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# A test of the mutagenicity of cooked meats *in vivo*

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**There is a correlation between intestinal cancer and diets high in meat, so fried beef, chicken, lamb, pork and fish were tested for their ability to induce mutations in the small intestine of mice. The mice were bred to be heterozygous at the *Dlb-1* locus so that loss of the dominant *Dlb-1*$^b$ allele by mutation could be detected. Mice were fed the AIN-76A diet (which contains 50% of the calories in the form of sucrose) or an isocaloric diet in which the sucrose was replaced by meat or fish, for 5 or 9 weeks. Manifestation of mutants requires ~1 week in this system, so this corresponds to an effective exposure of 4 and 8 weeks, respectively. There was no significant difference in the weights of animals on the different diets, and no difference in mutant frequency. Several food mutagens were present, but at low levels. These results, when considered in the light of tests of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine and amino(α)carboline at much higher doses (Zhang,X.-B., Tao,K.S., Urlando,C., Shaver-Walker,P. and Heddle,J.A. (1996) *Mutagenesis*, 11, 43–48), indicate that there is no highly mutagenic compound missed by previous testing with bacterial assays and that mixtures of heterocyclic amines at low levels do not show great synergy.**

## Introduction

The correlation between meat consumption and intestinal cancer (Haenszel *et al.*, 1973), especially colonic cancer, has yet to be satisfactorily explained in mechanistic terms. Two general hypotheses have been advanced, namely that high consumption of meat is associated with a dietary factor that causes cancer or that high consumption of vegetables is associated with a factor that protects against cancer (Doll and Peto, 1981). Dietary fat has been the most common putative cause investigated (Carroll *et al.*, 1986), usually as a promoter of cancer rather than an initiator, as it is in mammary cancer (Carroll and Braden, 1984). We have not detected any effect of high fat diets on the mutation frequency in the colon or small intestine of mice (Zhang *et al*., 1996b), which is in accord with epidemiological evidence (Giovannucci *et al*., 1994; Willett, 1994). Food mutagens have been suggested as an alternative possible cause. Several food mutagens have been detected by means of the Ames *Salmonella*/mammalian microsome test for mutagenicity, a number of them in cooked meat (Felton *et al.*, 1986). Of these, the heterocyclic amines—formed by partial pyrolysis of amino acids, sugar or creatinine at high temperatures—have been shown to be carcinogenic and mutagenic in mice and rats (Ohgaki *et al.*, 1991; Brooks *et al.*, 1994; Zhang *et al.*, 1996a).

Bacterial assays do not correlate as well with carcinogenicity as once believed (Tennant *et al.*, 1987), so it is possible that there may be other mutagens in meat, particularly cooked meat, that are mutagenic *in vivo*. It is conceivable that the combination of food mutagens present in cooked meat may be more effective in their original state or in combination than are the isolated compounds. This would be important, since the concentrations of these compounds in food are quite low compared with that required to produce cancer or mutation in laboratory animals (Felton and Knize, 1991; Wakabayashi *et al.*, 1993). The purpose of this study was to determine if cooked meat containing only moderate concentrations of the known food mutagens would be detectably mutagenic.

## Materials and methods

All treatment protocols were reviewed and approved in advance by the York University Animal Care Committee and conformed to Canadian guidelines for animal care. The mice used were the $F_1$ progeny from a cross between male Big Blue mice (*Dlb-1*$^b$/*Dlb-1*$^b$), which carries the bacterial *lacI* gene in a recoverable λ vector (Kohler *et al.*, 1991), and SWR females (*Dlb-1*$^a$/*Dlb-1*$^a$). It was our intention to measure mutations of the *Dlb-1*$^b$ allele in the small intestine and of the *lacI* gene in the colon, but the DNA samples from the colon were accidentally destroyed and could not be used.

The meat and fish were purchased at a supermarket in Toronto. They were fried at 203°C until they appeared to be well done (4 min for fish, 5 min for pork, 5 min for chicken, 5.5 min for lamb, and 15 min for beef) They were then ground and incorporated into the AIN-76A diet without sucrose at an isocaloric level. The caloric content was calculated from the Canadian Nutrient File. The food was changed twice weekly; animals were allowed to eat *ad libitum*. The animals were weighed regularly to check that the diet was not having an unexpectedly adverse effect upon them. Food consumption was similar in all groups. Animals were left on these diets for 5 or 9 weeks. Before the start of the experiment, the animals of each gender were assigned at random among the treatment groups and then these groups were assigned to a treatment at random.

At the end of the exposure, the animals were killed by cervical dislocation. The small intestines were prepared for examination as described by Winton *et al.* (1988) with minor modifications as described by Tao *et al.* (1993) and stained for the presence of the lectin-binding site determined by the *Dlb-1*$^b$ allele. Mutants were observed as non-staining ribbons on the surface of the villus. There are ~10 stem cells per villus, so each villus examined represents 10 mutable loci (Cosentino *et al*., 1996) and the mutant frequency is given as mutants/100 000 stem cells, which is the equivalent of ribbons observed/10 000 villi. About 10 000 villi, corresponding to 100 000 stem cells, were examined from each mouse.

Heterocyclic amines were measured by standard methods (Knize *et al.*, 1994).

## Results

The diets were well tolerated and all groups showed similar weight gains during the experiment (data not shown). The results obtained from the mutation analysis are given in Table I and shown graphically in Figure 1. Most treatment groups showed a very small increase in mutations from the 5 week sample to the 9 week sample, but this never exceeded the

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**Table I.** *Dlb-1* mutation frequency in the intestines of mice fed various cooked meats

| Treatment | Period (weeks) | Animal | Gender | No. of villi examined | Mutant ribbons | Mutants per $10^5$ stem cells | Individual mean ± SEM |
|---|---|---|---|---|---|---|---|
| AIN-76A | 5 | 1609 | female | 9775 | 0 | 0 | 1 ± 0.4 |
| | | 1648 | female | 9949 | 2 | 2 | |
| | | 1616 | male | 8606 | 1 | 1.2 | |
| | | 1631 | male | 15 363 | 1 | 0.7 | |
| | 9 | 1626 | female | 13 634 | 3 | 2.2 | 2 ± 0.1 |
| | | 1663 | female | 11 051 | 2 | 1.8 | |
| | | 1619 | male | 10 192 | 2 | 2 | |
| Fried beef | 5 | 1614 | female | 13 620 | 0 | 0 | 8 ± 3 |
| | | 1633 | female | 14 475 | 1 | 0.7 | |
| | | 1613 | male | 9480 | 1 | 1.1 | |
| | | 1641 | male | 15 720 | 2 | 1.3 | |
| | 9 | 1618 | female | 14 330 | 2 | 1.4 | 2 ± 0.3 |
| | | 1627 | male | 11 384 | 3 | 2.6 | |
| | | 1658 | male | 12 870 | 2 | 1.6 | |
| | | 1645 | female | 13 154 | 3 | 2.3 | |
| Fried lamb | 5 | 1622 | male | 10 178 | 1 | 1 | 0.7 ± 0.2 |
| | | 1649 | female | 9937 | 1 | 1 | |
| | | 1617 | male | 14 560 | 1 | 0.7 | |
| | | 1637 | male | 10 248 | 0 | 0 | |
| | 9 | 1632 | female | 12 022 | 1 | 0.8 | 2 ± 0.3 |
| | | 1615 | male | 16 907 | 3 | 1.8 | |
| | | 1652 | male | 15 650 | 4 | 2.6 | |
| | | 1662 | female | 12 186 | 2 | 1.6 | |
| Fried pork | 5 | 1606 | female | 11 716 | 2 | 1.7 | 1.5 ± 0.3 |
| | | 1642 | female | 10 701 | 2 | 1.9 | |
| | | 1644 | male | 11 675 | 1 | 0.9 | |
| | 9 | 1660 | female | 14 254 | 3 | 2.1 | 1.8 ± 0.2 |
| | | 1628 | male | 15 078 | 2 | 1.3 | |
| | | 1661 | male | 15 985 | 3 | 1.9 | |
| | | 1630 | female | 16 271 | 3 | 1.8 | |
| Fried chicken | 5 | 1635 | female | 12 540 | 1 | 0.8 | 0.1 ± 0.3 |
| | | 1638 | male | 12 169 | 2 | 1.6 | |
| | | 1639 | male | 14 770 | 1 | 0.7 | |
| | 9 | 1640 | female | 11 498 | 2 | 1.7 | 1.6 ± 0.1 |
| | | 1655 | female | 12 920 | 2 | 1.5 | |
| | | 1656 | male | 13 120 | 2 | 1.5 | |
| Fried fish | 5 | 1623 | female | 13 680 | 2 | 1.5 | 1 ± 0.2 |
| | | 1643 | female | 12 330 | 1 | 0.8 | |
| | | 1610 | male | 12 770 | 1 | 0.8 | |
| | 9 | 1611 | female | 14 706 | 2 | 1.4 | 1.7 ± 0.2 |
| | | 1659 | female | 15 950 | 3 | 1.9 | |
| | | 1621 | male | 13 720 | 3 | 2.2 | |
| | | 1651 | male | 16 178 | 2 | 1.2 | |
| ENU (200 mg/kg) | 5 | 1612 | female | 12 850 | 160 | 124.5 | 140.8 ± 11.2 |
| | | 1629 | female | 12 570 | 204 | 162.3 | |
| | | 1620 | male | 12 550 | 170 | 135.5 | |
| | 9 | 1605 | female | 14 278 | 185 | 129.6 | 130.3 ± 2.8 |
| | | 1653 | female | 12 784 | 177 | 138.5 | |
| | | 1625 | male | 14 083 | 178 | 126.4 | |
| | | 1646 | male | 14 996 | 190 | 126.7 | |

increase observed in the controls. There is, therefore, no need for statistical analysis of the results to determine if the treatments induced mutations. Power analysis of the data showed that the power to detect a doubling in the mutant frequency was essentially 1.0, i.e. that there was virtually no chance that a real doubling would have been missed with these samples. It is noteworthy that the samples were large enough to detect the small but significant increase in mutant frequency with age. Evidently ageing has a greater impact on the mutant frequency than the consumption of 50% of calories from fried meat or fish.

An analysis of the concentration of some common food mutagens in our samples is included in Table II. It can been seen that the conditions used in cooking these food samples, although they produced some food mutagens, were not extreme enough to produce the high levels that can be found under some conditions.

## Discussion

Epidemiological studies have established that colon cancer is caused by environmental factors, since migrants from Japan (where there is a low rate of colon cancer) to the USA (where the rate is high) have rates that approximate the high rate (Haenszel, 1973). In many studies, consumption of meat and fat has been found to be correlated with high rates of colonic cancer, but the mechanism responsible is unknown. Many studies have been conducted on the effect of diets upon the frequency of colon cancer induced by chemical carcinogens, 1,2-dimethylhydrazine in particular, in mice and rats (McIntosh

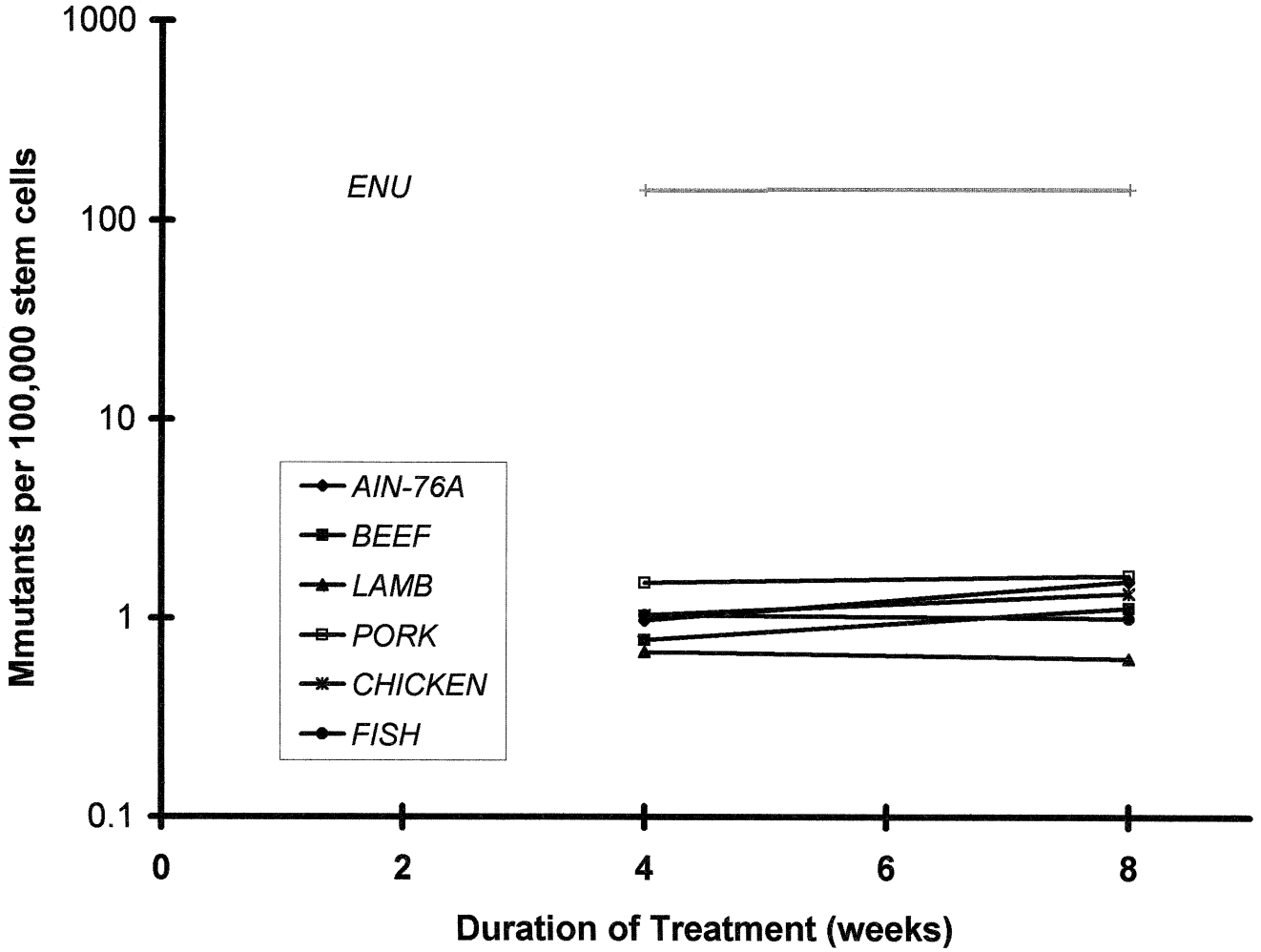


**Fig. 1.** Mean frequencies of mutations observed at the *Dlb-1* locus in mice fed the control (AIN-76A) diet or diets containing various cooked meats or fish. Note that the scale is logarithmic and that the positive control (ENU at 250 mg/kg) is ~100-fold higher than the other curves. The effective duration of the treatment, shown as the abscissa, is the duration of feeding minus 1 week, since 1 week is required for manifestation of mutations in this tissue.

**Table II.** Analysis of food mutagens in the samples of cooked meat

| Dietary substitution | $MeIQ_x$ | | $DiMeIQ_x$ | | PhIP | | IQ | | MeIQ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | p.p.b. | % Recovery | p.p.b. | % Recovery | p.p.b. | % Recovery | p.p.b. | % Recovery | p.p.b. | % Recovery |
| None | 0 | 49 | 0 | 44 | 0 | 50 | 0 | 32 | 0 | 22 |
| | 0 | 61 | 0 | 58 | 0 | 70 | 0 | 43 | 0 | 30 |
| Beef (3.5 min)[a] | 2.3 | 56 | 0 | 58 | 0.9 | 35 | 0 | 44 | 0 | 42 |
| | 5.5 | 20 | 0 | 39 | 0 | 21 | 0 | 31 | 0 | 41 |
| Beef (15 min) | 17.3 | 41 | 2.2 | 57 | 28.5 | 24 | 0 | 30 | 0 | 38 |
| | 18.0 | 45 | 7.0 | 50 | 40.1 | 18 | 0 | 35 | 0 | 34 |
| Fish (4 min) | 0 | 39 | 0 | 41 | 0 | 21 | 0 | 28 | 0 | 23 |
| | 0 | 59 | 0 | 60 | 0 | 64 | 0 | 33 | 0 | 34 |
| Lamb (5.5 min) | 5.2 | 42 | 3.4 | 44 | 9.4 | 26 | 0 | 32 | 0 | 31 |
| | 7.7 | 46 | 3.3 | 43 | 27.4 | 16 | 0 | 35 | 0 | 30 |
| Chicken (4 min) | 2.3 | 44 | 1.2 | 41 | 18.6 | 17 | 0 | 37 | 0 | 29 |
| | 2.0 | 43 | 2.0 | 44 | 18.2 | 15 | 0 | 37 | 0 | 33 |
| Pork (5 min) | 8.5 | 51 | 2.2 | 51 | 21.5 | 18 | 0 | 47 | 0 | 41 |
| | 7.7 | 51 | 2.0 | 48 | 17.7 | 17 | 0 | 47 | 0 | 39 |

[a]Cooking time.

*et al*., 1998). It is not clear, however, that this model is particularly relevant to the human situation where no acute exposure to a chemical carcinogen is likely and the only known chronic exposures are to ionizing radiations (UNSCEAR) and heterocyclic amines (Layton *et al.*, 1995). Heterocyclic amines are mutagenic in mice in the intestinal epithelium when animals are treated subacutely with high doses (Brooks *et al*., 1994) or chronically with lower doses (Zhang *et al*.,

1996a). For 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the mutation rate induced is proportional to the product of dose and time (Zhang *et al*., 1996a). If the murine mutation rate induced by PhIP is extrapolated to people, the mutation rate seems too low to account for the difference in cancer rates from one country to another, even with the much longer period of exposure. This is one of the attractions of using somatic mutations as a model: mutations are known to be involved in colon cancer and they are frequent enough to be measured in control animals; no carcinogenic treatment is necessary to investigate dietary effects.

The *Dlb-1* locus seems to be ideal for measuring mutations *in vivo* since it seems to be a neutral locus, i.e. one that has no influence on cell survival (Heddle *et al.*, 1995). There is experimental evidence for its neutrality in the small intestine, in that the mutation frequency is stable for many weeks following both acute and chronic exposures (Tao *et al.*, 1993; Shaver-Walker *et al.*, 1995). The mutation frequency observed is thus the integral of the mutation rate from conception (Zhang *et al.*, 1995) and a chronic exposure protocol should be, and is, the most sensitive method (Shephard *et al.*, 1993, 1994; Tao and Heddle, 1994; Zhang *et al.*, 1996a; Staedtler *et al*., 1999). Results from similar exposures to PhIP at much higher concentrations show that the accumulation of mutants approximated a linear increase as a function of dose, where dose was defined as the product of concentration and duration of exposure (Zhang *et al*., 1996a). The slope of that curve was ~0.0037 mutants/100 000 stem cells/p.p.m.-day. The highest exposure to PhIP was from fried beef at ~0.04 p.p.m. for 56 days, that is 2 p.p.m.-days. Clearly, this would not be expected to produce a detectable response. Since the other heterocyclic amines are present at similar or lower levels, they would have had to be much more mutagenic than PhIP or to have been synergistic in their effects to have produced a detectable response.

A previous study of isocaloric high fat diets in mice of the same genotype showed that such diets did not increase the mutation rate in the colon or the small intestine (Zhang *et al.*, 1996b). We have similar data for cooked fats (unpublished). Heterocyclic amines do increase the mutation rate in proportion to the product of concentration in the diet and exposure time, but the levels tested were high. The current results indicate that there is no unknown potent mutagen in cooked meats that might be responsible for the epidemiological results. They also indicate that the combination of food mutagens present at low levels does not interact to produce a large effect on the mutant frequency. Since the samples of colonic epithelium were lost, the results for the colon are not definitive. Although in most cases colonic epithelium responds very much like the small intestine, that limited study showed that one food mutagen, amino(α)carboline, seemed to be specific for the colonic epithelium. Our experiments did not address the possibility that the response of the colon might be different.

An alternative explanation for the correlation between consumption of meat or fat and colon cancer is that high fibre diets are protective (Ferguson, 1994). This is just as good an explanation, since high fat diets are almost always low in fibre and vice versa. The AIN-76A diet contains 5% fibre in the form of α-cellulose and 15% complex carbohydrate in the form of corn starch. It is a sufficient diet, with both minerals and vitamins at the recommended levels. More recent recommendations, incorporated into the AIN-93G diet, are for still higher levels of vitamins. Possibly these diets are significantly better than many human diets and protect against cancer in some unknown way.

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