Association between Diet and Age-related DNA Modifications (I-Compounds) in Rat Liver and Kidney

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ABSTRACT

Mammalian tissue DNA has recently been found, by 32P-postlabeling, to contain complex profiles of age-dependent and tissue-specific bulky carcinogen adduct-like modifications, which have been termed I-compounds since they appeared to arise indigenously, in the absence of exposure to exogenous carcinogens. I-compounds are presumably formed by reaction of metabolically produced, yet unidentified, electrophiles with DNA. In order to shed light on the origin of I-compounds, we have examined whether diet affects the levels and profiles of I-compounds. Weanling female Sprague-Dawley rats were provided with either one of three natural ingredient diets (rodent chows) or a purified diet (AIN-76A) for up to 6 months. Liver and kidney DNAs were analyzed after 0, 3, and 6 months of feeding, by a nuclease P1-enhanced 32P-postlabeling assay. Rats fed natural ingredient diets showed a greater complexity and 2.5-6.4-fold higher levels of I-compounds in the DNA of both tissues than rats fed purified diet. In addition, less marked qualitative and quantitative differences were noted among rats fed different chow diets. Three classes of I-compounds were identified: class A, I-spots common to both kinds of diet; class B, chow-specific spots; and class C, AIN-76A-specific spots. Liver and kidney shared some I-compounds, mostly belonging to class A, but there were also tissue-specific spots. These observations indicate a novel intimate link between diet and DNA modifications and are consistent with the hypothesis that the formation of I-compounds proceeds via normal metabolism of nutrients and other natural dietary components, leading to the production of small amounts of DNA-reactive electrophiles. Because of their DNA adduct-like character, I-compounds may play a critical role at the interface between nutrition and cancer.

INTRODUCTION

It has been estimated (1) that 30-40% of cancers in men and 60% of cancers in women are influenced by normal constituents of the diet and by chemicals that are normal cell products or metabolites. This indicates that a large proportion of human cancers could possibly be prevented by dietary modifications. Therefore, an understanding of mechanisms of dietary effects on carcinogenesis, especially at the molecular level, is essential in cancer prevention. It is known that dietary composition and caloric intake can modify chemical-induced or spontaneous tumorigenesis (2-7). Although there is strong evidence identifying genes as essential substrates for aging and carcinogenesis (8), it is not clear whether human genes are directly affected by dietary constituents or specific nutrients. Further research is needed to explore mechanisms underlying relationships between nutrition, gene expression, and carcinogenesis. In the present work, we have studied the effects of diet on a recently discovered class of covalent DNA modifications termed I-compounds (9).

I-compounds are age-dependent, tissue-, sex-, and species-specific, putative indigenous covalent DNA modifications in untreated laboratory animals (10). The total amount of I-compounds is substantial, e.g., in 8-10-month-old rat liver, this value is as high as 2 adducts in 10^7 normal nucleotides, resembling the levels of persistent carcinogen-DNA adducts during chemical carcinogenesis (9-12). While the small amounts of individual I-compounds present in tissue DNA have thus far prevented chemical identification, chromatography has provided evidence of great structural diversity among I-compounds (11). I-compounds resemble carcinogen adducts but not 5-methylcytosine in terms of their chromatographic properties and behavior during tissue regeneration (11). Markedly reduced levels of I-compounds have been observed in tissues of animals treated with one of several hepatocarcinogens (12-14). Lower levels of I-compounds have also been linked to strains of mice with high incidences of spontaneous tumor and degenerative renal diseases (15). As suggested previously (9, 16), the formation of I-compounds may be mediated by environmental factors or by endogenous DNA-reactive metabolites and may play a role in spontaneous tumorigenesis and aging. Nevertheless, I-compounds could also function in normal gene expression, regulation of DNA replication and tissue growth, or other processes. The hypothesis that formation of I-compounds is affected by diet has been tested in the present paper.

MATERIALS AND METHODS

Materials. AIN3 purified diet (20% casein, 65% corn starch and sucrose, 5% corn oil, 5% cellulose, vitamin mix, mineral mix, methionine, and choline) (17, 18) was obtained from Dyets (Bethlehem, PA). Animals and Teklad diet were purchased from Harlan Sprague-Dawley Inc. (Houston, TX). Purina and Wayne diets were from Alief and Pearlard Feeding Co., respectively (Houston, TX). Diets were stored at 4°C for a maximum period of 3 months. Materials for DNA analysis have been described previously (19, 20), except that cloned T4 poly-nucleotide kinase was obtained from US Biochemicals (Cleveland, OH) and [32P]phosphate (acid-free) from ICN Radiochemicals (Irvine, CA).

Animals. Weanling (about 1-month-old) female Sprague-Dawley rats were divided into four groups of eight animals each. One group received AIN purified diet. The other three groups were fed either Teklad, Purina, or Wayne natural ingredient diets. Rats were housed four/cage and allowed free access to food and water. Light was maintained on a 12-h cycle (7 a.m. to 7 p.m.). Food consumption and body weight were measured every week. Animals were killed after 3 and 6 months of feeding. A group of five weaning rats from the same shipment was sacrificed before the feeding started. Livers and kidneys were excised, minced, and frozen immediately for storage at −80°C until DNA isolation.

DNA Isolation and 32P Analysis of I-Compounds. DNA was isolated by a procedure involving solvent extraction and enzymatic digestion of protein and RNA (21). I-compounds were measured by the nuclease P1-enhanced 32P-postlabeling assay (22). Briefly, 10 μg DNA were digested with a mixture of micrococal nuclease and spleen phosphodiesterase to deoxiribonucleoside 3'-monophosphates. After the digest had been treated with nuclease P1 to convert the normal 3'-nucleotides to nucleosides, the modified 3'-nucleotides were converted to 5'-32P-labeled deoxyribonucleoside 3',5'-bisphosphates by T4 polynucleotide kinase-catalyzed transfer of [32P]phosphate from [γ-32P]ATP. Labeled

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3The abbreviations used are: AIN, AIN-76A; chow, natural ingredient (cereal-based) diet; Teklad, Teklad LM485 rodent chow; Purina, Purina 5001 rodent chow; Wayne, Wayne MKH 22/5 Rodent Blox; TLC, thin layer chromatography; RAL, relative adduct labeling.

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products were purified and separated by multidirectional polyethylene-imine-cellulose TLC exactly as described before (11). This mapping included the resolution of I-compounds into lower, central, and upper groups in the order of increasing polarity (11). For chromatographic analysis, $5\mu g$ each of two test DNAs were mixed before hydrolysis and $^{32}P$-labeled and were examined by the same chromatographic procedure, along with individual DNA samples. Autoradiograms from mixed and single DNAs were compared.

For quantitative analysis, TLC fractions ("spots" or incompletely resolved clusters of spots) were located through screen-enhanced autoradiography using Kodak XAR-5 X-ray film, excised from the maps, and subjected to scintillation (Cerenkov) assay. Data were expressed as RAL values, which represented a minimum estimate of the level of DNA modification, since 100% recovery of all I-compounds in $^{32}P$-labeled form was presumably not achieved by these procedures (22). Unpaired Student’s $t$ test and one-way analysis of variance were employed for statistical analysis.

Histology. Two pieces each of liver and kidney from rats fed different diets for 6 months were fixed in phosphate-buffered formalin and examined at Texas Veterinary Medical Diagnostic Laboratory (College Station, TX).

**RESULTS**

**General.** Average food intake (g/rat/day) and body weight of rats are illustrated in Fig. 1. Although rats fed chow diets consumed about 30–35% more food than rats fed AIN diet, growth rates of the two dietary groups were identical. Diminished food intake by the AIN group was probably related to lack of bulk in the purified diet. No abnormal histological features were observed in the livers. Rats fed AIN or Purina diet showed a mild toxic tubular nephropathy.

Liver I-Compounds. A total of nine individual spots and clusters of I-spots of varying polarities were noted in weanling rat liver DNA (Fig. 2). The total RAL $\times 10^9$ value was 36.35 ± 1.05. Two spots (L24 and L25) present in weanling rat liver were not observed at 3 and 6 months. Spots with numbers prefixed by L were detected only in liver and not kidney DNA. The intensities and patterns of liver I-compounds were remarkably different for rats fed purified (AIN) diet or chow (Teklad) diets for 3 and 6 months (shown for the 6-month group only in Fig. 2). Liver I-spots of rats fed purified diet for 3 months were qualitatively and quantitatively similar to those in weanling rats (data not shown); at 6 months, however, most spots were intensified, and four additional spots (L4, L7, L9, and L10) appeared (Fig. 2). Spots L22 and L23 were detected in AIN-fed rats only. In rats fed Teklad chow, lower, central, and upper maps combined yielded a total of 19 spots or clusters of spots at 3 months and 21 spots at 6 months, due to the appearance of spots 17 and 18 (Fig. 2). Compared with rats fed AIN diet (Fig. 2, middle), Teklad-fed rats showed eight additional spots or clusters of spots and greatly intensified labeling of most fractions (Fig. 2, right).

As shown by quantitative analysis, age-dependent increases in total liver I-compound levels were evident in all three chow diet groups at both time points, while purified diet led to such increases only at the 6-month time point (Fig. 3, upper). The 2.5–3.5-fold increase in total I-compound levels at 3 and 6 months was due not only to additional spots elicited by chow diet but also to much higher levels of spots that were common to both diets (Table 1). Similar increases were detected in all three chromatographic polarity groups analyzed (Tables 1 and 2).

I-spot patterns from Purina diet were identical to those from Teklad diet (data not shown), but the amounts were somewhat lower (Table 2). On the other hand, three major spots on Teklad and Purina lower maps (spots L2, L3, and L4) were absent in the Wayne group (data not shown), and those spots detected on lower maps of the Wayne group were weaker at 3 and 6 months than those in the other chow groups (Table 2). Total RAL values were similar, however, among the three chow diet groups after 6 months of feeding, and only the Teklad values were elevated at 3 months (Table 2; analysis of variance, $P < 0.01$).

Kidney I-Compounds. A total of 11 $^{32}P$-postlabeled I-compound fractions were discernible on maps derived from weanling rat kidney DNAs (Fig. 4), giving an RAL $\times 10^9$ value of 41.88 ± 2.29. The same I-compounds were also seen in rats fed AIN or Teklad diets for 3 or 6 months (see examples in Fig. 4). Qualitatively, the patterns of these two dietary groups resembled each other, but three relatively weak spots (K4, K7, and 15) were seen in Teklad diet-fed animals only (Fig. 4). As shown by chromatography experiments, the spots given the prefix K in Fig. 4 were detected only in kidney and not liver DNA.

All three chow diets were associated with clear age-dependent increases in kidney I-compound levels of each polarity group (Table 3, Fig. 3). Only spot K3 was decreased at 6 months compared with 3 months (data not shown). No age-dependent increase in I-compound levels was seen for kidney DNA of rats fed purified diet (Table 3, Fig. 3). This phenomenon occurred because the age-related increase of certain fractions (spots 5 and 6) was negated by the decline of others (spots K3 and 16) (Table 1; quantitative data for individual spots at 6 months only shown).

Total RAL values for kidney DNA of chow diet groups were 2.5–4.2 and 3–6.4 times higher, compared with the purified diet group, after 3 and 6 months of feeding, respectively (Table 3). These differences were mainly due to intensification of most individual spots (Fig. 4, Table 1). Among the chow diets, Teklad consistently gave the highest levels of kidney I-compounds (Table 3, Fig. 3).

**Cochromatographic Comparisons of I-Compound Fractions.** As shown in Fig. 5 (left), cochromatographic analysis revealed that both liver and kidney DNA contained common (chromatographically but not necessarily chemically identical) as well as
tissue-specific compounds. Liver and kidney DNAs shared eight spots, i.e., spots 5, 6, 14–17, 19, and 20 in rats fed Teklad diet. Ten liver-specific (L1–L4, L7, L9, L11–L13, and L21) and two kidney-specific (K3 and K4) fractions were observed.

In AIN diet-fed rats, five common spots of liver and kidney comigrated in the two-dimensional TLC system, i.e., spots 5, 6, 14–16, and 20 (data for cochromatography not shown; see Figs. 2 and 4 for spot locations). Three liver-specific (L11, L22, and L23) and three kidney-specific (K2, K3, and K18) spots were identified.

Tissue-specific spots amounted to 71 and 12% of total RAL values for liver and kidney DNAs, respectively, of rats fed Teklad diet (Table 1). Most spots common to both tissues were more intense in the kidney (Table 1). In AIN-fed rats, tissue-specific spots contributed 44 and 18% of total RAL values for liver and kidney DNA, respectively (Table 1). Absolute and relative intensities of common spots varied between tissues (Table 1). In addition to tissue-specific spots, cochromatography also revealed the existence of diet-specific spots. In liver DNA, 12

![Liver I-compounds profiles of female Sprague-Dawley rats at the time of weaning (left) and after 6 months of feeding either AIN purified diet (middle) or Teklad chow diet (right), as determined by 32P-postlabeling and polyethylene-imine-cellulose TLC. Experimental conditions are given in “Materials and Methods.” Film exposure was 14 h at -80°C with Kodak XAR-5 film and DuPont Lightning Plus intensifying screen. B, background spot which was present in all samples to similar intensities. Prefix L, liver-specific spot or cluster of spots. L24 and L25 were identified only in weanling rats. L22 and L23 were reproducibly detected only in AIN diet-fed rats. Note marked differences in spot patterns between rats fed purified or chow diet. U, upper; C, central; L, lower.

![Age dependence of mean total (lower plus central plus upper) I-compound levels in liver and kidney DNA of weanling rats and rats fed one of four different diets for 3 or 6 months. SE were <11% of the means.](image-url)

**Table 1** Individual I-compounds of rats fed AIN or Teklad diet for 6 months

<table>
<thead>
<tr>
<th>Class*</th>
<th>Spot</th>
<th>AIN</th>
<th>Teklad</th>
<th>Spot</th>
<th>AIN</th>
<th>Teklad</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>7.61 ± 1.14⁺</td>
<td>11.22 ± 1.35</td>
<td>5</td>
<td>2.73 ± 0.30</td>
<td>3.95 ± 0.82</td>
</tr>
<tr>
<td>6</td>
<td>0.21 ± 0.09</td>
<td>1.89 ± 0.40</td>
<td>6.04 ± 0.36</td>
<td>14</td>
<td>1.77 ± 0.09</td>
<td>1.65 ± 0.24</td>
</tr>
<tr>
<td>14</td>
<td>8.64 ± 0.91</td>
<td>9.56 ± 1.72</td>
<td>1.60 ± 0.44</td>
<td>17</td>
<td>1.80 ± 0.12</td>
<td>1.43 ± 0.24</td>
</tr>
<tr>
<td>19</td>
<td>7.35 ± 2.10</td>
<td>8.72 ± 0.82</td>
<td>1.70 ± 0.78</td>
<td>20</td>
<td>10.22 ± 2.26</td>
<td>NA</td>
</tr>
<tr>
<td>Subtotal</td>
<td>35.80 (49%)</td>
<td>49.76 (22%)</td>
<td>25.20 (82%)</td>
<td>47.66 (86%)</td>
<td>167.88 (86%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Liver I-compounds of rats fed different diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Months*</th>
<th>Lower</th>
<th>Central</th>
<th>Upper</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>3</td>
<td>5.87 ± 0.67⁺</td>
<td>12.20 ± 1.32</td>
<td>10.75 ± 0.70</td>
<td>28.82 ± 2.28</td>
</tr>
<tr>
<td>Purina</td>
<td>3</td>
<td>23.73 ± 1.96</td>
<td>31.63 ± 2.03</td>
<td>31.31 ± 1.32</td>
<td>76.67 ± 4.58</td>
</tr>
<tr>
<td>Teklad</td>
<td>3</td>
<td>33.47 ± 1.90</td>
<td>32.79 ± 1.53</td>
<td>33.49 ± 2.61</td>
<td>99.74 ± 2.64</td>
</tr>
<tr>
<td>Wayne</td>
<td>3</td>
<td>15.42 ± 1.90</td>
<td>25.43 ± 2.52</td>
<td>31.34 ± 0.74</td>
<td>72.20 ± 4.51</td>
</tr>
<tr>
<td>F (P)</td>
<td>22.37 (&lt;0.001)</td>
<td>1.43 ± 0.29</td>
<td>13.94 (0.002)</td>
<td>12.90 (0.002)</td>
<td></td>
</tr>
</tbody>
</table>

| AIN      | 6       | 17.97 ± 1.63 | 37.43 ± 3.95 | 17.57 ± 2.26 | 72.97 ± 5.35 |
| Purina   | 6       | 73.24 ± 8.05 | 64.38 ± 7.15 | 62.05 ± 6.94 | 199.67 ± 21.40 |
| Teklad   | 6       | 86.18 ± 7.48 | 67.23 ± 2.64 | 75.35 ± 9.29 | 228.97 ± 16.00 |
| Wayne    | 6       | 42.93 ± 5.15 | 77.65 ± 6.32 | 71.96 ± 4.30 | 192.54 ± 13.90 |
| F (P)    | 8.81 (0.008) | 1.49 (0.277) | 0.50 (0.624) | 0.58 (0.578) |

*Months of feeding.
⁺Total RAL values are the sums of RAL values from lower, central, and upper maps.
*Mean ± SE (n = 4).
*F and P values were from one way analysis of variance for the three chow diet groups. Differences between AIN group and chow groups in all columns were highly significant (t test; P values not given).
Table 3 Kidney I-compounds of rats fed different diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Months</th>
<th>Lower (RAL x 10^4)</th>
<th>Central</th>
<th>Upper</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>3</td>
<td>12.37 ± 0.63^a</td>
<td>4.30 ± 0.18</td>
<td>14.45 ± 2.20</td>
<td>31.12 ± 3.00</td>
</tr>
<tr>
<td>Purina</td>
<td>3</td>
<td>25.99 ± 1.83</td>
<td>21.42 ± 1.38</td>
<td>31.72 ± 1.43</td>
<td>79.13 ± 2.90</td>
</tr>
<tr>
<td>Teklad</td>
<td>3</td>
<td>37.77 ± 3.50</td>
<td>44.29 ± 1.78</td>
<td>49.25 ± 7.30</td>
<td>131.31 ± 8.07</td>
</tr>
<tr>
<td>Wayne</td>
<td>3</td>
<td>24.33 ± 0.91</td>
<td>24.51 ± 0.90</td>
<td>29.65 ± 2.37</td>
<td>78.49 ± 1.90</td>
</tr>
<tr>
<td>F (P)</td>
<td></td>
<td>20.75 (&lt;0.01)</td>
<td>129.90 (&lt;0.001)</td>
<td>5.62 (0.026)</td>
<td>34.56 (&lt;0.001)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Months</th>
<th>Lower (RAL x 10^4)</th>
<th>Central</th>
<th>Upper</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN</td>
<td>6</td>
<td>9.33 ± 1.25</td>
<td>5.46 ± 0.43</td>
<td>15.77 ± 1.41</td>
<td>30.56 ± 3.26</td>
</tr>
<tr>
<td>Purina</td>
<td>6</td>
<td>30.08 ± 1.97</td>
<td>40.25 ± 2.56</td>
<td>32.97 ± 2.25</td>
<td>103.30 ± 5.25</td>
</tr>
<tr>
<td>Teklad</td>
<td>6</td>
<td>46.25 ± 6.13</td>
<td>89.47 ± 6.24</td>
<td>59.29 ± 6.95</td>
<td>195.01 ± 12.80</td>
</tr>
<tr>
<td>Wayne</td>
<td>6</td>
<td>22.36 ± 1.05</td>
<td>45.54 ± 1.12</td>
<td>21.35 ± 1.21</td>
<td>93.34 ± 3.12</td>
</tr>
<tr>
<td>F (P)</td>
<td></td>
<td>10.52 (0.004)</td>
<td>58.14 (&lt;0.001)</td>
<td>19.79 (0.001)</td>
<td>61.05 (&lt;0.001)</td>
</tr>
</tbody>
</table>

* Months of feeding.

\[ RAL \text{ (Relative Activity Level)} \]

class A (common to both diet groups), 9 class B (chow-specific), and 2 class C (AIN-specific) spots were identified (Fig. 5, right; Table 1). In kidney, there was barely any qualitative difference between the dietary groups (data not shown). Only three weak chow-specific spots (K4, K7, and 15) were detected. The chow diet-specific (class B) spots contributed 64% and 3% of the total RAL values for liver and kidney, respectively, in Teklad diet-fed rats, while AIN-specific spots (class C) amounted to 7% in liver and were not detected in kidney (Table 1). Most class A spots exhibited much higher intensities in both liver and kidney of chow diet-fed rats (Table 1).
Could I-compounds be primarily derived from dietary contaminants? The occurrence of contaminants, including low levels of carcinogens, has been reported in commercial chow diets (26-28). The following observations argue against the possibility that I-compounds are products of dietary carcinogens. First, I-compound patterns illustrated in this report were distinct from DNA-adduct patterns found after exposure of rodent liver to potential contaminants, i.e., aflatoxins, safrole and other alkenylbenzenes, nitrosamines, benzo(a)pyrene and other polycyclic aromatic hydrocarbons, and food pyrolysis products (19, 20, 22, 29, 30). Second, rats fed Purina 5002, a diet certified for minimal contaminants (31), have been found to give hepatic I-compound levels and patterns virtually identical to those of the rats fed Teklad or Purina 5001 chow diets in this study (12). Another type of purified diet, i.e., choline-devoid diet, which was found to contain no carcinogens and barely detectable levels of mutagens (32), produced I-compound patterns very similar to those of the rats fed AIN-76A diet in this study (13). Third, most genotoxic carcinogens are known to elicit qualitatively similar or identical major DNA adduct profiles in various organs of the same animal (30, 33) or in the same organ of different species, but substantial tissue and species differences in I-compound patterns have been observed in the current as well as previous studies (9, 10). These observations imply that I-compounds are formed by a mechanism different from that of DNA adduct formation and, therefore, are not derived from dietary genotoxic contaminants.

Natural ingredient but not purified diets contain large amounts of unidentified nonnutrient natural products (34). Many of these products, e.g., flavones, are mutagenic and carcinogenic (35), and these substances may react directly with DNA, giving rise to class B I-spots preferentially in liver via a first-pass effect. On the other hand, some of these products are enzyme inducers (36) and, thus, may affect the levels of endogenous electrophilic precursors of class A I-spots.

What could be the source of such electrophiles? It is possible that they are formed in the course of normal metabolism of dietary macronutrients because (a) I-compound profiles are highly reproducible in animals of the same kind (9-12) and on the same type of diet (this paper); (b) I-compounds are strongly affected by sex-, tissue-, and species-dependent metabolic activities (10); and (c) variation of dietary macronutrient content has been linked to quantitative I-compound changes in rat liver and kidney DNA. As to effects of micronutrients, while AIN and chow diets contain similar levels of carbohydrate, protein, and fat, AIN diet contains markedly lower levels of minerals and vitamins than the chow diets (Table 4). However, this difference cannot be responsible for the lower levels of I-compounds in AIN-fed rats, since supplementation of the AIN diet with vitamins and minerals to match the chow composition did not affect I-compound levels. In addition to alteration of metabolism by nonnutrient products (see above), the complex composition of nutrients from various sources (Table 5) may itself contribute to the higher levels of class A I-spots and to formation of class B I-spots in chow diet-fed rats, but the mechanisms remain unknown.

As to I-compounds among animals fed different chow diets, three female-specific liver I-spots (L2-L4) (16) were missing in Wayne diet-fed rats, and RAL values on lower maps (Table 2) were significantly lower in this group. These observations were presumably related to differences in specific dietary ingredients. As illustrated in Table 5, Teklad and Purina diets contain alfalfa meal and ground oats, while Wayne diet contains corn and wheat flakes instead. It is not clear if and how absence or presence of any of these ingredients could be associated with I-compound formation. It was also noted that the presence (Purina) or absence (Teklad) of animal products (Table 5) made no apparent difference in liver I-spot patterns or levels. Lower levels of kidney I-compounds in Purina- versus Teklad-fed rats may be secondary to renal tissue damage (see “Results”).

Although the potential biological significance of I-compounds has not been determined, results from other studies indirectly suggest a beneficial role of these DNA derivatives. For example, feeding purified diet alone induces hepatic lesions (37), nephrocalcinosis (38), myocardial damage (39), and tubular nephrosis (present study) in laboratory rodents. The etiology of these lesions has not been elucidated. Animals fed purified rather than natural ingredient diets have also been found frequently to exhibit higher sensitivity to toxic agents (40-45) and higher tumor incidences (46-48). Diet-related alteration of xenobiotic metabolism is probably one of the mechanisms involved, but impairment of DNA functions potentially resulting from reduced I-compound levels in animals fed purified diet could also play a role. In line with previous findings (12-15), it is thus possible that I-compounds represent normal DNA modifications that are important for normal gene function and DNA replication (16). Under certain conditions, how-

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ever, some of these DNA derivatives may conceivably act as adduct-like premutagenic lesions and contribute to mutation and cancer (15).

In conclusion, we have shown significant associations between diet and age-dependent DNA modifications (I-compounds). Studies of nutrition-mediated changes in DNA modifications may eventually help to clarify the molecular basis of diet-related effects in carcinogenesis, aging, and certain non-neoplastic diseases and may define new approaches to human cancer prevention.

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