Invited Paper 3

NUTRITIONAL FACTORS INFLUENCING THE RESULTS OF TOXICOLOGY EXPERIMENTS IN ANIMALS

Yuzo HAYASHI, Michihito TAKAHASHI and Takeshi KOKUBO

Division of Pathology, National Institute of Hygienic Sciences, Biological Safety Research Center, Kamiyoga 1-18-1 Setagaya-ku, Tokyo 158

Accepted June 18, 1984

Abstract······The purpose of this paper is to present experimental data illustrating the importance of a toxicopathological basis necessary for understanding the role of nutrition on drug toxicity. Nutritional status constitutes an important factor capable of modulating the results of animal experiments in toxicology. Therefore, it is necessary in any toxicity testing to consider the effects of nutrition on drug toxicity and, at the same time, the effect of drug toxicity on the nutritional state of animals.

Key words: Diet, high protein-diet, low calorie intake, modulation of drug toxicity, monocrotaline

INTRODUCTION

Drugs or chemicals may manifest toxicity by specific individual mechanisms such as covalent binding with cellular macromolecules, lethal syntheses, liberation of endogenous vasoactive substances into the blood stream or lipid-peroxidation of membranes. Various factors, both endogenous and exogenous to animals are known to modulate such toxic manifestation. Endogenous factors may vary with species, strain, sex or age of animals while exogenous factors include housing, bedding, seasonal or diurnal variation in light, temperature and humidity, handling, methods of treatment, exposure to other organisms, and, of particular importance, diet.

Dietary factors affecting drug toxicity can be classified into the following categories; 1) Direct contamination with toxic substances, 2) specific nutrient-drug interaction, 3) general qualitative or quantitative inadequacy of nutrients and 4) increased or decreased caloric intake. The aim of the present paper is to focus attention on the...
latter 2 variables with illustration taken from some of our recent findings and to discuss
general principles for interpretation of nutritional effects on drug toxicity.

INHIBITORY EFFECTS OF REDUCED CALORIE INTAKE ON
MONOCROTALINE INTOXICATION IN RATS

Monocrotaline is a toxic pyrrolizidine alkaloid isolated from the legume, Crotalaria
spectabilis. A single subcutaneous injection of this substance to rats results in pro-
gressive interstitial pneumonitis with thickening of the pulmonary arteries and right
ventricular hypertrophy of the heart (Hayashi, et al. 1967). Mattocks (1968) and
Butler et al (1970) have shown that monocrotaline is converted into a pyrrolic compound
before exerting its toxic effect on the lung. In a series of experiments aimed at
elucidating the pathogenesis of monocrotaline intoxication, we found that diet-reduc-
tion could significantly inhibit the development of cardiopulmonary changes in rats
receiving the alkaloid (Hayashi et al. 1979).

Five-week-old male Sprague-Dawley rats were given a single subcutaneous inject-
ion of 60 mg/kg of monocrotaline and were equally divided into 3 groups. Group 1 rats
had continuous free access to the diet and water. Group 2 rats were housed in auto-
matic-feeding cages (FAG 72K, Hofer Equipment for Animal Experimental Research,
Austral-Seengraebe, Switzerland) and maintained with a daily supply of 8 g/animal of the
diet and a free supply of water. Group 3 rats were similarly housed in automatic-
feeding cages receiving the supply of 8 g/day during the first 30 days after injection and,
thereafter, were transferred to standard individual cages and allowed free access to
both diet and water. The experiment was terminated 90 days after injection, and
throughout this period each rat was checked at regular intervals for body weight, daily
diet intake and survival.

The mortality and growth data for each group are shown in Fig. 1. Ad libitum-fed
rats (group 1) daily consumed 17.8±1.7 g/rat of the diet, and their body weights in-
creased to 185.5±10.6% of the initial values within 20 days. Thereafter, they began to
manifest labored breathing with gradual decline of growth rate, and 11 out of 12 rats
died between the 22nd and the 40th day. In the diet-reduced group (group 2), all rats
survived 90 days without showing any respiratory distress. Throughout this period,
their growth was almost completely suppressed (Fig. 1). Similarly, in group 3 growth
was also suppressed during the first 30 days. Subsequent allowing them of free access
to the diet resulted in a significantly higher rate of growth, with body weights reaching
170 ± 13.4% of initial values in 40 days. Interestingly, from this period their breathing
then became labored, and 7 of 12 rats died between the 45th and 84th day. This finding
indicates that the pulmonary alterations occurring in rats after monocrotaline treat-
ment progress in association with increase of body weight.

In order to confirm the effect of diet-reduction on monocrotaline intoxication, an
additional experiment was performed. Twenty four rats were injected with 60 mg/kg
of monocrotaline, and as the control group, another 24 rats received an injection of 2
Nutritional factors in toxicology experiment.

Fig. 1. Survival and body weight of rats after a single subcutaneous injection of 60 mg/kg of monocrotaline. ○, group 1-ad libitum feeding throughout the experimental period; △, group 2-reducing supply of the diet (8 g/rat/day) throughout the experimental period; ■, group 3-reducing supply of the diet for the first 30 days followed by ad libitum feeding. Hayashi Y. et al.: Toxicology Letters 3(1979). 151 155.
ml/kg of the solvent physiological saline alone. Thereafter, they were divided into 4
groups: 12 monocrotaline-treated rats and the same number of controls were allowed
free access to the diet and water: the remaining 12 treated and control animals were
housed in the automatic-feeding cages, in which they were given 8 g/rat/day of the diet
and a free supply of water. Four weeks after injection, all rats were killed under
Nembutal anesthesia for pathological examination of the lung and heart.

In the groups fed ad libitum, 2 monocrotaline-treated rats died with labored
breathing on the 20th and the 25th day respectively, while all control rats survived the
4 weeks. In the diet-reduced groups, all rats, whether monocrotaline-treated or
control, survived 4 weeks. The pathological findings of the lung and heart are shown
in Table 1. Monocrotaline-treated, ad libitum-fed rats invariably showed severe pul-
monary alterations such as multiple petechial or massive hemorrhage, swelling or focal
necrosis of the alveolar walls with capillary thrombi, dilatation of the alveolar ducts
with occasional hyaline membrane formation, and thickening of the pulmonary arteries.
In all these cases, there were various grades of right ventricular hypertrophy assessed
by estimating the cross-sectional area of ventricles and septum at the widest cardiac
dimension (Table 1). In the monocrotaline-treated, diet-reduced group, the gross
appearance of the lung was normal, and only focal swelling of the alveolar walls was
noted on histological examination. Right ventricular hypertrophy did not occur in any
animal in this group. In control rats, either ad libitum-fed or diet-reduced, no cardio-
pulmonary alterations were apparent.

From these results, it is concluded that diet-reduction or reduced calorie intake can
inhibit monocrotaline intoxication in rats in terms of either prolongation of survival or
improvement of the cardiopulmonary alterations. The mechanism underlying such

| Table 1 Pathological Alterations of the Lung and Heart in Rats 28 Days After a Single
Subcutaneous Injection of 60 mg/kg of Monocrotaline |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Feeding</td>
<td>Body weight (g)</td>
<td>Lung</td>
<td>Heart</td>
<td>Cross-sectional area of right ventricle (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of rats examined</td>
<td>Initial</td>
<td>Final</td>
<td>Relative weight</td>
<td>Initial</td>
<td>Final</td>
<td>Relative weight</td>
</tr>
<tr>
<td>Control Ad libitum</td>
<td>12</td>
<td>149.3</td>
<td>353.1</td>
<td>0.34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>±5.6</td>
<td>±37.8</td>
<td>±0.02</td>
<td>±0.00</td>
<td>±3.5</td>
<td></td>
</tr>
<tr>
<td>Test Ad libitum</td>
<td>12</td>
<td>150.3</td>
<td>267.3**</td>
<td>0.78**</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>±7.9</td>
<td>±27.5</td>
<td>±0.15</td>
<td>±0.05</td>
<td>±4.0</td>
<td></td>
</tr>
<tr>
<td>Control Reduced</td>
<td>12</td>
<td>149.9</td>
<td>156.7</td>
<td>0.43</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>±5.0</td>
<td>±4.8</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±3.0</td>
<td></td>
</tr>
<tr>
<td>Test Reduced</td>
<td>12</td>
<td>148.4</td>
<td>158.4</td>
<td>0.48*</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>±4.8</td>
<td>±3.7</td>
<td>±0.08</td>
<td>±0.03</td>
<td>±3.8</td>
<td></td>
</tr>
</tbody>
</table>

*Statistical difference from the paired control (*p<0.05, **p<0.01).
1 g/100 g body weight.
2 Hearts were cut transversely at their widest dimension. After processing and staining slides with the heart
specimens, the sections were inserted into a “Fuji Enlarger S-69” so that the myocardium was magnified 10
times. Outlines of ventricles and septum were then traced onto paper of uniform thickness. Approximation
of the cross-sectional area of both ventricles and septum was estimated by weighing the paper of the
ventricular silhouettes.
3 The figure in parentheses denotes the number of rats which died within 25 days.
Nutritional factors in toxicology experiment.

inhibitory effects is unclear at present. Reduced functional loading to the lung which could be associated with reduced calorie intake is raised as a possibility.

ENHANCING EFFECTS OF HIGH-PROTEIN DIET ON SPONTANEOUS CHRONIC NEPHROPATHY IN F344 RATS

Various diseases, both neoplastic and non-neoplastic, are known to occur spontaneously in aged rats and their consideration plays an increasing role in the interpretation of long-term studies. The incidence and progression of each inherent disease differs from strain to strain and with regard to spontaneous chronic nephropathy, Sprague-Dawley and Wistar rats appear to be particularly susceptible. In addition to depending on such genetically determined factors, the development of this renal lesion is influenced by hormonal and nutritional state (Gray, 1974). For example, the lesion occurs more severely in males than in females and administration of androgens can enhance the incidence of its occurrence in the latter. Lalich et al (1970) have shown that feeding of diet with a high concentration of casein enhances the renal lesion in Sprague-Dawley rats. In order to confirm and extend their observations, we studied the effects of diet supplemented with various concentrations of white fish meal on development of the renal lesion in F344 rats. White fish meal has been used as the protein ingredient of rat chow in Japan, and the F344 rat is the strain most frequently adopted for carcinogenicity tests and long-term toxicity studies because of its long survival and relatively low incidence of spontaneous diseases.

One hundred and fifty F344 rats each of both sexes were divided into 3 equal groups at an age of 7 weeks and fed diets containing white fish meal at concentrations of 8, 32 and 64% for 2 years. At the 112th week, they were subjected to biochemical analyses of blood and urine as well as estimation of arterial blood pressure. Pathological examinations were performed on all rats found dead, killed when moribund or sacrificed at the end of the study (112 weeks).

Growth in the 64% groups, most particularly in the males, was retarded as compared with that of other groups (Fig. 2). Survival rates at the 112th week were more than 70% in the 8% and 32% groups, and less than 30% in the 64% groups (Fig. 3). Urinalyses revealed that the 64% and 32% groups excreted larger amount of total protein and albumin than those maintained on the diet containing 8% white fish meal (Table 2). Urinary concentrations of total lipids and triglyceride were also higher in the 64% and 32% groups (Table 2). Serum levels of total cholesterol, triglyceride, urea nitrogen, creatinine, uric acid and A/G ratio were estimated to increase in parallel with dietary concentrations of fish meal (Table 3). Blood pressure of rats also tended to be elevated with increasing concentration of protein in the diet (Fig. 4).

Histopathological examination of the kidney invariably revealed glomerular and tubular alterations. The glomerular lesions were characterized by an increase of mesangial PAS positivity, thickening of basement membranes, fibrous thickening of
Fig. 2. Mean Body Weight of F344 Rats Fed Diets Supplemented with Fish Meal at Concentrations of 8, 32 or 64%

Fig. 3. Survival Rate of F344 Rats Fed Diets Supplemented with Fish Meal at concentrations of 8, 32 or 64%
Nutritional factors in toxicology experiment.

Table 2  Urinalyses of F344 Rats Fed Diets Supplemented with Fish Meal at Concentrations of 8, 32 or 64%

<table>
<thead>
<tr>
<th>Conc. of fish meal:</th>
<th>Male 64%</th>
<th>Male 32%</th>
<th>Female 8%</th>
<th>Female 64%</th>
<th>Female 32%</th>
<th>Female 8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml/day)</td>
<td>13.7±1.7</td>
<td>10.4±5.0</td>
<td>10.7±2.6</td>
<td>9.8±2.6</td>
<td>8.3±1.6</td>
<td>8.5±2.3</td>
</tr>
<tr>
<td>Total protein (mg/day)</td>
<td>484.0±65.4**</td>
<td>234.4±120.9</td>
<td>126.5±101.3</td>
<td>204.6±99.0</td>
<td>98.0±42.0**</td>
<td>21.4±9.9</td>
</tr>
<tr>
<td>Albumin (mg/day)</td>
<td>324.6±52.7**</td>
<td>159.2±83.1</td>
<td>81.7±66.8</td>
<td>194.6±63.5**</td>
<td>82.6±24.8**</td>
<td>15.8±10.7</td>
</tr>
<tr>
<td>Total lipid (mg/dl)</td>
<td>466±68**</td>
<td>322±84**</td>
<td>187±37</td>
<td>466±256*</td>
<td>271±84*</td>
<td>162±37</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>213±29</td>
<td>204±52</td>
<td>192±53</td>
<td>290±74*</td>
<td>159±23</td>
<td>164±40</td>
</tr>
</tbody>
</table>

Mean±SD  * p<0.05  ** p<0.01

Table 3  Biochemical Analysis of Serum in F344 Rats Fed Diets Supplemented with Fish Meal at Concentrations of 8, 32 or 64%

<table>
<thead>
<tr>
<th>Conc. of fish meal:</th>
<th>Male 64%</th>
<th>Male 32%</th>
<th>Female 8%</th>
<th>Female 64%</th>
<th>Female 32%</th>
<th>Female 8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>6.7±0.3</td>
<td>6.6±0.5</td>
<td>6.7±0.6</td>
<td>7.5±1.7</td>
<td>7.5±0.9</td>
<td>7.4±0.7</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>0.92±0.05**</td>
<td>1.03±0.17**</td>
<td>1.23±0.18</td>
<td>0.98±0.46**</td>
<td>1.22±0.19**</td>
<td>1.39±0.19</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>117±17**</td>
<td>103±25</td>
<td>78±18</td>
<td>170±63**</td>
<td>130±71*</td>
<td>93±37</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>530±175**</td>
<td>398±150**</td>
<td>242±122</td>
<td>546±229**</td>
<td>364±176*</td>
<td>255±145</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>57.5±49.1**</td>
<td>22.1±6.0**</td>
<td>14.6±2.7</td>
<td>30.6±5.7**</td>
<td>18.7±6.1</td>
<td>17.6±8.7</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>3.7±0.6*</td>
<td>2.9±3.8</td>
<td>1.4±0.7</td>
<td>6.0±3.8*</td>
<td>3.9±3.2</td>
<td>2.8±2.4</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2.4±0.8</td>
<td>2.5±0.7*</td>
<td>1.9±0.7</td>
<td>11.8±15.4**</td>
<td>3.7±2.3**</td>
<td>2.5±1.1</td>
</tr>
</tbody>
</table>

Mean±SD  * p<0.05  ** p<0.01

Bowman's capsules, proliferation of epithelial cells, crescent formation and hyalinization, while the tubular lesions consisted of luminal dilatation with flattening of epithelia and colloid-like hyaline casts (Fig. 6,7). Electron microscopically there were cytoplasmic fusions of podocytes and thickening of capillary basement membranes in glomeruli (Fig. 8). These findings correspond with the morphological criteria for spontaneous chronic nephropathy designated in the literature (Working Group Report, 1980).

Scoring of the magnitude of renal lesions (Fig. 5 and table 4), taken together with biochemical findings in the urine and serum in this study, indicates that spontaneous

— 225 —
chronic nephropathy deteriorates with increasing dietary protein concentration. The mechanism is not clear at present since the pathogenesis of chronic nephropathy itself has not been elucidated. However, it is raised as a possibility that feeding of high protein diet accelerates the progression of the lesions by functional overloading of the kidneys.

— 226 —
Nutritional factors in toxicology experiment.

Fig. 5. Grading of Chronic Nephropathy in Rats According to the Rate of Appearance of Tubules with Hyaline Casts on Histological Sections

Fig. 6. A histological view of grade +++ chronic nephropathy in a rat fed diet supplemented with fish meal at a concentration of 64%. Hyaline casts are seen in more than 50% of the tubules.
Fig. 7. Glomerular lesions of a rat fed diet supplemented with fish meal at a concentration of 64%, showing thickening of capillary basement membranes and adhesion of tufts with Bowmann’s capsule.

Fig. 8. An area of the glomerulus from a rat fed diet supplemented with fish meal at a concentration of 64%, showing a thickening of capillary basement membranes and Bowmann’s capsule. 7800×.
INHIBITORY EFFECTS OF RESTRICTED FEEDING ON SPONTANEOUS CHRONIC NEPHROPATHY AND MYOCARDIAL FIBROSIS IN SPRAGUE–DAWLEY RATS

Restricted feeding to rats is known to reduce the incidence and severity of various spontaneous diseases and eventually prolong their life-span (Simms et al, 1957). Correlative pathological and biochemical studies are now being carried out in our Institute to examine the inhibitory effects of restricted feeding on the development of spontaneous lesions in rats. Data concerning the effects on chronic nephropathy and myocardial fibrosis will be briefly described here.

A total of 72 male Sprague–Dawley rats aged 5 weeks were divided into 2 groups. Group 1 animals were housed in regular individual cages with a free supply of diet and water, whereas the group 2 rats were housed in automatic-feeding cages as previously described where they were given 10.5 g/day of the diet and a free supply of water. Urinalyses were performed periodically. Eight rats each of both groups were killed at the 24th and 44th week; the remainder were sacrificed at the 72nd week. Pathological examination of the kidney and heart was performed.

Growth curves of both groups are shown in Fig. 9. Body weight increase from the initial value reached about 600% in the ad libitum-fed group but was limited to approximately 100% in the diet-reduced group. Table 5 shows the urinalysis data of the 2 groups. Excretion of total protein and albumin was much higher in the ad libitum-fed group than the diet-reduced group throughout the experimental period. In the ad libitum-fed group, daily excretion of total protein was estimated to be near 200 mg/day at the 27th week and reached more than 300 mg/day by the 72nd week while in the diet-reduced group the value was less than 100 mg/day throughout the experimental period. As shown in Table 6, the incidence and severity of renal lesions were also lower after reduced feeding. In the diet-reduced group, only 3 out of 20 rats were observed to have mild renal lesions at the 72nd week while in the ad libitum-fed group, 16 out of 18 rats exhibited various grades of the renal lesion at this time.

The incidence of myocardial fibrosis was also significantly different between the 2 groups. In the ad libitum-fed group, this lesion appeared in 1 out of 6 rats at the 24th week, and at the 72th week, the incidence had reached nearly 80%. In the diet-reduced group on the other hand, the lesion had not appeared by the 44th week, and even at the 72nd week, it was limited to a mild form occurring in only 4 out of 20 rats (Fig. 10). These data clearly indicate that diet-reduction can inhibit the development of spontaneous chronic nephropathy and myocardial fibrosis in rats (Wilens et al, 1938).

TOXICOPATHOLOGICAL CONSIDERATION

There is an increasing amount of evidence suggesting a relationship between nutrition and drug toxicity. Mechanistically, many of the findings can be explained as the result of nutritional or dietary effects on the fate of drugs in animals. A drug
Fig. 9.  Body Weight of Male Sprague-Dawley Rats Kept on Ad Libitum or Restricted Feeding

Table 5  Urinary Excretion of Protein in Male Sprague-Dawley Rats Kept on Ad Libitum or Restricted Feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Item</th>
<th>27</th>
<th>34</th>
<th>45</th>
<th>52</th>
<th>61</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td>Total protein (mg/day)</td>
<td>173.3 ± 52.1</td>
<td>180.7 ± 50.1</td>
<td>164.7 ± 56.1</td>
<td>172.8 ± 49.7</td>
<td>227.4 ± 10.7</td>
<td>327.1 ± 25.2</td>
</tr>
<tr>
<td>feeding</td>
<td>Albumin (%)</td>
<td>35.9 ± 18.8</td>
<td>38.4 ± 16.9</td>
<td>47.1 ± 15.7</td>
<td>53.7 ± 14.9</td>
<td>55.1 ± 13.9</td>
<td>60.7 ± 12.3</td>
</tr>
<tr>
<td>Restricted</td>
<td>Total protein (mg/day)</td>
<td>79.4 ± 6.9</td>
<td>91.5 ± 21.5</td>
<td>80.9 ± 11.1</td>
<td>73.6 ± 10.2</td>
<td>91.9 ± 23.1</td>
<td>85.8 ± 27.7</td>
</tr>
<tr>
<td>feeding</td>
<td>Albumin (%)</td>
<td>13.7 ± 4.1</td>
<td>15.5 ± 6.6</td>
<td>15.3 ± 4.0</td>
<td>17.6 ± 3.9</td>
<td>21.0 ± 7.6</td>
<td>22.1 ± 6.2</td>
</tr>
</tbody>
</table>

follows a route of absorption, distribution, metabolism and excretion which depends on a number of systems that can be influenced and modified through changes in the diet (Angeli Greaves et al, 1979). For example many drugs are transported in an albumin-bound form. Long-term feeding of a protein-calorie deficient diet results in a decrease in the level of plasma protein which can enhance the toxicity of some drugs such as frusemide or sulfonamides since saturation of drug-binding sites in the albumin fraction takes place at lower doses than normal. The levels of cytochrome P-450 and other microsomal metabolizing enzymes of experimental animals are also known to depend on their nutritional and hormonal states. Low-protein or protein-free diets decrease the microsomal enzymes in rats; the effect is greater in males than females for aminopyrine N-demethylase and hexobarbital hydroxylase (Dauterman, 1980). The content
Nutritional factors in toxicology experiment.

Table 6  Grading of Chronic Nephropathy in Male Sprague-Dawley Rats Kept on Ad Libitum or Restricted Feeding

<table>
<thead>
<tr>
<th>Group</th>
<th>Experimental period (weeks)</th>
<th>No. of rats examined</th>
<th>Grades of renal lesions</th>
<th>Total incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ad libitum</td>
<td>24</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>feeding</td>
<td>44</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>18</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Restricted</td>
<td>24</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>feeding</td>
<td>44</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>20</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

Grades of renal lesions

Fig. 10. Occurrence of Myocardial Fibrosis in Male Sprague-Dawley Rats Kept on Ad Libitum or Restricted Feeding

of cytochrome P-450, NADPH oxidase and NADPH-cytochrome reductase decreases after feeding of low-protein or protein-free diet. Moreover, the feeding of a high-carbohydrate in place of a standard diet to rats results in a marked decrease in the activities of aminopyrine N-demethylase, phenobarbital oxidase and p-nitrobenzoic acid reductase (Dauterman, 1980).

However, it must be borne in mind that, as shown in our experiments previously mentioned, there are many examples which can not be explained on the basis of pharmacokinetic or metabolic alterations and the complexity of the relationship between nutrition and drug toxicity allows few generalizations to be made. In routine
toxicity testing, we frequently encounter cases where it is necessary to consider whether visceral alterations seen in animals are primarily related to the drug toxicity alone or are attributable to the combined effects of the drug and nutritional factors. In coping with such situations, it might be helpful to analyze the problem from the standpoint of toxicopathology before specific mechanistic studies are conducted.

On the basis of toxicopathology, nutritional effects on drug toxicity can be divided into the following groups; 1) modulation of animal susceptibility to drug toxicity, 2) modulation of progression of drug-induced lesions, 3) induction of lesions by nutritional factors and 4) alterations of nutritional state of animals by drug toxicity. Practically, most examples of nutritional effects can be categorized into one of these groups although a few cases remain equivocal or transitional.

Modulation of animal susceptibility to drug-toxicity

Historically, it is well known that the feeding of a low-protein, low-vitamin diet can increase the susceptibility of rats to the hepatocarcinogenicity of 4-dimethylaminoazobenzene. Later, it was found that protein and vitamin B, promoted the detoxication of the dye by microsomal enzymes (Weisburger et al, 1967). In contrast, decreasing the protein content of the diet, which reduces cytochrome P-450 levels in the liver protects the animals against damage by carbon tetrachloride, by inhibiting its metabolism to the active form (Angeli-Greaves et al, 1979). As a rule, most compounds will appear to be somewhat more toxic and less well tolerated on a semisynthetic diet than on a diet composed of natural ingredients. A number of heavy metal ions such as copper, nickel and cobalt inhibit liver carcinogenesis in rats with azo dyes but not with 2-fluorenlylaceticamide. Supplementation of the diet with a given concentration of selenium is known to inhibit the toxic effects of mercuric compounds. Mechanistically, many of these examples might be related to modulation of a process or processes involved in the disposition of drugs in the body such as absorption of drug from its site of entry, distribution of drug in the body, biotransformation of drug in the body, interaction of drug with receptor sites or interaction of drug with elimination mechanism.

Modulation of progress of drug-induced lesions

Nutritional effects of this group and the previous one are indistinguishable in some cases. The inhibitory effect of diet-reduction on monocrotaline intoxication is a typical example of this group. Tannenbaum reported that poor nutritional conditions during the initial treatment with a chemical carcinogen did not affect the ultimate occurrence of tumors when the animals were subsequently placed on a nutritionally adequate diet. In contrast, exposure to carcinogen under good dietary conditions followed by a regimen lacking in essential factors gave a poorer tumor yield. Thus, the development of tumors required an adequate supply of nutrients, whereas the early action of carcinogen could take place under less favorable conditions. Dietary conditions are also known to modulate the progression of age-associated visceral lesions in
Nutritional factors in toxicology experiment.

animals; As already discussed, reduced-calorie intake can retard the progression of spontaneous neoplastic and non-neoplastic lesions in rats while a high-protein diet can enhance the development of chronic glomerular lesions.

Induction of lesions by nutritional factors

Various visceral lesions are known to occur in animals due to either deficiency or excess of some vitamins. Long-term feeding of low-protein diet to rats can induce atrophy of the intestinal mucosa. These visceral lesions may alter the susceptibility of animals to drug toxicity.

Alteration of nutritional state of animals by drugs

Administration of drugs to animals may result in various morphological or functional changes in organs or tissues. Such changes can induce change in nutritional state by disturbing dietary intake, absorption of nutrients from the intestine or metabolism of nutrients in the body. Inversely, the altered nutritional conditions can modulate the susceptibility of animals to drugs.

REMARKS FOR ROUTINE TOXICITY TESTING

Nutrition is regarded as an important factor which can modulate the results of animal experiments in toxicology. Therefore, it is indispensable for routine toxicity testing that animals be fed on diet with an adequate composition of nutrients and also that an estimate of daily intake of the diet by each animal or animal group be made periodically throughout the course of the experiment. Pair feeding is helpful to exclude differences due to nutritional effects on animals among the groups. Drug-nutrient interactions should be taken into consideration when the drug is administered as a dietary supplement. For final interpretation of test results, it is necessary to consider the nutritional effect on drug toxicity and, at the same time, the effect of toxicity on the nutritional state of the animals.

Acknowledgment: This work was supported in part by a Research Grant-in-Aid for Special Project (5824033) from the Ministry of Education, Science and Culture, and a Grant-in-Aid for Cancer Research (Designated Project 56-2) from the Ministry of Health and Welfare.
REFERENCES


SIMMS, H. S. and BEARG, B. N. (1957): Longevity and the onset of lesions in male rats. J. Gerontol., 12, 244-252.

VESELL, E. S. and PASSNANTI, G. T. (1977): Genetic and environmental factors affecting host response to drugs and other chemical compounds in our environment. Environmental Health Perspectives, 20, 159-182.
