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# Dietary Oat Lipids-Induced Novel DNA Modifications and Suppression of Altered Hepatic Foci Formation

**Donghui Li, Mianying Wang, Greg Paul, Henry C. Pitot, and Yvonne Dragan**

***Abstract:*** *Previous studies have shown that the presence of several hepatic I-compounds, i.e., age-dependent covalent DNA modifications, is related to the presence of a natural ingredient, i.e., oats, in the diet. To demonstrate the biological significance of these novel DNA modifications, the effect of oat lipids on tumor initiation and promotion was examined in a rat liver tumor model. Female Sprague-Dawley rats were treated with a single dose of diethylnitrosamine, a hepatic carcinogen, 24 hours after a 70% partial hepatectomy, then subjected to dietary phenobarbital promotion. Diets containing 10% oat lipids or corn oil were given during the initiation or the promotion stage of the tumorigenesis. At the end of the feeding, hepatic I-compounds were measured by $^{32}P$ postlabeling, and the number and volume of enzyme-altered hepatic foci, which served as preneoplastic markers, were measured in serial sections of liver by the method of quantitative stereology. Rats receiving oat lipids-supplemented diets had five- to sixfold higher levels of I-compounds in their liver DNA than those receiving control diets. Meanwhile, rats receiving diets containing oat lipids during promotion had significantly smaller numbers and reduced volume of altered hepatic foci compared with those fed the control diet containing corn oil. These observations support the hypothesis that some I-compounds, e.g., the oats-specific I-compounds, are novel DNA modifications related to nutrient metabolism. The diet containing oat lipids may have chemopreventive activities, as demonstrated in this model system.*

## Introduction

Epidemiological studies suggest that ≥70% of human cancers are related to environmental factors such as smoking and diet (1). Diet may contribute to carcinogenesis as a source of mutagens, carcinogens, and anticarcinogens; alternatively, diet may act as a modulator of carcinogen metabolism and tumor promotion. A series of previous studies have shown that diet has a significant influence on the endogenous covalent DNA modifications, i.e., I-compounds, in laboratory animals (2–5). In general, natural-ingredient diets induce a significantly higher level and a much more complicated pattern of I-compounds than a purified diet (2). In addition, purified diets high in fat induce a lower level of I-compounds than those high in carbohydrate or protein (3). Interestingly, a natural dietary ingredient, oats, induces the formation of three specific I-compounds in female rat liver (4). The component responsible for this effect was traced to a methanol-extractable lipid fraction rather than to the fractions containing carbohydrate, protein, or fiber (5). There is no clear answer to the question, Are the endogenous DNA modifications, i.e., I-compounds, premutagenic DNA lesions or normal DNA modifications? Certain I-compounds seem to be derived from oxidative stress and lipid peroxidation; others may have a functional role in physiological processes.

To shed light on the biological significance of I-compounds, we have conducted a short-term feeding study investigating the effect of dietary oat lipids on tumor initiation and promotion in a well-established rat tumorigenesis model. These studies indicate that although a diet containing oat lipids induced a significantly higher level of DNA modifications, i.e., I-compounds, this diet reduced the formation of preneoplastic lesions in rat liver compared with the control diet containing corn oil.

## Materials and Methods

### Diet Preparation

Oat lipids were prepared by methanol extraction of ground oats, as previously described (5). Briefly, 650 g of whole oat flour were extracted with 3.2 liters of methanol for three hours. The flour was separated by filtration and reextracted with another 3.2 liters of methanol. The process was repeated until 2 kg of oat flour had been extracted.

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**Table 1.** Analytic Values of the Oat Lipids in Comparison With Corn Oil

| | Oat Lipids[a] | Corn Oil[b] |
|---|---|---|
| Fat, % | | |
| AH fat | 94.48 | |
| Free fatty acids | 8.31 as oleic acid | |
| Peroxide value | None | |
| Fatty acid profile, % | | |
| Myristic | 0.2 | Trace |
| Palmitic | 16.0 | 10.8 |
| Stearic | 2.0 | 2.1 |
| Oleic | 36.9 | 26.5 |
| Linoleic | 42.5 | 60.0 |
| Linolenic | 1.5 | 0.6 |
| Unknown | 1.7 | |
| Vitamins, mg/100 g | | |
| Carotene | 5.6 | |
| α-Tocopherol | 12.9 | 15.0 |
| β-Tocopherol | 1.4 | |
| γ-Tocopherol | 0.09 | |
| Other tocopherols | | 70.0 |
| Sterols, mg/100 g | | |
| β-Setosterol | 217 | |
| Campesterol | 33.1 | |
| Stigmasterol | 19.3 | |
| Other miscellaneous | | |
| Unsaponifiable matter | 0.07% | |
| Total phosphorus | 1,409.5 ppm | |
| AOM[c] | 63 h | |

*a:* Oat lipids were analyzed by John Stuart Research Laboratories of Quaker Oats Company and Hazelton Laboratory (Madison, WI).
*b:* Information for corn oil was provided by Dyets (Bethlehem, PA).
*c:* Active oxygen method (AOM) or time to reach 100 meq/kg peroxide value with exposure to oxygen is an indicator of stability.

Methanol was evaporated in a rotary evaporator until the methanol residue was <0.2%. The extract was then mixed with trioctanoin, a $C_8$ medium triglyceride, at a 1:1 (wt/wt) ratio. The composition of the oat lipids (Table 1) was analyzed (John Stuart Research Laboratories, Quaker Oats Company), and the sterols were determined (Hazelton Laboratory, Madison, WI). A modified AIN-76A diet was supplemented with 10% corn oil or 10% oat lipids as the source of fat (Dyets, Bethlehem, PA). The AIN-76A diet used in this study contained 20% casein, 15% cornstarch, 45% sucrose, and 10% corn oil or oat lipids. The supplementation of DL-methionine, choline bitartrate, salt, and vitamins was the same as in the original AIN-76A diet.

### Animal Treatment

Weanling female Sprague-Dawley rats (130 ± 10 g) were obtained from Harlan Sprague Dawley (Houston, TX) and divided into four dietary groups of 10 rats each. The experimental design is shown in Figure 1. Two groups of rats received the corn oil diet and two groups received the oat lipids diet from the beginning of the study. All rats were subjected to a 70% partial hepatectomy under ether anesthesia after three weeks of feeding. At the peak of cell proliferation

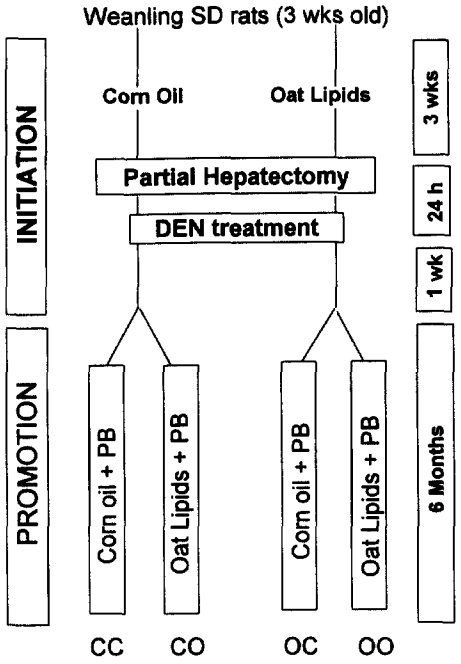


**Figure 1.** Schematic presentation of experimental design. DEN, diethylnitrosamine; SD, Sprague-Dawley; PB, phenobarbital; CC, corn oil diet only; CO, corn oil during initiation and oat lipids during promotion; OC, oat lipids during initiation and corn oil during promotion; OO, oat lipids only.

24 hours after surgery, a single dose of diethylnitrosamine (DEN, 10 mg/kg) was administered by gastric intubation. One week after the DEN treatment, each dietary group was further divided into two groups that were given corn oil or the oat lipids-containing diet supplemented with 0.05% phenobarbital (PB). The feeding continued for another five months. The initiation stage is defined as the period beginning three weeks before DEN dosing and ending one week after DEN exposure. The promotion stage started one week after DEN dosing and ended at the termination of the study. The final four dietary groups included corn oil only (Corn-Corn), oat oil only (Oat-Oat), oat oil during initiation and corn oil during promotion (Oat-Corn), and corn oil during initiation and oat oil during promotion (Corn-Oat). Rats were housed three per cage and provided food and water ad libitum. The body weights of rats were measured biweekly.

All rats were sacrificed five months after carcinogen (DEN) treatment by $CO_2$ exposure. Livers were excised and weighed. Sections of each of the three lobes of the liver remaining after the partial hepatectomy were placed on filter paper and immediately frozen on dry ice as a tissue block. The remaining liver tissues were frozen for DNA isolation.

### Altered Hepatic Foci Assay

Four serial sections of each tissue block were obtained by cryostat section and stained immunohistochemically with the placental isozyme glutathione *S*-transferase (GST) or histochemically with γ-glutamyl transpeptidase (GGT), canalicular adenosine triphosphatase (ATPase), or glucose 6-

phosphatase (G6Pase), as previously described (6). The number and volume fraction of the liver occupied by altered hepatic foci (AHF) were determined by the method of quantitative stereology, as previously described (7,8). Differences between groups were compared with two-way analysis of variance, and $p < 0.05$ was considered significant.

### DNA Adduct Analysis

DNA was isolated from liver tissues of four individual rats of each dietary group by a phenol-chloroform procedure. DNA adducts were analyzed by the nuclease P1-enhanced version of $^{32}P$ postlabeling (9). Chromatography conditions were as previously reported (4,5). Briefly, DNA was digested with nucleases into mononucleotides. The unmodified normal nucleotides were dephosphorylated by nuclease P1, and the modified nucleotides were labeled by polynucleotide kinase in the presence of [$\gamma$-$^{32}P$]ATP. The labeled products were then separated by multiple-dimensional chromatography. The $^{32}P$-labeled adducted nucleotides were excised from the chromatography thin layer, and radioactivity was determined by Cerenkov counting. The level of DNA adducts is expressed as a relative adduct labeling value, which is a ratio of counts of modified nucleotides to counts of total nucleotides in the reaction (9).

## Results

### Growth of the Rats

Replacement of corn oil with oat lipids did not affect the growth of the rats. Body weights of rats in all four dietary groups were similar during the study (data not shown). The final body weights and liver weights of rats in different dietary groups were also comparable (Table 2).

### DNA Modifications

The hepatic I-compound patterns in rats of different dietary groups are shown in Figure 2. Eight I-compound spots, including the three previously identified oats-specific I-compounds (Spots 2–4) with substantial intensities, were observed in rats that were given oat lipids throughout the feeding period (Oat-Oat group) or during the promotion stage (Corn-Oat group). A number of spots with very low intensities were seen in rats consuming predominantly the corn oil diet

**Table 2.** Body Weight and Liver Weight of Rats Fed Different Diets[a]

| Group | Body Wt, g | Liver Wt, g | Liver Wt/ Body Wt, % |
|---|---|---|---|
| Corn-Corn | 281 ± 31 | 9.88 ± 0.58 | 3.5 |
| Corn-Oat | 295 ± 18 | 11.06 ± 0.50 | 3.7 |
| Oat-Corn | 315 ± 27 | 10.90 ± 0.52 | 3.5 |
| Oat-Oat | 316 ± 29 | 11.64 ± 0.50 | 3.7 |

*a:* Values are means ± SE.

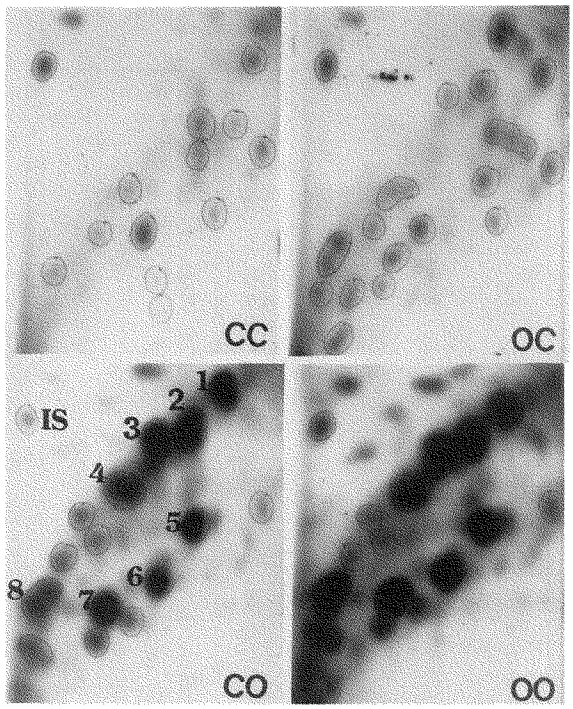


**Figure 2.** Typical $^{32}P$-labeled DNA adduct profiles in liver from rats fed different diets. Film exposure was at −80°C for 16 h. For clarity, adduct spots with strong intensities were numbered for CO group only. IS, adduct derived from an internal standard DNA of mouse skin exposed to a weak carcinogen, dibenzo[*a,j*]acridine. See Figure 1 legend for explanation of diet groups.

(Corn-Corn and Oat-Corn groups). These weak spots were not individually quantified because of the inconsistent recovery. The total I-compound levels in these two groups were obtained from summation of radioactive counts of any detectable spots on the chromatogram. Oat lipids in the diet resulted in the formation of a significantly greater amount of I-compounds than in animals fed the corn oil-containing diet (Table 3). The level of total I-compounds was five- to sixfold higher in rats fed the oat lipids diet (Oat-Oat and Corn-Oat groups) than in those fed the corn oil diet (Corn-Corn and Oat-Corn groups) (Table 3). Two-way analysis of variance showed that the levels of total I-compounds are significantly different between the groups fed diets containing corn oil (Corn-Corn and Oat-Corn groups) and those fed diets containing oat lipids (Oat-Oat and Corn-Oat groups) during the promotion stage ($F = 55.92, p < 0.001$). This difference was not present when the two dietary groups were compared during the initiation stage ($F = 1.42, p > 0.05$). There is no interaction in the dietary effects between initiation and promotion ($F = 2.78$).

### AHF

With four enzyme markers, 15 different AHF phenotypes can be detected, i.e., foci expressing GST, GGT, ATPase, G6Pase, GST-GGT, GST-ATPase, GST-G6Pase, GGT-G6Pase, GGT-ATPase, ATPase-G6Pase, GST-GGT-ATPase, GST-GGT-G6Pase, GST-ATPase-G6Pase, GGT-ATPase-G6Pase, and

**Table 3.** Hepatic I-Compounds in Rats Fed Different Diets[a]

| Spot No. | Group | | | |
|---|---|---|---|---|
| | Corn-Corn | Oat-Corn | Corn-Oat | Oat-Oat |
| 1 | | | 22.5 ± 24.0 | 39.5 ± 5.6 |
| 2 | | | 34.3 ± 33.6 | 58.0 ± 10.5 |
| 3 | | | 27.7 ± 22.9 | 43.8 ± 11.5 |
| 4 | | | 12.8 ± 10.2 | 20.0 ± 5.6 |
| 5 | | | 18.8 ± 12.4 | 27.5 ± 10 |
| 6 | | | 15.2 ± 13.9 | 25.1 ± 5.3 |
| 7 | | | 31.1 ± 22.4 | 46.9 ± 15.2 |
| 8 | | | 12.2 ± 9.8 | 19.1 ± 5.3 |
| Other | | | 22.1 ± 19.4 | 35.9 ± 8.4 |
| Total | 47.8 ± 15.8 | 58.9 ± 36.6 | 191.9 ± 175.4 | 315.9 ± 67.9 |

*a:* Values are means ± SD. Spots 1–8 were the same I-compounds in Corn-Oat and Oat-Oat groups and were not individually quantified in Corn-Corn and Oat-Corn groups.

GST-GGT-ATPase-G6Pase. Figure 3 shows the effects of dietary oat lipids on the number and volume fraction of AHF expressing the individual enzyme markers GST, GGT, ATPase, and G6Pase as well as the total number or volume percentage of the liver occupied by AHF (any phenotype). Apparently GST-expressing AHF constituted the majority of the detectable AHF in these animals. Two-way analysis of variance showed that feeding of oat lipids-containing diets during the promotion stage resulted in a significantly smaller number and reduced volume of foci expressing GST (Table 4). A similar trend was observed for foci expressing any phenotypes at borderline significance (Table 4). This effect was not seen when different diets were given during the initiation stage (Table 4). An interaction was not detected between the dietary manipulation during initiation and promotion (Table 4). Data on other phenotypes are not shown, since significant differences were not detected in these comparisons.

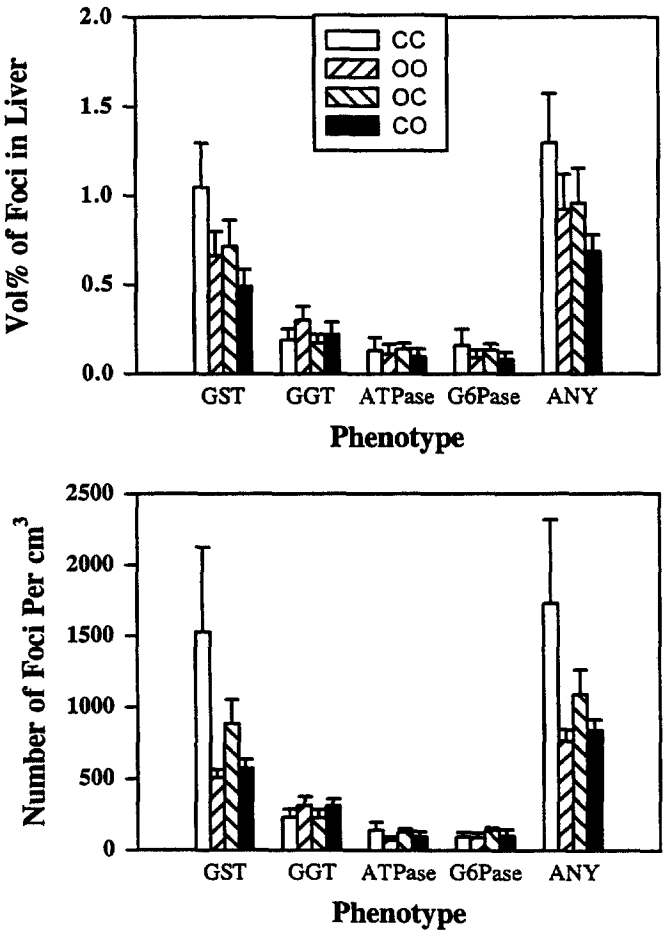


**Figure 3.** Phenotypic distribution of altered hepatic foci expressed as volume percentage of liver (top) and number of foci/cm$^3$ liver (bottom) in rats fed different diets (means ± SEM, $n$ = 8–10). GST, glutathione *S*-transferase; GGT, γ-glutamyl transpeptidase; G6Pase, glucose 6-phosphatase; ATPase, adenosine triphosphatase. ANY, total number of altered hepatic foci that could be detected with all 4 markers of hepatic preneoplasia. See Figure 1 legend for explanation of diet groups. Results of statistical analysis are shown in Table 4.

## Discussion

This study has shown that oat lipids in the diet resulted in a significantly higher level of I-compounds in rat liver than corn oil. Formation of these DNA modifications was associated with a suppression of the formation of DEN-induced and PB-promoted preneoplastic AHF. These oats-

**Table 4.** Comparison of Corn Oil- to Oat Lipids-Containing Diets on Stereological Parameters of Multistage Rat Liver Carcinogenesis[a]

| | Initiation | | | Promotion | | | Interaction |
|---|---|---|---|---|---|---|---|
| | Corn | Oats | *P* value | Corn | Oats | *P* value | *P* value |
| GST | | | | | | | |
| Mean vol% | 0.79 ± 0.63 | 0.71 ± 0.44 | 0.66 | 0.91 ± 0.63 | 0.59 ± 0.37 | 0.08 | 0.20 |
| Foci/cm | 1,077 ± 1,365 | 694 ± 448 | 0.23 | 1,214 ± 1,330 | 536 ± 176 | 0.04 | 0.24 |
| Foci/liver | 10,082 ± 10,076 | 7,716 ± 5,228 | 0.35 | 11,593 ± 10,341 | 6,073 ± 2,092 | 0.04 | 0.47 |
| Any | | | | | | | |
| Mean vol% | 1.01 ± 0.69 | 0.97 ± 0.60 | 0.86 | 1.16 ± 0.73 | 0.82 ± 0.50 | 0.11 | 0.24 |
| Foci/cm | 1,311 ± 1,339 | 930 ± 458 | 0.23 | 1,422 ± 1,313 | 797 ± 235 | 0.06 | 0.40 |
| Foci/liver | 12,591 ± 9,714 | 10,395 ± 5,472 | 0.39 | 13,749 ± 10,092 | 9,116 ± 3,137 | 0.08 | 0.47 |

*a:* Values are means ± SD. *P* values are from 2-way analysis of variance. Mean vol%, volume percentage of liver occupied by altered hepatic foci. GST, glutathione *S*-transferase.

induced I-compounds belong to a class of I-compounds that are reduced by tumor-promoting agents and enhanced by caloric restriction.

The biological significance of I-compounds is not clearly understood. Studies in the laboratory of Randerath have shown that these DNA derivatives can be classified into two categories (10). The type I I-compounds are primarily related to normal metabolism of nutrients and hormones. The type II I-compounds represent DNA lesions derived from oxidative stress (11). The type I I-compounds are species, sex, tissue, and diet specific, and their levels are significantly reduced by carcinogen exposure and enhanced in calorie-restricted animals (12). A circadian variation in the formation of type I I-compounds is observed in rat liver, in contrast to the consistent level of carcinogen-DNA adducts in the same tissues (13). Interestingly, a recent study shows that type I I-compound formation in mouse liver may be related to isoprenoids and cholesterol (14). Whether the formation of the oats-specific I-compounds in rat liver is related to an effect on cholesterol metabolism is unknown. Oats-related I-compounds are detected primarily in female rats and are observed in male rats only after two years of age (12). In addition, their formation is reduced by oophorectomy and induced by castration (15), implicating sex hormones in their formation or maintenance. Oats contain a number of sterols, including oats-specific $\Delta^5$-avenasterol, which are structurally similar to cholesterol and cholesterol precursors (16). Furthermore, the avenanthramides, a group of phenolic antioxidants, are found only in oats (17,18). It is possible that the oat sterols and/or avenanthramides or their metabolites are responsible for the formation of the oats-related DNA derivatives. Further fractionation of the oat lipids and studies on individual components may reveal the source and structural properties of these novel DNA modifications. The available evidence supports the hypothesis that oat lipids-induced I-compounds represent DNA modifications derived from normal intermediate metabolites.

An oat lipids-containing diet given during promotion significantly suppressed formation of AHF induced by DEN and promoted by PB in rat liver. Whether the reduced formation of AHF is directly related to the formation of oats-specific DNA derivatives is unknown. Previous studies show that natural-ingredient diets act to promote or enhance promotion by other agents but inhibit initiation compared with a purified diet (19–21). These effects may be related to the enzyme-inducing activity of many natural plant products present in the natural-ingredient diets. In the current study, purified AIN-76A diet was supplemented with corn oil or oat lipids; therefore, the effect of other natural products should not have been an appreciable factor. Other factors that may affect the AHF formation involve the levels of linoleic acid and antioxidant in the diet. The linoleic acid level (42.5%) in oat lipids was lower, whereas the oleic acid level (36.9%) was higher, than the corresponding levels in corn oil (60% and 26.5%, respectively). Previous studies show that the level of fatty acids in purified diet can affect the initiation of DEN-induced AHF without significantly affecting promotion of AHF (22,23). In the current study the effect of oat lipids was seen during the promotion rather than the initiation stage. In addition, the growth rate of rats was not affected by the dietary manipulation in this study. Thus it is unlikely that the reduced formation of AHF was due to variation in the fatty acid composition or calorie intake. It is possible, however, that oat lipids suppressed the formation of AHF through an antioxidant mechanism. Earlier work demonstrates that antioxidants, such as those found in soybean isoflavone extract, suppress AHF formation (24). Oat lipids contain unique phenolic antioxidants, which may be responsible for the formation of I-compounds and may also contribute to the reduced formation of AHF observed in the current studies. Further studies to explore these possibilities and the effects of oat lipids on AHF formation in uninitiated rats are warranted.

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## References

1. Randerath, K, Reddy, MV, and Disher, RM: Age- and tissue-related DNA modifications in untreated rats: detection by $^{32}$P-postlabeling assay and possible significance for spontaneous tumor induction and aging. *Carcinogenesis* **7,** 1615–1617, 1986.
2. Li, D, and Randerath, K: Association between diet and age-related DNA modifications (I-compounds) in rat liver and kidney. *Cancer Res* **50,** 3991–3996, 1990.
3. Li, D, and Randerath, K: Modulation of DNA modification (I-compound) levels in rat liver and kidney by dietary carbohydrate, protein, fat, vitamin, and mineral content. *Mutat Res* **275,** 47–56, 1992.
4. 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.
5. Li, D, Chen, S, Randerath, E, and Randerath, K: Oat lipids-induced covalent DNA modifications (I-compounds) in female Sprague-Dawley rats, as determined by $^{32}$P-postlabeling. *Chem Biol Interact* **84,** 229–242, 1992.
6. Hendrich, S, Campbell, H, and Pitot, HC: Quantitative stereological evaluation of four histochemical markers of altered hepatic foci in multistage hepatocarcinogenesis in the rat. *Carcinogenesis* **8,** 1245–1250, 1987.
7. Campbell, H, Pitot, HC, Potter, V, and Laishes, B: Applications of quantitative stereology to the evaluation of enzyme altered foci in rat liver. *Cancer Res* **42,** 465–472, 1982.
8. Campbell, H, Xu, Y, Hanigan, M, and Pitot, HC: Application of quantitative stereology to the evaluation of phenotypically heterogeneous enzyme-altered foci in the rat liver. *JNCI* **76,** 751–767, 1982.
9. Reddy, MV, and Randerath, K: Nuclease P1-mediated enhancement of sensitivity of $^{32}$P-postlabeling test for structurally diverse DNA adducts. *Carcinogenesis* **7,** 1543–1551, 1986.

10. Randerath, K, Randerath, E, Zhou, G, and Li, D: Bulky endogenous DNA modifications (I-compounds)—possible structural origins and functional implications. *Mutat Res*. In press.
11. Randerath, K, Yang, PF, Danna, TF, Reddy, R, Watson, WP, et al.: Bulky adducts detected by $^{32}$P-postlabeling in DNA modified by oxidative damage *in vitro*. Comparison with rat lung I-compounds. *Mutat Res* **250,** 135–144, 1991.
12. Randerath, K, Li, D, and Randerath, E: Age-related DNA modifications (I-compounds): modulation by physiological and pathological processes. *Mutat Res* **238,** 245–253, 1990.
13. Nath, RG, Vulimiri, SV, and Randerath, K: Circadian rhythm of covalent modifications in liver DNA. *Biochem Biophys Res Commun* **189,** 545–550, 1992.
14. Randerath, K, Li, D, Nath, RG, and Randerath, E: Exogenous and endogenous DNA modifications as monitored by $^{32}$P-postlabeling: relationships to cancer and aging. *Exp Gerontol* **7,** 533–549, 1992.
15. Randerath, E, Randerath, K, Reddy, R, and Lucier, GW: Sexual dimorphism of the chromatographic profiles of I-compounds (endogenous deoxyribonucleic acid modifications) in rat liver. *Endocrinology* **129,** 3093–3100, 1991.
16. White, PJ, and Armstrong, LS: Effect of selected oat sterols on the deterioration of heated soybean oil. *J Am Oil Chem Soc* **63,** 525–529, 1986.
17. Dimberg, LH, Theander, O, and Lingnert, H: Avenanthramides—a group of phenolic antioxidants in oats. *Cereal Chem* **70,** 637–641, 1993.
18. Collins, FW: Oat phenolics: avenanthramides, novel substituted *N*-cinnamoylanthranilate alkaloids from oat groats and hulls. *J Agric Food Chem* **37,** 60–66, 1989.
19. Pitot, HC, Campbell, HA, Maronpot, R, Bawa, N, Rizvi, TA, et al.: Critical parameters in the quantitation of the stages of initiation, promotion, and progression in one model of hepatocarcinogenesis in the rat. *Toxicol Pathol* **17,** 594–612, 1989.
20. Hendrich, S, Glauert, HP, and Pitot, HC: A semipurified diet that suppresses phenobarbital promotion of hepatocarcinogensis in the rat. *Nutr Cancer* **12,** 249–258, 1989.
21. Russell, JJ, Staffeld, EF, Wright, BJ, Prapuolenis, A, Carnes, BA, et al.: Effects of rat strain, diet composition, and phenobarbital on hepatic γ-glutamyl transpeptidase histochemistry and on the induction of altered hepatocyte foci and hepatic tumors by diethylnitrosamine. *Cancer Res* **47,** 1130–1134, 1987.
22. Glauert, HP, and Pitot, HC: Effect of dietary fat on the promotion of diethylnitrosamine-induced hepatocarcinogenesis in female rats. *Proc Soc Exp Biol Med* **181,** 498–506, 1986.
23. Glauert, HP, Lay, LT, Kennan, WS, and Pitot, HC: Effect of dietary fat on the initiation of hepatocarcinogenesis by diethylnitrosamine or 2-acetylaminofluorene in rats. *Carcinogenesis* **6,** 991–995, 1991.
24. Lee, KW, Wang, HJ, Murphy, PA, and Hendrich, S: Soybean isoflavone extract suppresses early but not later promotion of hepatocarcinogenesis by phenobarbital in female rat liver. *Nutr Cancer* **24,** 267–278, 1995.