Metabolic Effects of Dietary Purine and Pyrimidine Bases in Rats

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The effect of dietary nucleic acid constituents (purine and pyrimidine bases) on the metabolism of serum and urinary uric acid, allantoin, creatinine, urea nitrogen and urea was examined in the rat. The results were compared with the morphological changes. It was found that guanine is readily converted to uric acid and allantoin, whereas adenine, due to its unique metabolism, is metabolized quite differently from guanine and pyrimidine bases. Furthermore, an increase of creatinine, urea nitrogen and urea in the serum as well as a reduction in their urine excretion was observed in rats fed on the adenine diet. Dietary adenine produced a nephrotoxic condition as reflected in the histopathological changes. That is, deposits of amorphous birefringent crystal (2,8-dihydroxyadenine) with formation of foreign body granuloma were demonstrated in the tubular lumina and the interstitium of the kidney, and also in the urinary bladder and urine. However, there were not seen in the glomeruli and other organs. On the other hand, in the kidneys of rats fed on guanine, uracil, cytosine and yeast RNA diets, these histopathological changes were not noticed.

Hyperuricemia is associated with the development of gout and it may be due to enhanced endogenous production or reduced excretion of uric acid. Although hyperuricemia is exacerbated by diets high in fat,¹ protein²,³ or nucleic acid,³⁻⁵ the nucleic acid intake has the greatest influence. For example, a purine-free diet results in an average reduction of the blood uric acid level,⁴ while feeding of nucleic acid results in an average elevation in blood uric acid.⁵ On the other hand, the previous reports from our laboratory have shown that the administration of nucleic acid to rats caused a significant rise in the urinary output of uric acid. On the contrary, the feeding of an adenine diet has exhibited a decrease of the uric acid excreted in the urine.⁶⁻¹¹ From these results, it seemed probable that an evaluation of the individual components in foods may enhance the understanding of the hyperuricemic state induced by nucleic acid intake.

To investigate this problem, the present studies were undertaken to evaluate the metabolism of rats fed on the components of ribonucleic acid (purine and pyrimidine bases). The report also describes the histopathological changes produced by the feeding of individual purine and pyrimidine bases.
MATERIALS AND METHODS

Animals and diets. Male rats of the JCL: Wistar strain (Hokuriku Labour, Ltd., Toyama, Japan), initially weighing 90~100 g, were placed in a metabolic chamber under a conventional lighting regimen with a dark night. The animals were fed on commercial feeds (CLEA Japan Inc., Tokyo, type CE-2) for one week after arrival. They were then divided into 6 groups of 6 animals each and fed ad libitum on a control, purine base, pyrimidine base or yeast RNA diet for 6 days. The control diet consisted of the following composition (in 100 g): casein 18 g, α-cornstarch 57.9 g, sucrose 15 g, soybean oil 2 g, salt mixture[12] 4 g, vitamin mixture[12] 1 g, cellulose powder 2 g and choline chloride 0.1 g. To this control diet, adenine, guanine, uracil or cytosine was added at 0.75 g/100 g of diet. Yeast RNA was added at 3 g/100 g of diet. The body weight, food intake and water intake of each rat were recorded daily, and during the experimental period, the urine was collected daily in a 50 ml Erlenmeyer flask. On the 6th day of the experimental diet, the rats were sacrificed by cutting the carotid artery and the blood was collected. The serum was separated by centrifugation immediately after collecting the blood.

Chemical analyses. Uric acid was determined by a modification of the method of Caraway[13]; allantoin, by the method of Young and Conway[14]; creatinine, by a modification of the method of Folin-Wu[15]; urea nitrogen, using a commercial reagent ("Urea NB-Test Wako" obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the urease-indophenol method; urea, by the method of Archibald.[16]

Histopathological studies. Both kidneys were obtained by autopsy from each animal and fixed in 10% buffered neutral formalin. Paraffin embedded sections were stained with haematoxylin and eosin and other stains performed for an analysis of the renal lesion.

RESULTS

Body weight, food intake, water intake and urine volume

The changes in body weight, food intake, water intake and urine volume during the experimental period are summarized in Table I. The feeding of an adenine diet showed a decrease of body weight on the 6th day. The reduction of body weight was calculated to be 92% of that before adenine feeding. However, the rats on this diet remained overtly healthy and active. Their behavior suggested a continuous craving for food. Indeed, when offered an adenine-free diet, they evinced an active

### Table I. Body Weight, Food Intake, H₂O Intake and Urine Volume

<table>
<thead>
<tr>
<th>Diet</th>
<th>Changes in body wt. (g/6 days)</th>
<th>Food intake (g/day)</th>
<th>H₂O intake (ml/day)</th>
<th>Urine volume (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>29.5±2.5</td>
<td>17.0±2.0</td>
<td>21.5±2.8</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>Adenine</td>
<td>-8.4±3.0*</td>
<td>6.4±0.9*</td>
<td>24.6±1.8</td>
<td>15.6±2.5*</td>
</tr>
<tr>
<td>Guanine</td>
<td>30.0±1.5</td>
<td>18.8±1.4</td>
<td>23.3±0.8</td>
<td>10.6±0.9</td>
</tr>
<tr>
<td>Uracil</td>
<td>23.7±0.9</td>
<td>16.5±1.3</td>
<td>18.3±1.1</td>
<td>9.2±0.6</td>
</tr>
<tr>
<td>Cytosine</td>
<td>26.0±2.7</td>
<td>15.8±1.2</td>
<td>15.5±1.4</td>
<td>7.3±0.6</td>
</tr>
</tbody>
</table>

Values are mean ±S.E. of 6 rats.
* Significantly different from the value of rats fed on a control diet, p<0.05, b p<0.01, c p<0.001.

### Table II. Uric Acid, Allantoin, Creatinine and Urea Nitrogen in the Serum

<table>
<thead>
<tr>
<th>Diet</th>
<th>Uric acid (mg/100 ml)</th>
<th>Allantoin (mg/100 ml)</th>
<th>Creatinine (mg/100 ml)</th>
<th>Urea-N (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.94±0.10</td>
<td>2.33±0.14</td>
<td>0.84±0.02</td>
<td>20.1±0.8</td>
</tr>
<tr>
<td>Adenine</td>
<td>2.30±0.09*</td>
<td>7.25±0.37*</td>
<td>1.28±0.03*</td>
<td>47.7±1.6*</td>
</tr>
<tr>
<td>Guanine</td>
<td>2.00±0.06</td>
<td>3.48±0.13*</td>
<td>0.85±0.02</td>
<td>22.3±0.9</td>
</tr>
<tr>
<td>Uracil</td>
<td>1.89±0.09</td>
<td>2.16±0.11</td>
<td>0.89±0.02</td>
<td>21.6±1.0</td>
</tr>
<tr>
<td>Cytosine</td>
<td>1.75±0.05</td>
<td>2.30±0.16</td>
<td>0.85±0.02</td>
<td>17.4±1.2</td>
</tr>
</tbody>
</table>

Values are mean ±S.E. of 6 rats.
* Significantly different from the value of rats fed on a control diet, p<0.05, b p<0.001.
Metabolic Effects of Dietary Purine and Pyrimidine Bases

Table III. Uric Acid, Allantoin, Creatinine and Urea in the Urine

<table>
<thead>
<tr>
<th>Diet</th>
<th>Uric acid (mg/day)</th>
<th>Allantoin (mg/day)</th>
<th>Creatinine (mg/day)</th>
<th>Urea (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.39 ± 0.16</td>
<td>23.8 ± 2.4</td>
<td>2.81 ± 0.29</td>
<td>264 ± 39</td>
</tr>
<tr>
<td>Adenine</td>
<td>1.47 ± 0.04*</td>
<td>40.4 ± 3.7*</td>
<td>1.97 ± 0.08*</td>
<td>155 ± 12*</td>
</tr>
<tr>
<td>Guanine</td>
<td>3.41 ± 0.13*</td>
<td>64.3 ± 3.9*</td>
<td>2.97 ± 0.22</td>
<td>271 ± 14</td>
</tr>
<tr>
<td>Uracil</td>
<td>2.75 ± 0.45</td>
<td>23.2 ± 1.5</td>
<td>3.12 ± 0.28</td>
<td>357 ± 19*</td>
</tr>
<tr>
<td>Cytosine</td>
<td>2.27 ± 0.19</td>
<td>22.1 ± 1.1</td>
<td>2.73 ± 0.15</td>
<td>297 ± 19</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 6 rats.

* Significantly different from the value of rats fed on a control diet, p < 0.05, " p < 0.01, ** p < 0.001.

Fig. 1. Macroscopic Findings of the Kidneys in 2 Experimental Rats of 5 Groups.
Nos. 171 & 172, the kidney of 2 rats fed on an adenine diet; Nos. 173 & 174, the kidney of 2 rats fed on a guanine diet; Nos. 175 & 176, the kidney of 2 rats fed on a uracil diet; Nos. 177 & 178, the kidney of 2 rats fed on a cytosine diet; Nos. 179 & 180, the kidney of 2 rats fed on a yeast RNA diet.

Fig. 2. Several Acicular Crystals in the Proximal Tubulous Lumina in the Adenine-fed Rat (No. 171). Haematoxylin-eosin stain, × 400.

Fig. 3. A Foreign Body Granuloma with Acicular Crystals in the Center of the Tubulus (No. 171). Haematoxylin-eosin stain, × 400.

Biochemical findings

Dietary adenine increased the level of serum allantoin, being about 3.1 times higher in rats fed on an adenine diet than in those fed on a control diet. As shown in Table II, the allantoin level increased up to 7.25 mg/100 ml on the average at the end of experiment. Although adenine increased the allantoin level most extensively among the experimental diets, guanine also produced a great influence. However, the value of serum uric acid for adenine and guanine was almost the same (Table II). On the other hand, dietary compositions produced

appetite. This was also evident in the rapid recovery of lost weight. Such observations suggest that the diet supplemented with a high concentration of adenine is actively rejected by rats. On the other hand, the food intake of adenine group was almost proportional to weight change, and was about 62% less in the adenine group as compared with the control group throughout the experimental period. Adenine also increased the urine volume to a greater extent. However, other dietary groups showed no appreciable changes in all respects.
changes in the urinary uric acid excretion. As shown in Table III, guanine most extensively increased the urinary uric acid among the diets tested. Conversely, feeding an adenine diet exhibited a decrease in the uric acid excretion in the urine. In the present study, the diets were ranked in the following order of the extent to which uric acid was excreted: guanine > uracil > cytosine > adenine.

The present data strongly confirmed that the urinary allantoin response showed differences according to the dietary compositions. As shown in Table III, feeding guanine to rats caused a significant rise in the urinary output of allantoin. In addition, a greater influence on the urinary excretion of allantoin was observed in rats fed on an adenine diet. However, there was no appreciable change in rats fed on a uracil or cytosine diet.

On the contrary, feeding rats on an adenine diet showed significant rises in creatinine and urea nitrogen in the serum. In addition, a decrease was observed in the amounts of urinary creatinine and urea when feeding with an adenine diet (Tables II, III). Dietary uracil also showed a rise in the urinary urea (Table III).

**Histopathological findings**

Macroscopically, only the kidney of rats fed on an adenine diet was markedly enlarged with

![Fig. 4. Infrared Spectrum of 2,8-Dihydroxyadenine (A) and Crystal (B).](image-url)
a pale grey colour (Fig. 1). In the cut surface, numerous scattered yellowish grains were observed in the parenchyma, especially in the cortex.

Microscopically, numerous acicular crystals were seen mainly in the tubular lumina, rarely in the tubular epithelia and in the interstitium of the kidneys, and the formation of foreign body granuloma was noticed by marked reaction of the tubular epithelia, histiocytes and foreign body giant cells against the acicular crystals (Figs. 2, 3). These crystals were birefringent and were also found in the urinary bladder and urine. However, in the glomeruli and other organs of the adenine-fed rats, the same crystals were not seen.

Consequently, a direct analysis of the HCl extract of the crystals by UV spectrometry was performed according to the method of Bendich et al., showing an absorption maximum at 305 nm and at 302 nm when the pH was changed to 10. This preparation had a UV spectrum similar to that reported by Cavaliere and Bendich for 2,8-dihydroxyadenine.

**Fig. 5.** Mass Spectrum of 2,8-Dihydroxyadenine (A) and Crystal (B).
and the spectrum at different pH was identical to that found with 2,8-dihydroxyadenine from Sigma Chemical Co. In addition, IR (KBr) and mass \([m/z: 167 (M^+, C_5H_{12}N_5O_2)]\) spectra were identical with those of an authentic sample as shown in Figs. 4 and 5.

On the other hand, in the kidneys of rats fed on guanine, uracil, cytosine and yeast RNA diets, these acicular crystals were absent and the tubular epithelia showed no marked changes.

**DISCUSSION**

The constituents of ribonucleic acid (RNA) are purine (adenine, guanine) and pyrimidine (uracil, cytosine) bases, a sugar (D-ribose) and phosphoric acid. Although considerable information is known about the effects of dietary RNA on blood and urinary uric acid in rats, little is known concerning the effects of individual constituents on these levels.

The present data confirmed that urinary uric acid response showed differences according to the purine and pyrimidine bases. As shown in Table III, guanine, when compared with adenine and pyrimidine bases, most extensively increased the urinary uric acid excretion. On the contrary, feeding adenine to rats exhibited a decrease of the uric acid excreted in the urine. This greater difference in the utilization of purine bases may, in part, reflect the metabolic pathways in purine degradation and reutilization, i.e., the administered guanine is directly converted to an end-product without phosphorylation, while adenine must first be phosphorylated to AMP prior to degradation. From the concept of these different pathways, it is conceivable that dietary purine would yield a differential response.

Another important observation was that the level of uric acid plus allantoin in the serum was remarkably high in rats fed on an adenine diet, while that of rats fed on a guanine, uracil and cytosine diet was low (Table II). This may be regarded as an influence of the renal function in the handling of uric acid and allantoin. Support for such a renal difference came from the results that an increase of creatinine, urea nitrogen and urate in the serum as well as a reduction in their urine excretion was observed in rats fed on the adenine diet (Tables II, III). Additional data examining the histopathological findings suggested that the kidneys from rats fed on an adenine diet were markedly enlarged and pale grey in colour compared with other diets (Fig. 1). Microscopically, numerous acicular crystals were seen mainly in the tubular lumina and the formation of foreign body granuloma (Figs. 2, 3). Furthermore, these crystals were identified as 2,8-dihydroxyadenine by UV, IR and mass spectrometry (Figs. 4, 5). From these results, it has been assumed that the administration of adenine may cause an impairment of the renal function by tubular obstruction with 2,8-dihydroxyadenine deposits. This impairment is thought to explain many of the biochemical features. In addition, it was clear in this experiment that the dose used was extremely high to produce crystals. Our interpretation is supported by the observation that a deposition of crystalline masses in the urinary bladder and urine was caused.

However, 2,8-dihydroxyadenine did not accumulate in the liver, a tissue known to contain relative high levels of xanthine oxidase. Blood also did not contain 2,8-dihydroxyadenine. Consequently the accumulation of 2,8-dihydroxyadenine did not correlate with tissue xanthine oxidase level. Thus, the accumulation of 2,8-dihydroxyadenine might be catalyzed by an other oxidase enzyme. This interpretation is supported by other data that electron dense material (2,8-dihydroxyadenine) accumulated only within the mitochondria in the proximal tubulous epithelia of the kidney since xanthine oxidase occurs in the cytoplasm and not in the mitochondria.

Our interpretation of the mechanism of dietary adenine is supported by the studies reported by Clifford and Story, Ho et al., i.e., dietary adenine increased the activities of hepatic adenine phosphoribosyltransferase (EC 2.4.2.7), 5'-nucleotidase (EC 3.1.3.5) and adenosine deaminase (EC 3.5.4.4), and the
concentration of free adenine in the liver. Furthermore, adenine was incorporated into tissues to a greater extent than was guanine, hypoxanthine or xanthine in both fed and starved rats. The majority of orally administered radioactively labeled adenine was excreted as allantoin. These observations suggest that once phosphorylated, adenine is readily converted to uric acid like the other purines. This information may also explain the possibility of adenine-induced hyperuricemia caused by the de novo biosynthesis of purine nucleotides and their catabolism to uric acid.

On the other hand, uracil-fed rats caused a significant rise in the urinary output of urea, although growth rate, kidney weight, liver weight, serum urea nitrogen and serum creatinine et al. showed no appreciable change throughout the experimental period (Tables I, II). In animal tissues, uracil may be degraded to β-alanine by way of the corresponding dihydropyrimidines and β-ureido acids. In a study of β-alanine catabolism it has been demonstrated that the carbon atoms 2 and 3 of β-alanine, in contrast to the carbon atom 1, appear in the fatty acid, cholesterol and excreted acetyl groups. Moreover, it has been demonstrated by Rutman et al. that when rats are given uracil-2-14C, at a dose level comparable to the endogenous replacement rate, the ureide carbon is rapidly and almost quantitatively excreted in the respiratory CO2, whereas significant amounts of labeled urea are formed when large doses of uracil-2-14C are given. These findings seem to suggest the possibility that, during the complete degradation of uracil, a correspondingly large fraction of the administered dose would have been recovered as urea. The greater excretion of uracil compared with adenine, guanine and cytosine may be interpreted by the unique metabolism.

The previous reports from our laboratory have shown that the administered nucleic acid caused a significant rise in the urinary output of uric acid plus allantoin. In the present study, it can be concluded that the purine and pyrimidine bases of the nucleic acid constituents are metabolized in different ways and produce different amounts of uric acid and allantoin, whereas adenine, due to its unique metabolism, is metabolized quite differently from the guanine and pyrimidine bases.

It is generally assumed that the hyperuricemic state is induced either by the overproduction of purine compound, elicited to remove feedback control on biosynthesis, or by impaired clearance in the kidneys. Based on the above observations, we have proposed two possible considerations for the induction of hyperuricemia by nucleic acid; one the overproduction of uric acid (dominant when the nucleic acid guanine produced by digestion was utilized) and the other an impaired clearance in the kidneys (dominant when the nucleic acid adenine produced by digestion was utilized). The biochemical mechanism through which these compounds are metabolized may be related to several factors such as the availability of substrates for de novo biosynthesis and reutilization, nucleotide pool size, and enzyme induction.

Several reports have suggested that the maximum safe limit of RNA in the diet is 2 g/day. This intake of RNA would result in an increase in serum uric acid of 1.13 to 1.80 mg/100 ml, and an increase in urinary uric acid of 226 to 328 mg/24 hr for normal humans. Although it has assayed a few of the common food items, the data show large differences in the amounts of individual purines making up the total purine content. Clifford and Story have reported that among the organ meats, liver generally had rather high levels of adenine and hypoxanthine while sardines and anchovies, although containing rather high levels of total purines and RNA, had very low levels of adenine and hypoxanthine. On the basis of these analyses, they have explained that the restriction of anchovies and sardines in the diet of hyperuricemic patients does not appear justified. Analysis of individual purine and pyrimidine bases are needed to classify foods that may be included in therapeutic diets in order to evaluate the relevance of our findings.
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REFERENCES