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# A Sucrose-rich Diet Induces Mutations in the Rat Colon

**Lars O. Dragsted,[1] Bahram Daneshvar, Ulla Vogel, Herman N. Autrup, Håkan Wallin, Lotte Risom, Peter Møller, Anne M. Mølck, Max Hansen, Henrik E. Poulsen, and Steffen Loft**

*Institute of Food Safety and Nutrition, Division of Biochemical and Molecular Toxicology, The Danish Veterinary and Food Administration, DK-2860 Søborg, Denmark [L. O. D., B. D., A. M. M., M. H.]; National Institute of Occupational Health, DK-2100 Copenhagen, Denmark [U. V., H. W.]; Institute of Environmental Medicine, University of Aarhus, Universitetsparken, 8000 Aarhus C, Denmark [H. N. A.]; Institute of Public Health, University of Copenhagen, DK-2200 Copenhagen, Denmark [L. R., P. M., S. L.]; and Department of Clinical Pharmacology, University Hospital Copenhagen, DK-2100 Copenhagen, Denmark [H. E. P.]*

## ABSTRACT

**A sucrose-rich diet has repeatedly been observed to have cocarcinogenic actions in the colon and liver of rats and to increase the number of aberrant crypt foci in rat colon. To investigate whether sucrose-rich diets might directly increase the genotoxic response in the rat colon or liver, we have added sucrose to the diet of Big Blue rats, a strain of Fischer rats carrying 40 copies of the λ-phage on chromosome 4. Dietary sucrose was provided to the rats for 3 weeks at four dose levels including the background level in the purified diet [3.4% (control), 6.9%, 13.8%, or 34.5%] without affecting the overall energy and carbohydrate intake. We observed a dose-dependent increase in the mutation frequency at the *cII* site in the colonic mucosa with increased sucrose levels, reaching a 129% increase at the highest dose level. This would indicate a direct or indirect genotoxic effect of a sucrose-rich diet. No significant increase in mutations was observed in the liver. To seek an explanation for this finding, a variety of parameters were examined representing different mechanisms, including increased oxidative stress, changes in oxidative defense, effects on DNA repair, or changes in the background levels of DNA adducts. Sucrose did not increase the number of DNA strand breaks or oxidized bases assessed as endonuclease III-sensitive sites or 8-oxodeoxyguanosine in colon or liver. DNA repair capacity as determined by expression of the *rERCC1* or *rOGG1* genes was not increased in colon or liver, but the background level of DNA adducts (I-compounds) as determined by $^{32}$P postlabeling was significantly decreased in colon. This decrease in colon I-compounds correlated inversely with both mutation frequency and ERCC1 DNA repair gene expression. Dietary sucrose did not change liver apoptosis or cell turnover as determined by the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end labeling assay and proliferating cell nuclear antigen. An increase in liver ascorbate was also observed, whereas oxidative damage was not observed in proteins or lipids in liver cytosol or in blood plasma. We conclude that a sucrose-rich diet directly or indirectly increases the mutation frequency in rat colon in a dose-dependent manner and concomitantly decreases the level of background DNA adducts, without a direct effect on the expression of major DNA repair enzyme systems. We also conclude that an oxidative mechanism for this effect of sucrose is unlikely. This is the first demonstration of a genotoxic action of increased dietary sucrose *in vivo*. Both sucrose intake and colon cancer rates are high in the Western world, and our present results call for an examination of a possible direct relationship between the two.**

## INTRODUCTION

High levels of sucrose are uncommon in vegetables and fruits. Sucrose may therefore be considered a relatively novel food in the sense that it has only contributed substantially to the human diet since the late 19th century, when sugar gradually became less of a luxury. Whereas sucrose constituted only <1% of total carbohydrates in the diet before the 20th century, its use peaked in the 1950s, and it presently constitutes about 14% of all carbohydrate consumed in Denmark, corresponding to about 40 kg annually *per capita*. Similar intake levels have been reported in the United States (1). The consumption of other disaccharides and of monosaccharides is comparatively much smaller.

Risk of colon cancer and intake of sucrose have been found to be associated in a cohort study (2) and in a few case-control studies (3–5), although it was recently concluded that there is still insufficient evidence to link refined sugar intakes with colon cancer (6). Several studies have pointed toward a higher rate of large bowel cancers among diabetics, indicating a link between blood glucose regulation and colon cancer risk (7–10). High levels of refined sugars can increase the number of aberrant crypt foci in rats treated with chemical carcinogens (11–13). There is also some evidence for a cocarcinogenic action of sucrose on dimethylhydrazine- and azoxymethane-induced tumorigenicity in the rat colon (12, 14). Although sucrose can increase the proliferative index of the colonic crypt cells (13, 15), sucrose dosed after an initiating carcinogen does not strongly influence the progression of the aberrant foci toward higher complexity (14, 16). This would point toward an initiating or cocarcinogenic action of sucrose rather than an effect on tumor promotion. We have therefore investigated the mutagenicity of dietary sucrose at four dose levels in the colonic mucosal cells and liver of the Big Blue rat. To explore mechanisms of action, a wide range of markers of oxidative stress, oxidative defenses, DNA damage, and DNA repair capacity were also measured in these target cells.

## MATERIALS AND METHODS

**Chemicals.** All chemicals were used as supplied, without further purification. Fluoresceinamine (isomer II), sodium cyanoborohydride, 4-morpholinoethane sulfonic acid, and SDS were from Aldrich Chemical Co. (Steinheim, Germany). If not otherwise stated, all other chemicals were from Merck (Darmstadt, Germany).

**Animals, Exposure Protocol, and Preparation of Subcellular Fractions.** Twenty-four male Big Blue (Fischer) rats (8–12 weeks of age; 180–250 g) from AH Diagnostics (Aarhus, Denmark) were acclimatized for 7 days while being maintained on a purified diet with a low sucrose content of 3.4%. The diet was formulated at the Danish Veterinary and Food Administration according to Meyer *et al.* (17), with some modifications (see Table 1). The feed was prepared as a powdered diet without heating to avoid caramelization of the sucrose. Animals were stratified by weight and randomly assigned to four groups (6 animals/group). One group served as the control group. Sucrose was added to the feed in the other groups at increasing levels while replacing all other components, resulting in final sucrose concentrations of 3.4% (control), 6.9%, 13.8%, or 34.8% (w/w). Animals were kept on a 12-h light/12-h dark cycle at an average temperature of 22°C and at 55% humidity. The diet was administered for a total of 21 days. Throughout the study, the animals had free access to the diet and water, and their food consumption was monitored. A 24-h urine sample was collected on day 20 and stored at −80°C. At day 21, all rats were healthy, and each individual animal was weighed, anesthetized in a mixture of 40% carbon dioxide and 60% oxygen, and decapitated. Blood was collected through a polyethylene funnel into heparin-coated tubes. The liver was removed and weighed, and the left lateral liver lobe was removed. The remainder was immediately frozen in liquid nitrogen and homogenized in a mortar under liquid nitrogen to pieces <0.05 g, and the crushed liver was stored at −80°C. A 0.5-cm-thick slice of the left lateral lobe was fixed in 4%

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[1] To whom requests for reprints should be addressed, at Institute of Food Safety and Nutrition, Division of Biochemical and Molecular Toxicology, The Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark.

Table 1 *Animal feed composition in the four dose groups*

| Feed component (g/kg feed) | Control group | Low sucrose | Medium sucrose | High sucrose |
|---|---|---|---|---|
| Protein (casein) | 180 | 174.6 | 162 | 126 |
| Fats | | | | |
| Soya oil | 50 | 48.5 | 45 | 35 |
| Carbohydrate | | | | |
| Potato starch | 306 | 296.8 | 275.4 | 252 |
| Corn starch | 306 | 296.8 | 275.4 | 252 |
| Dextrin | 34 | 33 | 30.6 | 23.8 |
| Sucrose | 34 | 64 | 134 | 334 |
| Fiber (cellulose) | 50 | 48.5 | 45 | 35 |
| Mineral mixture[a] | 40 | 38.8 | 36 | 28 |

[a] The mixture contains (mg/kg) $Ca^{2+}$ (4970), P (phosphate, 3100), $K^{22}$ (3610), S (sulphate, 300), $Na^{22}$ (2530), $Cl^{-}$ (1490), $Mg^{2+}$ (600), $Fe^{2+}$ (34), $Zn^{2+}$ (30), $Mn^{2+}$ (10), $Cu^{2+}$ (7), $I^{-}$ (200), Mb (molybdate, 0.15), Se (selenite, 0.15), Si (2.5), $Cr^{+4}$ (1.0), $F^{-}$ (1.0), $Ni^{2+}$ (0.5), B (borate, 0.5), $L^{+}i$ (0.1), V (vanadate, 0.1), $Co^{2+}$ (0.07), thiamine (5), riboflavine (6), pyridoxol (8), folate (2), biotin (0.3), cyanocobalamine (0.03), panthotenate (20), choline (hydrogentartrate, 2600), inositol (400), nicotine amide (40), phylloquinine (1000), *p*-aminobenzoic acid (40), methionine (1000), L-cysteine (2000) and (IU/kg) vitamin A (5000), vitamin $D_3$ (1000), and vitamin E (DL-α-tocopherol, 50).

neutral buffered formaldehyde for 24 h and embedded in paraffin. The colon was opened and rinsed in 0.15 M NaCl. It was cut longitudinally into two pieces, and one half was frozen in liquid nitrogen and stored at −80°C. From the other half, two pieces of 1 cm in length were removed about one-third and about two-thirds of the length of the colon from the distal end and fixed in 4% neutral buffered formaldehyde. The remaining pieces were immediately frozen in liquid nitrogen until storage at −80°C. The heparinized blood samples were separated into plasma, leukocytes (WBCs), and erythrocytes (RBCs) by centrifugation on Ficoll Paque. The washed RBCs were mixed 1:1 with water and stored at −80°C. WBCs and plasma were also stored at −80°C.

**Oxidized Protein and MDA.**[2] About 0.3 g of crushed liver was homogenized for 10 s with a Ultra-Turrax T25 homogenizer (Janke Kunkel GMBH, Staufen, Germany) in 3 ml of 0.25 M sucrose and centrifuged at 9000 × *g*, and the cytosolic fraction was obtained by calcium precipitation (18). Plasma and cytosolic fractions from liver were assayed for oxidized lysine residues (AAS) and for oxidized proline or arginine residues (γ-glutamyl semialdehyde) in proteins as described by Daneshvar *et al.* (19). Protein was determined on a Cobas Mira + analyzer using a commercial kit (catalogue number 0736783; Roche, Basel, Switzerland).

Total MDA in plasma was determined by HPLC as described by Lauridsen and Mortensen (20).

**Antioxidant Enzymes.** Automated assays for the antioxidant enzymes SOD, GPx, CAT, and GR in lysed RBCs were performed on a Cobas Mira analyzer. SOD (Randox catalogue number SD 125) and hemoglobin (Randox catalogue number HG 980) were determined using commercially available kits, whereas the activity of GR was determined by the method of Goldberg and Spooner (21). GPx activity, using *tert*-butylhydroperoxide as initiator, and CAT activity were determined according to a method described by Wheeler *et al.* (22). The enzymatic activities in RBCs were calculated relative to the amount of hemoglobin.

**Plasma and Liver Vitamin C.** Plasma (200 μl) and liver homogenate (300 mg) were treated immediately with metaphosphoric acid as described previously and stored for a maximum of 3 months at −80°C before HPLC analysis for ascorbate and dehydroascorbate (23).

**Isolation of Cells from Liver and Colonic Lining.** The isolation of liver cells was essentially carried out as described previously (24). Colonic mucosal cells were scraped off the thawed colon pieces with a glass microscope slide and placed in ice-cold Merchant-EDTA solution [0.14 M NaCl, 1.47 mM $KH_2HPO_4$, 2.7 mM KCl, 8.1 mM $Na_2HPO_4$, 10 mM $NaH_2PO_4$, and 10 mM NaEDTA (pH 7.4); Ref. 25].

**Mutation Analysis.** The colon and liver cells were suspended in 2 ml of Merchant-EDTA by pipetting up and down three times. About 20 million cells were filtered through a cell strainer (Falcon; Becton Dickinson, Franklin Lakes, NJ), and DNA was purified by the RecoverEase DNA isolation kit (Stratagene, La Jolla, CA). DNA from about 60 mg of frozen liver was prepared by the RecoverEase as described by the manufacturer (Stratagene). The DNA preparation (8 μl) was packaged with Transpack packaging extract (Stratagene). If the packaging mixture was viscous after the recommended standard packaging time of 180 min, the mixture was incubated for another 60 min. If the mixture was still viscous after this time, additional Transpack reagents were added, and the mixture was incubated for another 60 min. This phage preparation was used to infect *Escherichia coli* G1250 (*hfl*$^{-}$). Phages with mutations at the *cII* locus were identified by plaque formation under selective growth conditions at 24°C, and the total number of infective phages was determined by plaque formation under nonselective growth conditions at 37°C as described (λ Select-cII™ Mutation Detection System for the Big Blue Rodents; Stratagene).

**SCGE Assay.** The detection of DNA damage in single liver and colon cells was carried out as described previously (24). The level of EndoIII-sensitive sites was obtained as the difference in scores of parallel slides incubated with and without EndoIII enzymes at 37°C for 45 min [EndoIII enzyme was a kind gift from Serge Boiteux (UMR217 Centre National de la Recherche Scientifique et Commissariat a l'Energie Atomique, Fontenay aux Roses, France)]. A total of 50 images was scored for each sample, using the Kinetics Imaging Limited (Liverpool, United Kingdom) Version 4 software system to determine the amount of DNA that migrated from the comet head to the tail.

**Detection of 8-oxodG.** Levels of 8-oxodG relative to deoxyguanosine were measured in colonic mucosa cells and liver by means of HPLC with electrochemical detection after isolation and digestion of nuclear DNA as described elsewhere (26). Urinary concentrations of 8-oxodG were measured by HPLC with tandem mass spectrometry detection as described elsewhere (27).

**$^{32}$P Postlabelling Analysis.** DNA was extracted from crushed liver and from colonic mucosa cells by standard phenol/chloroform extraction procedure, and the $^{32}$P postlabeling assay was carried out as described previously (28), using butanol extraction as enrichment procedure. A standard consisting of *in vitro* benzo(*a*)pyrene-diol-epoxide-modified calf thymus DNA was used to correct for day-to-day variation in the assay. The results are expressed as adducts/$10^8$ nucleotides, based on the mean of two independent assays.

**Quantification of the *rERCC1* and *rOGG1* mRNA Levels in Colon and Liver.** Total RNA was purified from 10 mg of liver or from $5 \times 10^6$ colon cells using a Qiagen total RNA purification kit as recommended by the manufacturer. The RNA was treated with DNase as recommended by Qiagen. Subsequent quality control showed that all genomic contamination was removed by the DNase treatment. The integrity of the RNA was checked by gel electrophoresis as described previously (29). RNA (200 ng) was used for cDNA synthesis in a reaction volume of 10 μl using the Taqman Gold reverse transcription-PCR kit as recommended by PE Biosystems. For quantification of the mRNA levels, Taqman probes were used. For *rERCC1*, the following oligonucleotides were used: (*a*) forward primer (53F), 5′-cctgggaaggacgaggaaa-3′; (*b*) reverse primer (121R), 5′-tgggataacaaacttcttcctggt-3′; and (*c*) Taqman probe (74T), 5′-FAM-cggccacagccctcaggacc-TAMRA-3′ (TAG-Copenhagen). For *rOGG1*, the following oligonucleotides were used: (*a*) Taqman probe, 5′-FAM-TCATGCCCTGGCTGGTCCAGAAG-TAMRA-3′; (*b*) forward primer, 5′-ACTTATCATGGCTTCCCAAACC-3′; and (*c*) reverse primer, 5′-CAACTTCCTGAGGTGGGTCTCT-3′. This probe is not specific to rat *OGG1* and is likely to detect both nuclear and mitochondrial *OGG1* mRNA across species.

The PCR reactions were performed in duplicate or triplicate in an ABI 7700 Sequence Detection System in 15-μl reactions containing 200 nM primers, 300 nM Taqman probe, and 0.1 μl of cDNA in 1× Mastermix (PE Biosystems). For normalization, 18S mRNA was quantified in a separate PCR reaction using an endogenous control predeveloped assay reagent for 18S RNA quantification (PE Biosystems) in duplicate or triplicate. For each animal, the average value of *rERCC1* quantifications was divided by the average value of the 18S RNA quantifications.

The signals from *rERCC1*, *rOGG1*, and 18S RNA were linear over 100-fold dilution (data not shown). Likewise, normalization of the *rERCC1* signal to 18S RNA yielded the same signal over a 100-fold dilution (data not shown). Repeated measurements of the same sample yielded a SD of 20% between batches. The SD on triplicates was, on average, 15%.

[2] The abbreviations used are: MDA, malondialdehyde; AAS, 2-amino adipic semialdehyde; CAT, catalase; FAM, 6-carboxyfluoresceinamine succimidylester; GR, glutathione reductase; GPx, glutathione peroxidase; PCNA, proliferating cell nuclear antigen; SOD, superoxide dismutase; SCGE, single cell gel electrophoresis; TAMRA, 6-carboxytetramethylrhodamine succimidylester; TUNEL, terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end labeling; HPLC, high-performance liquid chromatography; 8-oxodG, 8-oxodeoxyguanosine; EI, energy index; NI, nutrient index; EndoIII, endonuclease III.

**Apoptosis.** The paraffin-embedded liver tissue was cut into 2-$\mu$m sections mounted on poly-L-lysine-coated slides. The sections were deparaffinized and incubated with 20 $\mu$g/ml proteinase K (code P6556; Sigma) for 5 min at room temperature to strip off nuclear proteins and then incubated in 3% $H_2O_2$ in PBS buffer for 5 min at room temperature to quench endogenous peroxidase. TUNEL was accomplished using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (code S-7100; Intergen Co.). After preincubation in equilibrium buffer for 30 min at room temperature, the sections were incubated with a mixture of terminal deoxynucleotidyltransferase enzyme and nucleotides linked with digoxigenin in a humidified chamber at 37°C for 1 h and then immersed in a stop/wash buffer at 4°C for 10 min. The slides were incubated with an antidigoxigenin antibody conjugated with peroxidase. Afterward, the sections were washed in PBS buffer and incubated in antidigoxigenin-peroxidase solution for 30 min at room temperature. 3,3′-Diaminobenzidine (Sigma Fast tablet sets, code 4168) was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin (code MHS-80; Sigma Diagnostics). Negative control sections were treated similarly but incubated in the absence of terminal deoxynucleotidyltransferase enzyme/nucleotides. Sections were compared with positive control slides from Intergen. Morphological criteria for apoptosis were evaluated because the TUNEL assay may also stain necrotic cells.

**Measurement of Cell Proliferation in the Liver.** Hepatocytes undergoing proliferation were identified by immunohistochemical staining of PCNA. The formaldehyde-fixed paraffin-embedded tissue sampled from the left lateral liver lobe was cut into sections, 4–6-$\mu$m thick, on slides. The sections were deparaffinized, rehydrated, and treated with 3% $H_2O_2$ in PBS for 10 min at room temperature to block endogenous peroxidase activity. Unspecific background staining was minimized by a 20-min incubation with 10% rabbit serum (X0902; DAKO, Roskilde, Denmark). After washing, the sections were incubated overnight with PCNA monoclonal antibody (PC10, M0879; DAKO) diluted 1:600 in a humidified chamber at 4°C. Subsequently, the sections were incubated with a secondary antibody (biotinylated rabbit antimouse antibody, E0354; DAKO) for 45 min at room temperature, followed by a 30-min incubation at room temperature with an avidin-biotin complex (ABC, K0335; DAKO). The complex was visualized by incubation with 3-amino-9-ethylcarbazol solution (Bie & Berntsen, Rødovre, Denmark) for 15 min at room temperature. Finally, the sections were lightly counterstained with Mayer's hematoxylin and mounted with Aquamont (Bie & Berntsen). Control sections were included to verify the specificity of the antibodies. As negative controls, the primary antibody was replaced by nonimmune serum from mouse. As positive controls, colon tissue was included and treated as described above.

The total number of hepatocytes and the number of cells with a clear nuclear PCNA staining were counted using a standard light microscope (Leica DMR, Wetzlar, Germany) connected to a digital camera (Leica DC 100) transmitting the picture on a 17-inch screen. Hepatocytes with a weak and granular staining of the nucleus or with cytoplasmic reaction were not included; therefore, the positive cells mainly represent cells in the S phase of mitosis (30). The liver samples were blinded to the observer. For each liver sample, the hepatocytes were counted in 15–20 systematic randomly chosen fields. In brief, each field was chosen moving through the tissue sample in a Meander pattern at ×100 magnification, taking care that the fields were not overlapping; the magnification was then changed to ×630 for counting of the cells. To avoid overestimation, only hepatocytes in focus and not touching the bottom and left border of the field were counted. Between 15 and 30 cells were counted/field, and approximately 360 cells were counted/animal. The percentage of hepatocytes staining positive for PCNA was determined as the number of positive hepatocytes/sample divided by total number of hepatocytes/sample × 100.

**Statistics.** All parameters were tested for normal distribution with the Kolmogorov-Smirnov test ($P > 0.01$). The homogeneity of variance among groups was evaluated by judgement of standard residual plots (General Linear Model procedure, SAS Statistical Package, release v8; SAS Institute Inc., Cary, NC). Some parameters had to be logarithmically transformed to meet either criterion. The groups were compared using one-way ANOVA. If significant differences were observed ($P < 0.05$), groups 2, 3, and 4 were compared with the control group using Dunnett's test. Pearson correlation analyses were performed separately for all colon markers and all liver markers.

## RESULTS

The animals in the control group and in the groups fed with sucrose had similar feed intake, total energy intake, total carbohydrate intake, weight gain, and final body weight during the 3-week feeding period. A significant difference in the liver weights between the groups was observed by ANOVA ($P = 0.046$), but none of the dosed groups differed from the control group (see Table 2). Due to the slightly higher actual feed intake in the low- and medium-dose groups, there was no decrease in the intakes of other feed components in these groups. Because all feed components except sucrose were consumed in proportional amounts by the animals in each group, an EI and a NI were calculated by setting the median of the control group to 100 (see Table 2). In the high-dose group, there was a significant decrease in the NI, indicating that the intake of all micronutrients, casein, starch, soy bean oil, and cellulose was decreased by almost 30% as compared with the controls ($P < 0.001$).

**Effects on Mutation, DNA Damage, and Repair.** A dose-dependent increase was observed in *cII* mutations in the colonic mucosa ($P = 0.0017$), whereas the liver was unaffected ($P = 0.61$; see Fig. 1). Individual phage titers and mutation frequencies are listed in Table 3. DNA adduct formation as determined by $^{32}$P postlabeling was dose-dependently and significantly reduced by increased dietary sucrose in the colon, whereas the liver was unaffected (see Fig. 2). There was no effect of sucrose on the level of 8-oxodG, strand breaks, or EndoIII-sensitive sites in DNA in colon or liver. The colon DNA repair capacity determined by *rERCC1* or *rOGG1* mRNA levels was unaffected by sucrose. Pearson correlation analysis of the colon markers revealed significant inverse associations of mutations to adducts ($r^2 = 0.56$; $P < 0.01$) and of *rERCC1* to adducts ($r^2 = 0.55$; $P < 0.01$), but no association of mutations to *rERCC1* ($r^2 = 0.13$; $P = 0.56$). A positive association in the expression of the two repair enzymes was also observed ($r^2 = 0.52$; $P = 0.01$).

Liver *rERCC1* but not *rOGG1* mRNA levels were significantly affected as determined by ANOVA, but only the lowest dose group differed from controls (see Table 4). There was no correlation between adduct formation, mutation frequency, and DNA repair enzyme expression in the liver.

No overall effect on oxidative damage to DNA was observed as determined by the urinary excretion of 8-oxodG ($P = 0.37$).

**Other Markers of Oxidative Stress.** In addition to the DNA-related markers, other markers of oxidative stress were also investigated in the liver and blood compartment (see Table 4). The markers

Table 2 *Feed intakes, body weight gain, and final body weights*

| | Feed intake (g/day) | | Body weight gain (g/day) | | Final body weights (g) | | Liver weights[a] (g) | | NI[b] (%) | | EI[b] (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Controls | 16.2 | 0.83 | 1.83 | 0.45 | 297 | 16 | 10.44 | 0.75 | 100 | 5.1 | 100 | 1.9 |
| Low-sucrose group | 16.4 | 1.47 | 1.79 | 0.69 | 287 | 35 | 9.41 | 1.12 | 98.4 | 8.8 | 106 | 12 |
| Medium-sucrose group | 17.7 | 1.04 | 2.02 | 0.48 | 281 | 39 | 9.31 | 1.23 | 98.2 | 5.8 | 119 | 21 |
| High-sucrose group | 16.6 | 0.62 | 2.00 | 0.45 | 304 | 30 | 11.11 | 1.51 | 71.7[c] | 2.7 | 104 | 6.4 |

[a] The liver weights differed by ANOVA ($P < 0.05$), but none of the dosed groups differed from controls.
[b] The NI and EI are the relative intake of all micronutrients and nutrients (except sucrose) and of energy relative to the control group, respectively.
[c] Significantly different from control ($P < 0.001$).

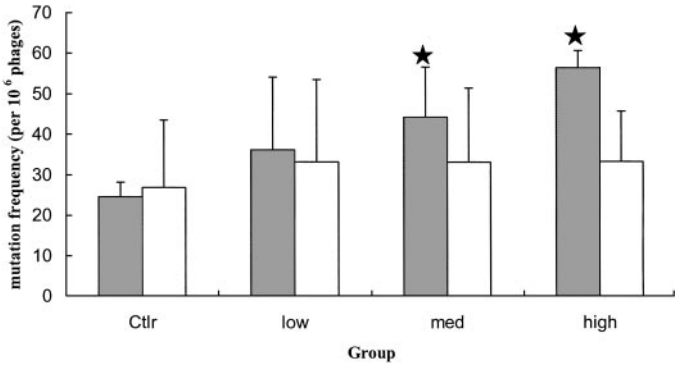


Fig. 1. Mutation frequency of *cII* in liver (□) and colon (▨) in the four groups. *Bars* represent means, and *lines* denote 1 SD. Groups that are significantly different ($P < 0.05$) from the control group (group 1) are denoted with a *star*.

represent damage to proteins and lipids. Liver cytosolic protein oxidation levels were unaffected by the sucrose treatment. Plasma protein oxidation and lipid oxidation were also unaffected.

**Markers of Oxidative Defense.** The hepatic level of ascorbate in the rats was significantly affected by sucrose treatment (see Fig. 3). Both reduced ($P = 0.04$) and total ($P = 0.02$) vitamin C levels were significantly elevated in the liver, and their difference, representing dehydroascorbic acid, was also elevated ($P = 0.02$). In the plasma compartment, oxidized, reduced, and total ascorbate levels were unaffected by sucrose.

The erythrocyte antioxidant enzyme activities, SOD, CAT, Gpx, and GR, were also unaffected by the sucrose treatments (data not shown).

**Other Markers.** Apoptosis was determined by the TUNEL assay, and cell proliferation was determined by PCNA analysis in the liver. Both were found to be unaffected by the sucrose dose (see Table 5). There was a significant correlation between apoptosis and liver weight ($r^2 = 0.53$; $P < 0.01$).

*Cox2* expression was determined in the colon to evaluate whether the arachidonate pathway may be involved in the sucrose-mediated effects in colon. There was no effect of sucrose exposure on *Cox2* expression in the present study (see Table 5), and Pearson correlation analysis revealed no interactions with any other marker in the colon.

## DISCUSSION

Sucrose has been observed to act as a cocarcinogen in rat colon and has also been observed to increase the number of aberrant crypt foci induced in the colon by carcinogens such as 2-amino-3 methylimidazo[4,5-*f*]quinoline, azoxymethane, and dimethylhydrazine (11–14). The present data support our hypothesis that sucrose is mutagenic in the colon epithelium as shown by a dose-dependent increase in mutations at the *cII* repressor site expressed in Big Blue rats. Chemicals generally induce very similar mutation frequencies in transgenes such as the *cII* gene and endogenous genes (31). In contrast, there was no increase in mutations in the liver, suggesting a possible site specificity of the effect. The duration of the sucrose exposure was chosen to optimize the number of end points that were likely to display effects. It takes some time before DNA damage is fixed into *cII* mutations, and the mutation frequency increases asymptotically with time. The time is dependent on the proliferation rate, and because

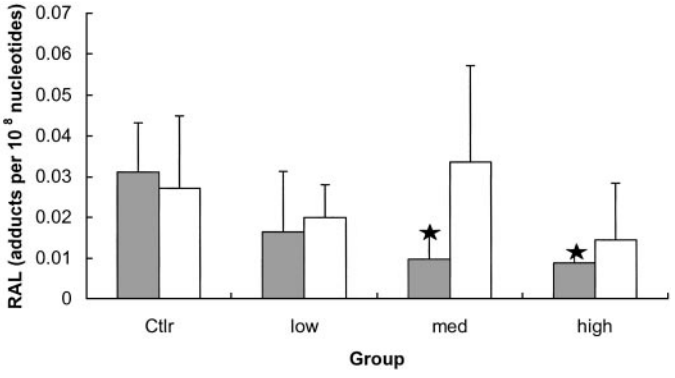


Fig. 2. DNA adduct levels in colon (▨) and liver (□) determined by $^{32}$P postlabeling. *Bars* represent means, and *lines* denote 1 SD. Groups that are significantly different ($P < 0.05$) from the control group (group 1) are denoted with a *star*. *RAL* (relative adduct level) is the observed level of unknown adducts relative to a standard of benzo(*a*)pyrene-diol-epoxide-modified calf thymus DNA.

Table 3 *The mutation frequency (MF) and total number of phages packed for each animal for colon and liver*

| | | Colon | | | Liver | | |
|---|---|---|---|---|---|---|---|
| Diet | Rat no. | Total no. of phages | MF × $10^{e6}$ | Average MF | Total no. of phages | MF × $10^6$ | Average MF |
| Control diet | BC 3 | 118,000 | 21 | 24.6 | 506,000 | 24.7 | 26.9 |
| | BC 4 | 795,000 | 20.4 | | 430,500 | 40.7 | |
| | BC 7 | 586,000 | 23.5 | | 535,000 | 9.3 | |
| | BC 8 | 365,500 | 27.4 | | 301,500 | 16.6 | |
| | BC 11 | 129,500 | 29 | | 438,000 | 17.1 | |
| | BC 12 | 660,000 | 26.5 | | 331,000 | 52.9 | |
| 3% (w/w) sucrose | BC 67 | 949,000 | 19.8 | 36.2 | 724,000 | 27.6 | 33.5 |
| | BC 68 | 120,000 | 62.5 | | 299,000 | 41.8 | |
| | BC 69 | 476,000 | 18.4 | | 786,000 | 17.5 | |
| | BC 70 | 202,000 | 30.9 | | 1,043,000 | 4.8 | |
| | BC 71 | 117,250 | 53.3 | | 175,000 | 50 | |
| | BC 72 | 348,500 | 32.3 | | 108,000 | 57.9 | |
| 10% (w/w) sucrose | BC 73 | 919,000 | 24.5 | 44.3[a] | 201,000 | 49.8 | 33.2 |
| | BC 74 | 464,500 | 59.2 | | 563,500 | 26.6 | |
| | BC 75 | 468,000 | 45.4 | | 403,500 | 31 | |
| | BC 76 | 649,000 | 50.1 | | 125,500 | 10 | |
| | BC 77 | 245,500 | 35.6 | | 274,500 | 59.2 | |
| | BC 78 | 221,750 | 50.7 | | 224,500 | 22.3 | |
| 30% (w/w) sucrose | BC 79 | 243,810 | 53.3 | 56.4[a] | 289,500 | 21.6 | 33.3 |
| | BC 80 | | | | 368,000 | 34 | |
| | BC 81 | 107,520 | 58.1 | | 435,000 | 46 | |
| | BC 82 | 270,000 | 55.6 | | 103,000 | 24.3 | |
| | BC 83 | 119,500 | 52.3 | | 588,500 | 23.4 | |
| | BC 84 | 119,500 | 62.8 | | 469,500 | 50.6 | |

[a] Significantly different from the control group, $P < 0.05$.

Table 4 *Markers of oxidative damage and defense*

| | Control | | +3.5% Sucrose | | +10.4% Sucrose | | +31.1% Sucrose | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *DNA damage and repair* | | | | | | | | |
| Urine 8-oxodG (pmol/24 h) | 167 | 54 | 124 | 64 | 170 | 34 | 131 | 50 |
| Colon 8-oxodG | 0.45 | 0.13 | 0.58 | 0.17 | 0.47 | 0.33 | 0.47 | 0.21 |
| Colon SCGE | 8.02 | 4.71 | 17.68 | 12.45 | 12.87 | 12.60 | 9.13 | 4.14 |
| Colon ENDO[a] | 5.27 | 3.23 | 5.54 | 5.43 | 1.95 | 3.34 | 4.28 | 2.89 |
| Colon *rOGG1* ($\times 10^{-6}$) | 76.0 | 28.0 | 70.1 | 53.2 | 47.3 | 26.2 | 58.8 | 27.8 |
| Colon *rERCC1* ($\times 10^{-6}$) | 13.9 | 6.14 | 20.8 | 17.0 | 18.4 | 3.62 | 17.8 | 27.8 |
| Liver 8-oxo-dG | 0.38 | 0.11 | 0.30 | 0.12 | 0.36 | 0.09 | 0.35 | 3.34 |
| Liver SCGE | 3.85 | 0.85 | 6.68 | 3.35 | 4.54 | 1.14 | 8.02 | 5.37 |
| Liver ENDO | 1.10 | 1.66 | 0.65 | 1.67 | 0.98 | 2.28 | 0.96 | 3.00 |
| Liver *rOGGl* ($\times 10^{-6}$) | 7.80 | 3.98 | 2.97 | 1.52 | 6.15 | 2.45 | 13.3 | 10.7 |
| Liver *rERCCl* ($\times 10^{-6}$) | 6.16 | 2.13 | 8.08 | 8.02 | 7.07 | 3.87 | 12.7 | 7.66 |
| *Oxidative damage to proteins and lipids* | | | | | | | | |
| Plasma AAS (pmol/mg) | 117 | 10 | 123 | 13 | 121 | 14 | 109 | 10 |
| Plasma MDA (nmol/mg) | 378 | 65 | 370 | 85 | 327 | 177 | 334 | 140 |
| Liver AAS (pmol/mg) | 76.5 | 10.1 | 102 | 42 | 96.0 | 9.1 | 94.8 | 6.7 |
| Liver GGS (pmol/mg) | 84.3 | 8.0 | 112 | 47 | 111 | 25 | 103 | 26 |

[a] ENDO, SCGE on EndoIII-treated cells; GGS, γ-glutamyl semialdehyde.

the liver proliferates slowly, the optimal fixation time may be as long as 1 month (32) or 5 weeks (33), whereas the fixation time is shorter in the colon (32, 33). It is therefore possible that a mutagenic effect in the liver may have been missed in this experimental set-up and that the mutagenic effect of sucrose in the colon may be even somewhat greater. A large number of biomarkers, including markers of oxidative stress, DNA damage, and DNA repair, did not point specifically to a mechanism of action.

The mutagenic effect of sucrose does not seem to be due to general or localized oxidative stress. There was no indication of oxidative damage to DNA as determined by 8-oxodG, strand breaks, or EndoIII-sensitive sites in colon or liver or by excretion of 8-oxodG-derived repair products into urine. The repair of 8-oxodG as determined by *rOGG1*expression was not affected in liver or in colon. It therefore seems unlikely that sucrose is mediating its mutagenic effect by a mechanism involving oxygen free radicals. This is corroborated by the observation that in plasma and liver, the protein and lipid oxidation markers were unaffected by sucrose treatment. However, liver vitamin C levels increased, indicating either an increased availability of carbohydrate precursors or an increased defense against reactive species. Increased rat liver ascorbate synthesis has been observed previously after exposure to either genotoxic hepatocarcinogens (34) or peroxisome proliferators (35). In the present study, we did not find evidence for any liver cytotoxicity because neither the apoptotic index nor the PCNA analysis revealed differences between the groups. Sucrose and other simple sugars can irreversibly glycosylate proteins, and the formation of complex glycation products in DNA may be partly responsible for sucrose-induced mutations (36, 37).

Table 5 *Effect of dietary sucrose on markers related to cell cycle regulation*

| | Control | | +3.5% Sucrose | | +10.4% Sucrose | | +31.1% Sucrose | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Apoptosis (%)[a] | 1.30 | 0.14 | 1.14 | 0.20 | 1.25 | 0.29 | 1.45 | 0.21 |
| PCNA (%)[a] | 0.39 | 0.43 | 0.57 | 0.66 | 0.50 | 0.56 | 0.40 | 0.44 |
| *Cox2* expression ($\times 10^{-6}$)[b] | 1.19 | 0.90 | 0.51 | 0.48 | 0.95 | 1.07 | 0.43 | 0.29 |

[a] Percentage of cells in the liver staining positive.
[b] Expression in colon relative to 18S mRNA.

The level of background bulky DNA adducts in colon as determined by $^{32}$P postlabeling analysis was decreased by sucrose treatment, indicating a change in their formation or repair. A similar effect was not observed in the liver. Colon strand breaks and colon *rERCC1* expression were not significantly affected overall by the sucrose treatment. The mRNA level of *rERCC1* has been shown to correlate with nucleotide excision repair capacity in human lymphocytes, bone marrow, and brain tissue (38–41). A correlation analysis of the colon markers indicates a significant negative association between *rERCC1* and adducts, *i.e.*, adduct removal was associated with increased excision repair. It is also noteworthy that *rOGG1* and *rERCC1* mRNA levels were positively correlated in colon, indicating that several repair pathways may be activated in concert in the animals. There was a negative association between colon mutations and colon adducts, but there was no apparent association between *rERCC1* and colon mutations. Factors related to colon mutation and to excision repair would therefore seem to be independent factors in adduct formation or removal. The background bulky adducts observed by $^{32}$P postlabeling analysis of DNA have been termed I-compounds due to their indigenous nature. A decrease in I-compounds has been observed in animals on a choline-deficient diet (42), after exposure to tumor promoters such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (43, 44) or phenobarbital (45), and after other treatments known to induce cancer without formation of DNA adducts (46). An increase in I-compounds has been observed in the rat liver after cancer-preventive treatments such as dietary restriction, or when more complex carbohydrates from oats and alfalfa were added to various rat feeds (47). The significant negative correlation between mutation frequency and DNA adducts in the colon indicates a strong rela-

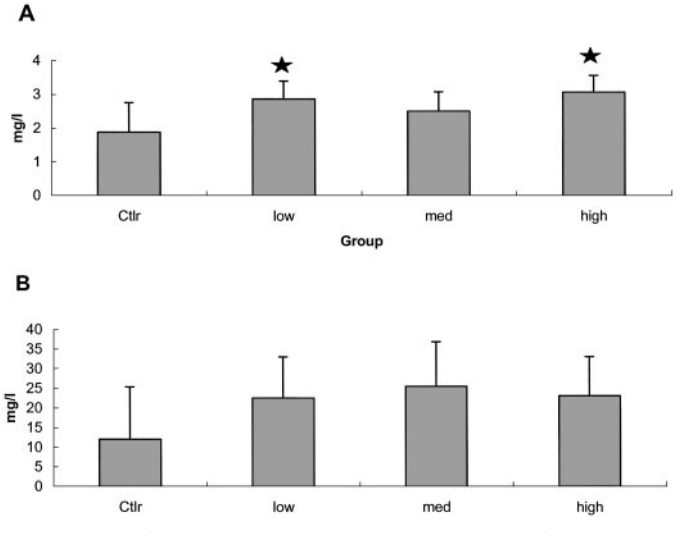


Fig. 3. Levels of total vitamin C in (*A*) liver and (*B*) plasma in the four groups. *Bars* represent means, and *lines* denote 1 SD. Groups that are significantly different ($P < 0.05$) from the control group (group 1) are denoted with a *star*.

tionship between the two observed effects. Therefore, the decrease of I-compounds in the colon might indicate that sucrose increases the mutation frequency by an indirect mechanism that does not involve the formation of DNA adducts.

Sucrose and fructose, as opposed to glucose or starch, have previously been found to cause increased rat liver weight and hyperplasia after a 90-day feeding period (48). After a 3-week feeding period, we observed no significant difference in liver weight, cell proliferation, or apoptosis between controls and the highest dose group. However, liver weight and apoptosis correlated significantly.

In the present study, sucrose displaced all other components in the feed, including potato starch, which is partly resistant to digestion in the small intestine. In the low- and medium-dose groups, the actual food intake was slightly increased, compensating for the decreased content of potato starch and nutrients in the feed, and even in these groups, the effect of sucrose was evident. In the high-dose group, the intake of all nutrients and micronutrients was decreased by 28% due to displacement by the sucrose. However, the overall energy intakes in the four groups were the same. It is therefore less likely that the effects observed on mutations and DNA adducts are due to the decrease in resistant starch or other protective dietary factors rather than to the increased sucrose intake *per se*. To see whether the sucrose-rich diet is causing mutations by a new type of lesions or rather by increasing the background rate of mutations, we are presently analyzing the mutational spectra of background and sucrose-induced mutations at the *cII* site in the colon.

We conclude that a sucrose-rich diet causes mutations in the rat colon epithelium and concomitantly causes a decrease in colon I-compounds. A similar effect in the liver was not observed, but a weak effect may have been masked by the relatively short feeding time in this study. We further conclude that although ascorbate levels increased in the liver with a sucrose-rich diet, an oxidative mechanism behind the actions of the sucrose-rich diet is unlikely.

## REFERENCES


1. Glinsman, W. H., Irausquin, H., and Park, Y. K. Evaluation of health aspects of sugars contained in carbohydrate sweeteners. J. Nutr., *116*: S1−S216, 1986.
2. Bostick, R. M., Potter, J. D., Kushi, L. H., Sellers, T. A., Steinmetz, K. A., McKenzie, D. R., Gapstur, S. M., and Folsom, A. R. Sugar, meat, and fat intake, and non-dietary risk factors for colon cancer incidence in Iowa women (United States). Cancer Causes Control, *5:* 38–52, 1994.
3. Slattery, M. L., Benson, J., Berry, T. D., Duncan, D., Edwards, S. L., Caan, B. J., and Potter, J. D. Dietary sugar and colon cancer. Cancer Epidemiol. Biomark. Prev., *6:* 677–685, 1997.
4. Franceschi, S., Favero, A., La Vecchia, C., Negri, E., Conti, E., Montella, M., Giacosa, A., Nanni, O., and Decarli, A. Food groups and risk of colorectal cancer in Italy. Int. J. Cancer, *72:* 56–61, 1997.
5. La Vecchia, C., Franceschi, S., Dolara, P., Bidoli, E., and Barbone, F. Refined-sugar intake and the risk of colorectal cancer in humans. Int. J. Cancer, *55:* 386–389, 1993.
6. Hill, M. J., and Caygill, C. P. Sugar intake and the risk of colorectal cancer. Eur. J. Cancer Prev., *8:* 465–468, 1999.
7. Hu, F. B., Manson, J. E., Liu, S., Hunter, D., Colditz, G. A., Michels, K. B., Speizer, F. E., and Giovannucci, E. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. J. Natl. Cancer Inst. (Bethesda), *91:* 542–547, 1999.
8. Kono, S., Honjo, S., Todoroki, I., Nishiwaki, M., Hamada, H., Nishikawa, H., Koga, H., Ogawa, S., and Nakagawa, K. Glucose intolerance and adenomas of the sigmoid colon in Japanese men (Japan). Cancer Causes Control, *9:* 441–446, 1998.
9. La Vecchia, C., Negri, E., Decarli, A., and Franceschi, S. Diabetes mellitus and colorectal cancer risk. Cancer Epidemiol. Biomark. Prev., *6:* 1007–1010, 1997.
10. Weiderpass, E., Gridley, G., Nyren, O., Ekbom, A., Persson, I., and Adami, H. O. Diabetes mellitus and risk of large bowel cancer. J. Natl. Cancer Inst. (Bethesda), *89:* 660–661, 1997.
11. Kristiansen, E., Meyer, O., and Thorup, I. Refined carbohydrate enhancement of aberrant crypt foci (ACF) in rat colon induced by the food-borne carcinogen 2-amino-3-methyl-imidazo[4,5-*f*]quinoline (IQ). Cancer Lett., *105:* 147–151, 1996.
12. Kristiansen, E., Thorup, I., and Meyer, O. Influence of different diets on development of DMH-induced aberrant crypt foci and colon tumor incidence in Wistar rats. Nutr. Cancer, *23:* 151–159, 1995.
13. Luceri, C., Caderni, G., Lancioni, L., Aiolli, S., Dolara, P., Mastrandrea, V., Scardazza, F., and Morozzi, G. Effects of repeated boluses of sucrose on proliferation and on AOM- induced aberrant crypt foci in rat colon. Nutr. Cancer, *25:* 187–196, 1996.
14. Poulsen, M., Molck, A. M., Thorup, I., Breinholt, V., and Meyer, O. The influence of simple sugars and starch given during pre- or post-initiation on aberrant crypt foci in rat colon. Cancer Lett., *167:* 135–143, 2001.
15. Caderni, G., Luceri, C., Lancioni, L., and Dolara, P. Dietary sucrose, glucose, fructose, and starches affect colonic functions in rats. Nutr. Cancer, *25:* 179–186, 1996.
16. Corpet, D. E., Peiffer, G., and Tache, S. Glycemic index, nutrient density, and promotion of aberrant crypt foci in rat colon. Nutr. Cancer, *32:* 29–36, 1998.
17. Meyer, O., Blom, L., and Sondergaard, D. The influence of minerals and protein on the nephrocalcinosis potential for rats of semisynthetic diets. Lab. Anim., *16:* 271–273, 1982.
18. Kim, K. B., and Lee, B. M. Oxidative stress to DNA, protein, and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(*a*)pyrene. Cancer Lett., *113:* 205–212, 1997.
19. Daneshvar, B., Frandsen, H., Autrup, H., and Dragsted, L. O. γ-Glutamyl semialdehyde and 2-amino-adipic semialdehyde: biomarkers of oxidative damage to proteins. Biomarkers, *2:* 117–123, 1997.
20. Lauridsen, S. T., and Mortensen, A. Probucol selectively increases oxidation of atherogenic lipoproteins in cholesterol-fed mice and in Watanabe heritable hyperlipidemic rabbits. Atherosclerosis, *142:* 169–178, 1999.
21. Goldberg, D. M., and Spooner, R. J. Glutathione reductase. *In:* H. U. Bergmeyer (ed.), Methods of Enzymatic Analysis, 3rd ed., pp. 258–265. Deerfield Beach, FL: Verlag Chemie, 1983.
22. Wheeler, C. R., Salzman, J. A., Elsayed, N. M., Omaye, S. T., and Korte, D. W., Jr. Automated assays for superoxide dismutase, catalase, glutathion peroxidase, and glutathion reductase. Anal. Biochem., *184:* 193–199, 1990.
23. Lykkesfeldt, J., Loft, S., Nielsen, J. B., and Poulsen, H. E. Ascorbic acid and dehydroascorbic acid as biomarkers of oxidative stress caused by smoking. Am. J. Clin. Nutr., *65:* 959–963, 1997.
24. Thein, N., Moller, P., Amtoft, H., Vogel, U., Korsholm, B., Autrup, H., and Wallin, H. A strong genotoxic effect in mouse skin of a single painting of coal tar in hairless mice and in MutaMouse. Mutat. Res., *468:* 117–124, 2000.
25. Brunborg, G., Holme, J. A., Soderlund, E. J., Hongslo, J. K., Vartiainen, T., Lotjonen, S., and Becher, G. Genotoxic effects of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX) in mammalian cells *in vitro* and in rats *in vivo*. Mutat. Res., *260:* 55–64, 1991.
26. Wellejus, A., and Loft, S. Receptor-mediated 17-ethynylestradiol-induced oxidative DNA damage in rat testicular cells. FASEB J., *16:* 195–201, 2002.
27. Weimann, A., Belling, D., and Poulsen, H. E. Measurement of 8-oxo-2′-deoxyguanosine and 8-oxo-2′-deoxyadenosine in DNA and human urine by high performance liquid chromatography-electrospray tandem mass spectrometry. Free Radic. Biol. Med., *30:* 757–764, 2001.
28. Autrup, H., Daneshvar, B., Dragsted, L. O., Gamborg, M., Hansen, Å. M., Loft, S., Okkels, H., Nielsen, F., Nielsen, P. S., Raffn, E., Wallin, H., and Knudsen, L. E. Biomarkers for exposure to ambient air pollution: comparison of carcinogen-DNA adduct levels with other exposure markers and markers for oxidative stress. Environ. Health Perspect., *10:* 233–238, 1999.
29. Pelle, R., and Murphy, N. B. Northern hybridization: rapid and simple electrophoretic conditions. Nucleic Acids Res., *21:* 2783–2784, 1993.
30. Kulkarni, S. G., Warbritton, A., Bucci, T. J., and Mehendale, H. M. Antimitotic intervention with colchicine alters the outcome of o-DCB-induced hepatotoxicity in Fischer 344 rats. Toxicology, *120:* 79–88, 1997.
31. Cosentino, L., and Heddle, J. A. Differential mutation of transgenic and endogenous loci *in vivo*. Mutat. Res., *454:* 1–10, 2000.
32. Nohmi, T., Suzuki, T., and Masumura, K. Recent advances in the protocols of transgenic mouse mutation assays. Mutat. Res., *455:* 191–215, 2000.
33. Heddle, J. A., Dean, S., Nohmi, T., Boerrigter, M., Casciano, D., Douglas, G. R., Glickman, B. W., Gorelick, N. J., Mirsalis, J. C., Martus, H. J., Skopek, T. R., Thybaud, V., Tindall, K. R., and Yajima, N. *In vivo* transgenic mutation assays. Environ. Mol. Mutagen., *35:* 253–259, 2000.
34. Braun, L., Kardon, T., El Koulali, K., Csala, M., Mandl, J., and Banhegyi, G. Different induction of gulonolactone oxidase in aromatic hydrocarbon-responsive or -unresponsive mouse strains. FEBS Lett., *463:* 345–349, 1999.
35. Braun, L., Mile, V., Schaff, Z., Csala, M., Kardon, T., Mandl, J., and Banhegyi, G. Induction and peroxisomal appearance of gulonolactone oxidase upon clofibrate treatment in mouse liver. FEBS Lett., *458:* 359–362, 1999.
36. Pischetsrieder, M., Seidel, W., Munch, G., and Schinzel, R. *N*(2)-(1-Carboxyethyl)deoxyguanosine, a nonenzymatic glycation adduct of DNA, induces single-strand breaks and increases mutation frequencies. Biochem. Biophys. Res. Commun., *264:* 544–549, 1999.
37. Mullokandov, E. A., Franklin, W. A., and Brownlee, M. DNA damage by the glycation products of glyceraldehyde 3-phosphate and lysine. Diabetologia, *37:* 145–149, 1994.
38. Dabholkar, M., Bostick-Bruton, F., Weber, C., Bohr, V. A., Egwuagu, C., and Reed, E. ERCC1 and ERCC2 expression in malignant tissues from ovarian cancer patients. J. Natl. Cancer Inst. (Bethesda), *84:* 1512–1517, 1992.
39. Dabholkar, M., Bostick-Bruton, F., Weber, C., Egwuagu, C., Bohr, V. A., and Reed, E. Expression of excision repair genes in non-malignant bone marrow from cancer patients. Mutat. Res., *293:* 151–160, 1993.
40. Dabholkar, M. D., Berger, M. S., Vionnet, J. A., Egwuagu, C., Silber, J. R., Yu, J. J., and Reed, E. Malignant and nonmalignant brain tissues differ in their messenger RNA expression patterns for ERCC1 and ERCC2. Cancer Res., *55:* 1261–1266, 1995.

41. Vogel, U., Dybdahl, M., Frentz, G., and Nexo, B. A. DNA repair capacity: inconsistency between effect of over-expression of five NER genes and the correlation to mRNA levels in primary lymphocytes. Mutat. Res., *461:* 197–210, 2000.
42. Li, D., Chandar, N., Lombardi, B., and Randerath, K. Reduced accumulation of I-compounds in liver DNA of rats fed a choline-devoid diet. Carcinogenesis (Lond.), *10:* 605–607, 1989.
43. Randerath, K., Putman, K. L., Randerath, E., Mason, G., Kelley, M., and Safe, S. Organ-specific effects of long term feeding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin on I-compounds in hepatic and renal DNA of female Sprague-Dawley rats. Carcinogenesis (Lond.), *9:* 2285–2289, 1988.
44. Randerath, K., Putman, K. L., Randerath, E., Zacharewski, T., Harris, M., and Safe, S. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on I-compounds in hepatic DNA of Sprague-Dawley rats: sex-specific effects and structure-activity relationships. Toxicol. Appl. Pharmacol., *103:* 271–280, 1990.
45. Randerath, K., van Golen, K. L., Dragan, Y. P., and Pitot, H. C. Effects of phenobarbital on I-compounds in liver DNA as a function of age in male rats fed two different diets. Carcinogenesis (Lond.), *13:* 125–130, 1992.
46. Randerath, E., Randerath, K., Reddy, R., Danna, T. F., Rao, M. S., and Reddy, J. K. Induction of rat liver DNA alterations by chronic administration of peroxisome proliferators as detected by $^{32}$P-postlabeling. Mutat. Res., *247:* 65–76, 1991.
47. Li, D., Chen, S., and Randerath, K. Natural dietary ingredients (oats and alfalfa) induce covalent DNA modifications (I-compounds) in rat liver and kidney. Nutr. Cancer, *17:* 205–216, 1992.
48. Bender, A. E., Damji, K. B., Khan, M. A., Khan, I. H., McGregor, L., and Yudkin, J. Sucrose induction of hepatic hyperplasia in the rat. Nature (Lond.), *238:* 461–462, 1972.