat diets on fecal peroxidation biomarkers (TBARs) in rats after 80 days on experimental diets (*values are means \pm SD, n = 5*)

* *Significantly different from CON diet, P = 0.01*

Significantly different from DCNO diet (P = 0.01)

Results 3 : Human volunteers' data fit rats data



TBARs & NOCs increased in stools of 17 volunteers given cured meat compared with meat-free period (Wilcoxon $P < 0.05$).

Calcium supplementation normalized fecal TBARs and NOCs in volunteers given cured meat ($P < 0.05$), but α -tocopherol normalized TBARs only.

*

Data shown in Stockholm KSLA clearly show that DCNO ham increased fecal NOCs and TBARs, in volunteers, but adding calcium to the diet fully prevented this DCNO effect. Data removed because they are not yet published.

Effect of cured meat diets on fecal biomarkers (TBARs) in stools of **volunteers** after 4 days on experimental diets (*values are means \pm SEM, n = 17*)

* *Significantly different from meat free period, $P < 0.05$*

° *Significantly different from DCNO period, $P < 0.05$*



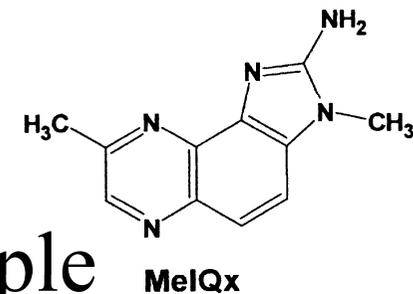
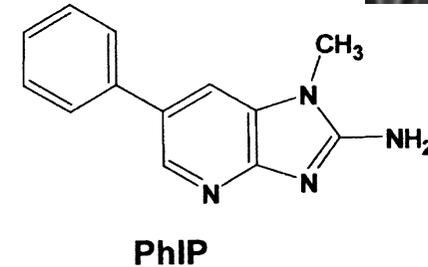
H3: Heterocyclic Aromatic Amines

Takashi Sugimura 1977 (Tokyo, Japan) ... Rashmi Sinha (NCI, Bethesda, MD)

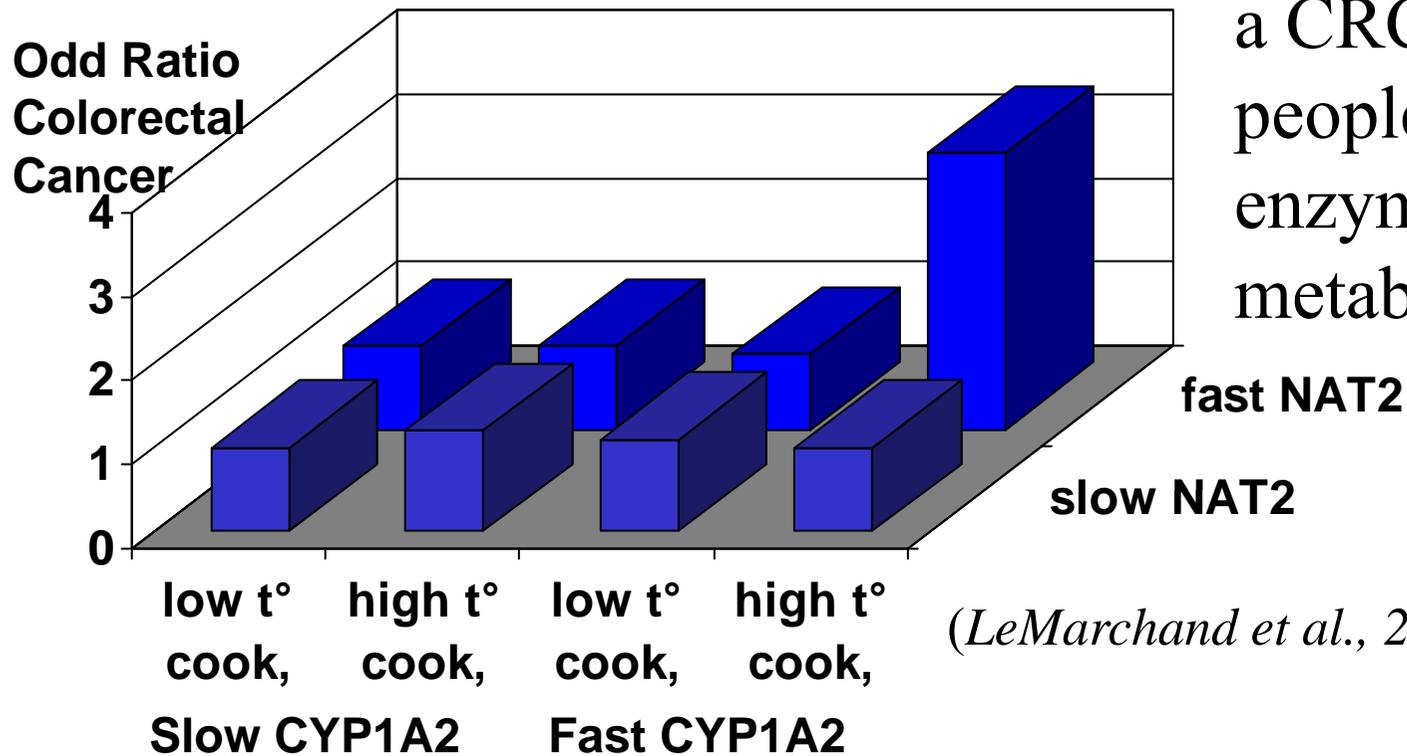


More than 20 HCA, e.g., MeIQx

- Origin: t°C+AA+ creatine+ sugar
- Very potent mutagens /bacteria
- Carcinogenic in animals: high dose induces colon & mammary tumors
- Extrapolating dose from rodents to people
Human risk seems very low (2 CRC/10 000)
- Roasted chicken, a major provider of PhIP, is **not** associated with colon cancer risk
- Only diMeIQx from beef meat would play a role



H3- Meat cooking, phenotypes & colorectal cancer in Hawaii



(LeMarchand et al., 2002)

Overcooked meat is a CRC risk only in people with "fast" enzymes for **HCA** metabolism

H4- Is Dietary Fat pro-cancer?

- Insulin resistance (long-term energy imbalance)
 - Promoting factors in blood (IGF-1, insulin, glucose, fatty acids)
 - Obesity: fatty cells aromatase=> more estrogens
- Secondary bile acids (e.g., Lithocholic acid) are aggressive detergents toward colon mucosa
- Oxidized fats are genotoxic
Fat PUFA + O₂ (+ heme) => MDA => DNA adducts **BUT**
- Meta-analysis: animal fat intake **not** a risk factor for CRC
- Intervention studies in humans: No reduction in adenoma recurrence or CRC incidence with low-fat diet
- → Fat not a major factor in CRC risk

Conclusion on the Hypotheses

- **Heme iron in red meat can promote cancer**, via two pathways:
 - **Fat peroxidation** (produces cytotoxic alkenals)
 - **Nitrosation** (produces potentially carcinogenic NOCs)
- **Nitrosation & NOCs: important role in cured meat**
- **Heterocyclic amines** (HCAs, surface of well-done meat), probably cause CRC in people with genetic predisposition.
- **HCAs, Heme and Nitrite of similar importance** on CRC burden, according to 3 observationnal studies
- **But** our experimental studies in rodents points towards **heme as a major player in CRC etiology**

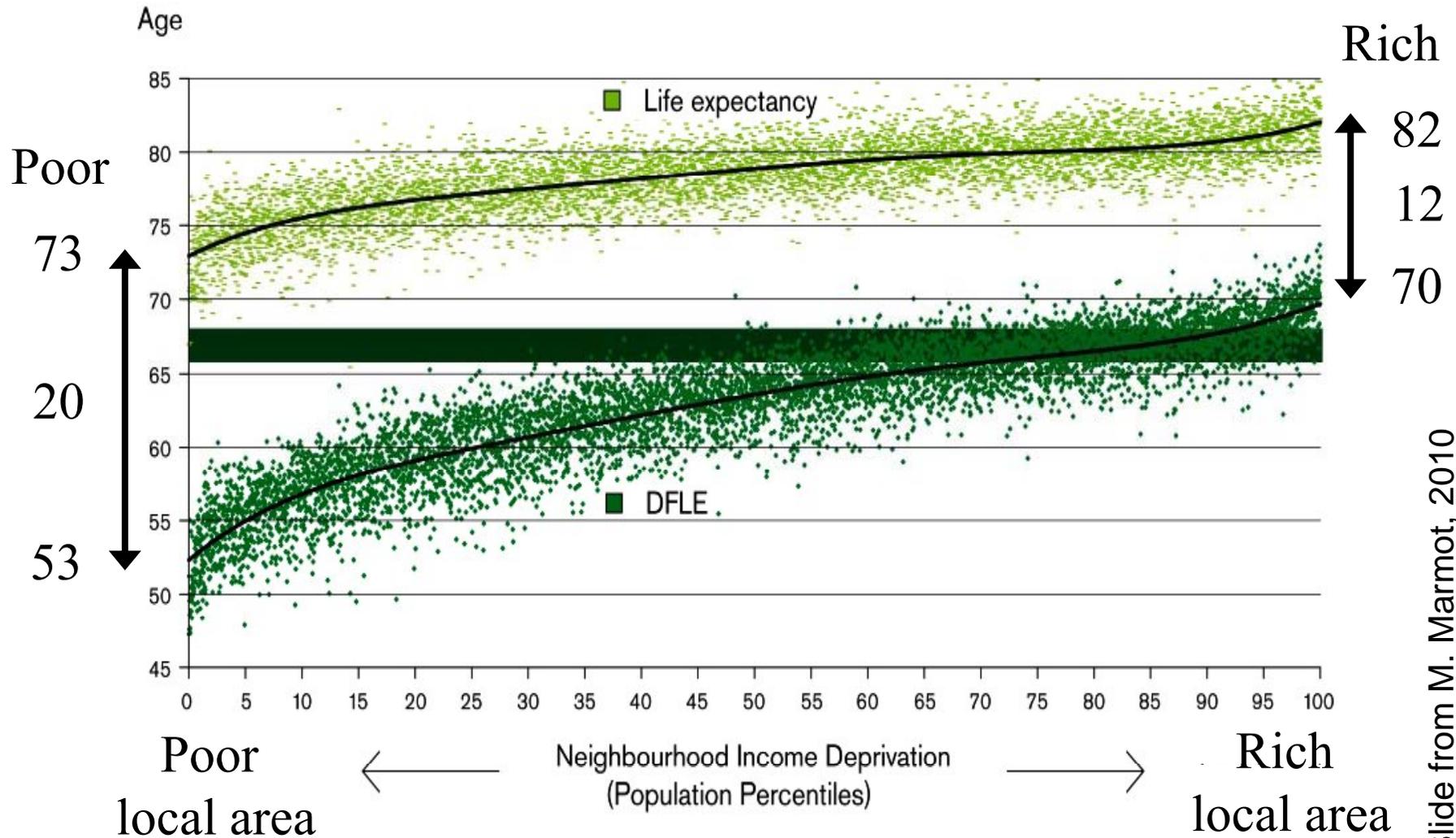
Conclusion of our prevention studies

- Promotion of colon carcinogenesis in rats by cured meat suppressed by diet calcium and by α -tocopherol.
- Calcium (and tocopherol) normalized associated fecal and urinary biomarkers in rats and in human volunteers given cured meat.
- Ongoing studies in my lab: prevention by polyphenols
- Many people are reluctant to change their diet: the advice “*avoid processed meat*” is badly adhered to, particularly by poor people. The addition of specific agents to cured meat may provide a new way to prevent colorectal cancer...

... and to move towards a fair society ?

Life Expectancy at Birth, and Disability-Free Life Expectancy (DFLE)

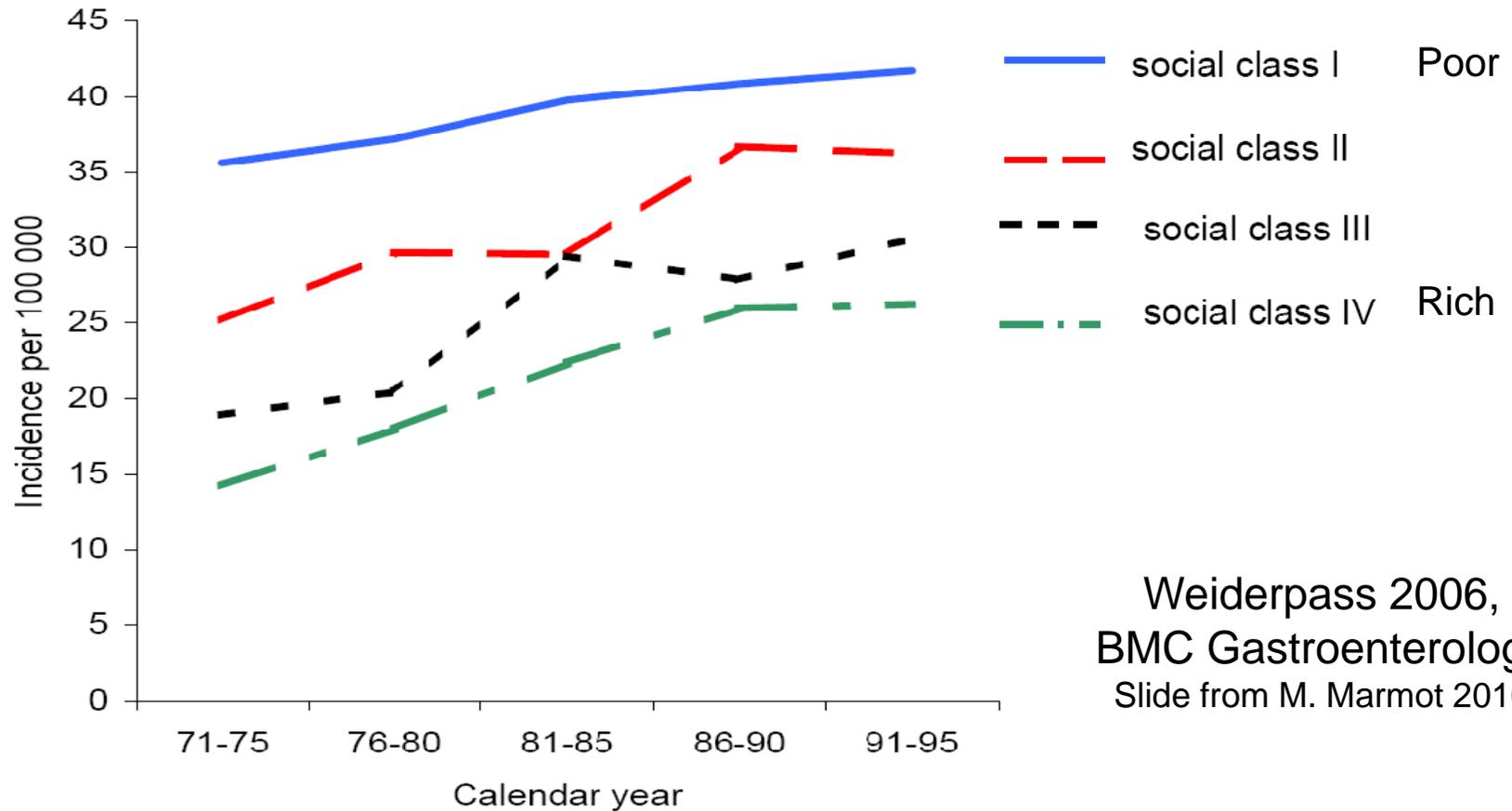
by Neighbourhood Income Deprivation, England, 1999-2003



Slide from M. Marmot, 2010

Age adjusted incidence of Colon cancers among male Finns

aged 45–64 at the beginning of each 5-years period between 1971–1995



Weiderpass 2006,
BMC Gastroenterology
Slide from M. Marmot 2010

Health inequalities

- The poor live shorter lives in good health than the rich. Colorectal cancer is a part of the inequality burden. Red meat & cured meat are a part of the colorectal cancer burden.
- Less educated people do not follow advices about smoking, exercise, sensible drinking and healthy eating.
- But are they really free to do so?
No, according to sir Michael Marmot (Marmot, 2010)
- Responsibility of agro-industry is to make a safer meat:

**More ethical, an easier, to change the food
than to change the consumer**



Eating Meat Promotes Cancer

Go Vegan 

Go vegan ... or make your meat safer!

Potential paths for a safer cured meat:

- Change diet (e.g., less meat, more calcium)
- Change process (e.g., without O₂ or NO₃)
- Use new additives (e.g., calcium, tocopherol)