Jpn. J. Cancer Res. 85, 26–31, January 1994

# Long-Evans A and C Rat Strains Susceptible to Clastogenic Effects of Chemicals in the Bone Marrow Cells

Yoshiaki Ito,[1] Kimiko Fujie,[2] Shuichi Matsuda,[3] Rei Takahashi[4] and Sakan Maeda[3]

[1]*Public Health Research Institute of Kobe City, 4-6, Minatojima-Nakamachi, Chuo-ku, Kobe 650,* [2]*Department of Natural Science, Osaka Women's University, Daisen-cho, Sakai 590,* [3]*Department of Pathology, Kobe University School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650 and* [4]*Department of Pathology, Faculty of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606*

**The clastogenic responses to direct- and indirect-acting carcinogens in bone marrow cells of LEA, LEC, Wistar and SD rats were compared. The frequency of chromosome aberrations (CA) induced by *n*-butyl-N-nitrosourea or methylmethanesulfonate (MMS), which does not need metabolic activation, was significantly higher in both LEA and LEC rats than in Wistar or SD rats. When bone marrow cells of each rat strain were exposed to MMS *in vitro*, they also showed the same tendency in CA frequency. Therefore, the high sensitivity of both LEA and LEC rats to the clastogenic effects of direct-acting carcinogens seems to result from the sensitivity of the bone marrow cells themselves. On the other hand, the CA frequency induced by 7,12-dimethylbenz[a]anthracene (DMBA) or aflatoxin $B_1$ ($AFB_1$), which requires metabolic activation, was lower in LEC rats than in the other 3 strains. The CA frequency induced by DMBA or $AFB_1$ in LEC rats fed Cu-free diet since birth (Cu-free LEC rats) was higher than that in LEC rats given normal diet and lower than that in LEA rats, although the difference was statistically significant only between Cu-free LEC rats and LEC rats treated with DMBA. The copper concentrations in the livers of LEA, Cu-free LEC and LEC male rats aged 4 weeks were $5.0 \pm 0.4$, $33 \pm 7.7$ and $106 \pm 3.4$ $\mu$g/g wet weight, respectively. These results suggest that the lower sensitivity of LEC rats to the clastogenic effects of indirect-acting carcinogens may be due to the effect of the large amount of copper accumulated in LEC rat liver.**

Key words: LEC rat — LEA rat — Chromosome aberration

Both LEC rat and LEA rat strains were isolated from a closed colony of LE rats and established at the Center for Experimental Plants and Animals, Hokkaido University. The LEC rat is a new mutant strain characterized by the spontaneous development of hepatitis with severe jaundice at about 4 months of age.[1,2] Although about 30% of rats die of fulminant hepatitis within 1 week after the onset of jaundice, the remaining animals survive with chronic hepatitis for more than 1 year and develop liver cancer. Another feature of LEC rats is that copper accumulates densely in the liver, while the serum levels of copper and ceruloplasmin are reduced.[3,4]

On the other hand, non-inbred LE rats which were kept at Ben May Cancer Institute, Chicago University, showed a high incidence of leukemia or mammary cancer when injected with carcinogens such as DMBA and TMBA. Furthermore, Sugiyama *et al.*[5–7] reported that specific chromosomal abnormalities, such as trisomy and elongation of the largest telocentric chromosome (#2 trisomy and long #2) were observed at a high frequency in the cells of rats with leukemia induced by DMBA or TMBA. We have also been studying CA induced by various chemicals in non-inbred LE rat bone marrow cells for more than 10 years.[8–11] In these studies, we detected the clastogenicity of 3-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF2) using non-inbred LE rat, although it could not be detected in other rat strains at other laboratories.[12]

In the present study, we compared the sensitivity to clastogenic effects of several chemicals in bone marrow cells of different rat strains. DMBA- or $AFB_1$-induced CA in bone marrow cells of Cu-free LEC rats was also investigated in connection with the effect of the copper accumulated in LEC rat liver. The bone marrow cells of each rat strain were also exposed to MMS *in vitro* and the results were compared.

Abbreviations: LE rat, Long-Evans rat; LEC rat, LE rat with a cinnamon-like coat color; LEA rat, LE rat with an agouti coat color; Cu-free LEC rat, LEC rat fed Cu-free diet since birth; CA, chromosome aberrations; DMBA, 7,12-dimethylbenz[a]anthracene; TMBA, 7,8,12-trimethylbenz[a]anthracene; $AFB_1$, aflatoxin $B_1$; BNU, *n*-butyl-N-nitrosourea; MMS, methylmethanesulfonate.

## MATERIALS AND METHODS

**Chemicals** BNU was purchased from Nacalai Tesque, Inc., Tokyo. MMS was obtained from Aldrich Chemical

Co., Milwaukee, WI, USA; DMBA was from Tokyo Chemical Synthetic Industry Co., Ltd., Tokyo; $AFB_1$ was from Makor Chemicals, Ltd., Tokyo; dimethyl sulfoxide (spectrophotometric grade) was from E. Merck A.G., Darmstadt, Germany; colchicine and colcemid were from Wako Pure Chemicals Co., Tokyo.

**Animal experiment** LEA and LEC rats were kindly donated by Dr. N. Takeichi from Hokkaido University. They were propagated and maintained in the animal facilities of Kobe University School of Medicine. Wistar and SD rats were purchased from Charles River Japan, Inc., Kanagawa. Male rats of each rat strain, aged 28–35 days and weighing 70–100 g, were used for the experiment. Each experimental group consisted of 6 rats. They were kept in an air-conditioned room and fed diet (Oriental MF; Oriental Yeast Co., Ltd., Tokyo) and water *ad libitum*. Cu-free LEC rats were fed Cu-free diet (Oriental Yeast Co., Ltd.). $AFB_1$, DMBA and MMS were dissolved in dimethyl sulfoxide and injected i.p. BNU was dissolved in sesame oil and given by gastric instillation. Colchicine (0.3 mg/rat) was injected i.p. 1 h before the animals were killed. Bone marrow cells collected from the femur at various times after the carcinogen treatment were incubated in hypotonic solution (0.075 *M* KCl) for 20 min at 37°C, and fixed with 3:1 methanol:acetic acid. Chromosome specimens were prepared by the conventional air-drying method, stained in 2% Giemsa solution (pH 6.8) for 15 min, and then analyzed microscopically.

**Chromosome analysis** Fifty well-spread metaphase cells per rat, that is, 300 metaphases in each experimental group were analyzed. CA were divided into gaps, breaks and exchanges. A gap was defind as a complete discontinuity of one or both chromatids not exceeding the width of a chromatid, and a break as a discontinuity greater than the width of a chromatid, irrespective of whether or not the distal fragment was dislocated. Multiple CA (more than 10) were defined as uncountable CA per cell.

The "incidence of aberrant cells" was defined as the percentage of cells with breaks, exchanges and multiple CA. The severity of damage was expressed in terms of "the number of aberrations per cell," and in this case multiple CA were counted as 10 aberrations. The mitotic index was expressed as percentage of mitotic cells among more than 1,000 cells per rat. Statistical significance of differences was determined by using Student's *t*-test.

**Assay of CA *in vitro*** The bone marrow cells were collected from the femur of male rats of each strain and seeded at a density of $2\times10^7$ cells/dishes in Dulbecco's modified Eagle's medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% fetal bovine serum (Intergen Company, NY). After culture for 1 h in a humidified atomosphere with 5% $CO_2$ at 37°C, MMS was added to the culture medium. After the cells had been treated with MMS for 3 h, they were washed to remove MMS and cultivated further for 18 h. Two h before fixation, colcemid (final concentration, $2\times10^{-7}$ *M*) was added. The following procedure was the same as that *in vivo*.

**Determination of copper** The copper concentrations in the livers were determined using an atomic absorption spectrophotometer (Model Z-6400; Hitachi, Tokyo).

## RESULTS

**BNU- or MMS-induced CA in bone marrow cells of various rat strains** The CA frequency in bone marrow cells of non-treated rats was almost the same in all rat strains. However, the mitotic index of rats without chemical treatment was slightly high in LEA rats and slightly low in LEC rats, compared with Wistar or SD rats (Table I). A statistically significant difference was observed in the mitotic index between LEA and LEC rats.

BNU-induced CA consisted mainly of gaps and breaks in all rat strains tested. Very few exchanges or multiple CA were observed. However, in all strains of rats injected with MMS, exchanges and multiple CA were also observed frequently, as well as gaps or breaks. Not only the percentage of cells with breaks but also the frequency of cells with exchanges or multiple CA was higher in rat strains in which the incidence of aberrant cells was higher, when compared between rat strains treated with the same chemical.

The incidence of aberrant cells induced by BNU or MMS was significantly higher in both LEA and LEC rats than in Wister or SD rats, as was the number of aberrations per cell. No clear strain difference was observed between LEA and LEC rats treated with BNU or MMS (Table I). The mitotic index of rats treated with BNU or MMS was about half as high as that of non-treated rats, although that of LEC rats treated with MMS was extremely low.

**DMBA- or $AFB_1$-induced CA in bone marrow cells of various rat strains** DMBA- or $AFB_1$-induced CA also consisted mainly of gaps and breaks in all rat strains. Very few exchanges or multiple CA were observed.

The incidence of aberrant cells induced by DMBA or $AFB_1$ was slightly higher in LEA rats than in Wistar or SD rats, although the difference was not statistically significant (Table II). However, LEC rats showed a lower frequency of CA induced by DMBA or $AFB_1$. The incidence of aberrant cells and the number of aberrations per cell of LEC rats treated with DMBA were significantly lower than those of LEA or Wistar rats with the same treatment. In the case of $AFB_1$ treatment, a statistically significant difference was observed only between LEA and LEC rats. Dose-related increase of aberrant cells induced by DMBA was observed in LEA, LEC and Wistar rats (Fig. 1). The number of aberrations per

Table I. Chromosome Aberrations Induced by BNU or MMS in Bone Marrow Cells of Various Rat Strains[a]

| Treatment | Percentage of cells with[b] | | | | Mitotic index | Number of aberrations per cell | Incidence of aberrant cells (%) |
|---|---|---|---|---|---|---|---|
| | Gap | Break | Ex. | Multi. | | | |
| Control | | | | | | | |
| LEA | 1.8±1.4 | 1.0±1.0 | 0±0 | 0±0 | 2.6±0.7[g] | 0.01±0.01 | 1.0±1.0 |
| LEC | 1.8±0.8 | 1.1±0.9 | 0±0 | 0±0 | 1.8±0.4 | 0.01±0.01 | 1.1±0.9 |
| Wistar | 1.7±0.8 | 0.7±1.0 | 0±0 | 0±0 | 2.1±0.4 | 0.01±0.01 | 0.7±1.0 |
| SD | 1.2±0.7 | 1.0±0.6 | 0±0 | 0±0 | 2.2±0.5 | 0.01±0.01 | 1.0±0.6 |
| BNU 200 mg/kg, 18 h | | | | | | | |
| LEA | 13.0±1.0 | 36.4±7.9 | 2.4±1.1 | 2.3±2.4 | 1.2±0.4 | 1.10±0.55[e,f] | 41.1±9.2[c,f] |
| LEC | 9.8±1.6 | 37.7±7.2 | 3.5±1.8 | 0.7±1.0 | 1.1±0.3 | 0.96±0.28[e,f] | 41.8±9.0[c,f] |
| Wistar | 10.8±1.0 | 22.2±5.2 | 0.7±0.8 | 0.7±1.6 | 1.0±0.4 | 0.50±0.29 | 23.5±5.9 |
| SD | 10.6±1.1 | 27.8±6.6 | 1.4±1.1 | 0.2±0.4 | 1.2±0.2 | 0.51±0.14 | 29.4±6.2 |
| MMS 75 mg/kg, 18 h | | | | | | | |
| LEA | 9.0±2.4 | 20.4±5.0 | 6.8±2.3 | 38.2±8.3 | 1.2±0.3[g] | 5.10±0.83[c,d] | 65.4±10.6[c,d] |
| LEC | 10.3±1.5 | 19.3±3.0 | 7.0±2.1 | 41.0±7.6 | 0.6±0.2 | 5.39±0.93[c,d] | 67.3±8.6[c,d] |
| Wistar | 9.3±1.4 | 13.5±3.6 | 2.7±1.2 | 18.8±5.9 | 1.2±0.3[g] | 2.48±0.66 | 35.0±7.7 |
| SD | 7.7±1.4 | 16.9±7.1 | 2.5±1.9 | 12.4±5.2 | 1.2±0.3[g] | 1.83±0.49 | 31.8±6.2 |

*a*) Chromosome specimens were prepared 18 h after the carcinogen treatment. Values are mean±SD for 6 rats.
*b*) Ex., exchange; Multi., multiple CA (cells having more than 10 aberrations).
*c, d*) Statistically significant compared with Wistar or SD rats with the same treatment ($P<0.01$).
*e, f*) Statistically significant compared with Wistar or SD rats with the same treatment ($P<0.05$).
*g*) Statistically significant compared with LEC rats with the same treatment ($P<0.05$).

Table II. Chromosome Aberrations Induced by DMBA or $AFB_1$ in Bone Marrow Cells of Various Rat Strains and Cu-free LEC Rat[a]

| Treatment | Percentage of cells with[b] | | | | Mitotic index | Number of aberrations per cell | Incidence of aberrant cells (%) |
|---|---|---|---|---|---|---|---|
| | Gap | Break | Ex. | Multi. | | | |
| DMBA 50 mg/kg, 24 h | | | | | | | |
| LEA | 9.8±1.7 | 15.8±3.8 | 0.5±0.6 | 0±0 | 1.5±0.6[e] | 0.30±0.12[d] | 16.3±4.2[d] |
| Cu-free LEC | 9.7±1.8 | 14.7±3.4 | 0.3±0.5 | 0±0 | 0.6±0.1 | 0.20±0.05[d] | 15.0±3.7[d] |
| LEC | 5.6±1.7 | 8.8±2.3 | 0±0 | 0±0 | 0.9±0.3 | 0.10±0.03 | 9.2±3.0 |
| Wistar | 9.0±2.1 | 15.2±3.7 | 0.2±0.4 | 0.2±0.4 | 1.4±0.7 | 0.26±0.06[c] | 15.6±3.2[d] |
| DMBA 100 mg/kg, 24 h | | | | | | | |
| LEA | 10.3±2.1 | 25.6±6.9 | 0.3±0.5 | 1.7±1.1 | 1.4±0.5[d] | 0.63±0.28[d] | 27.6±7.2[d] |
| LEC | 9.3±2.0 | 18.5±4.0 | 0.3±0.5 | 0±0 | 0.7±0.1 | 0.27±0.07 | 18.8±3.8 |
| Wistar | 11.7±4.2 | 23.2±5.8 | 0.4±1.0 | 0.4±0.8 | 1.2±0.6 | 0.41±0.17[d] | 24.1±6.3[d] |
| $AFB_1$ 10 mg/kg, 18 h | | | | | | | |
| LEA | 11.8±2.0 | 32.0±5.2 | 1.0±1.0 | 1.8±1.5 | 2.0±0.6[d,e] | 0.93±0.35[d] | 34.8±4.6[d] |
| Cu-free LEC | 9.3±1.5 | 29.3±5.1 | 1.0±1.0 | 0.4±0.8 | 1.3±0.4 | 0.60±0.21 | 30.7±5.3 |
| LEC | 9.6±2.1 | 21.1±6.7 | 1.6±1.4 | 1.1±1.6 | 1.1±0.2 | 0.54±0.22 | 23.9±6.7 |
| Wistar | 12.3±3.0 | 28.7±5.0 | 0.4±0.7 | 0.9±1.3 | 1.9±0.5[d] | 0.70±0.21 | 30.0±5.9 |
| SD | 8.0±3.3 | 31.7±6.0 | 0.2±0.4 | 0.7±0.8 | 1.7±0.5 | 0.86±0.25 | 32.5±6.7 |

*a*) Chromosome specimens were prepared 24 h after the DMBA treatment or 18 h after the $AFB_1$ injection. Values are mean±SD for 6 male rats.
*b*) Ex., exchange; Multi., multiple CA (cells having more than 10 aberrations).
*c*) Statistically significant compared with LEC rats with the same treatment ($P<0.01$).
*d*) Statistically significant compared with LEC rats with the same treatment ($P<0.05$).
*e*) Statistically significant compared with Cu-free LEC rats with the same treatment ($P<0.05$).

cell also increased in proportion to the DMBA dose. The mitotic index of rats injected with DMBA was also about half as high as that of rats without chemical treatment in all rat strains, but that of rats injected with $AFB_1$ did not decrease so much as that of rats treated with DMBA, BNU or MMS.

**DMBA- or $AFB_1$-induced CA in bone marrow cells and copper concentration in liver of Cu-free LEC rats** The data for Cu-free LEC rats (Table II) show that the CA frequency induced by DMBA or $AFB_1$ in Cu-free LEC rats was higher than that in LEC rats given normal diet and lower than that in LEA rats, although a statistically significant difference was observed only between Cu-free LEC rats and LEC rats treated with DMBA. The mitotic index of bone marrow cells in Cu-free LEC rats was at a low level, as was that in LEC rats. The values of copper concentration in the liver of LEA, Cu-free LEC and LEC male rats aged 4 weeks were 5.0±0.4, 33±7.7 and 106±3.4 μg/g wet weight (mean values for 3 rats), respectively.

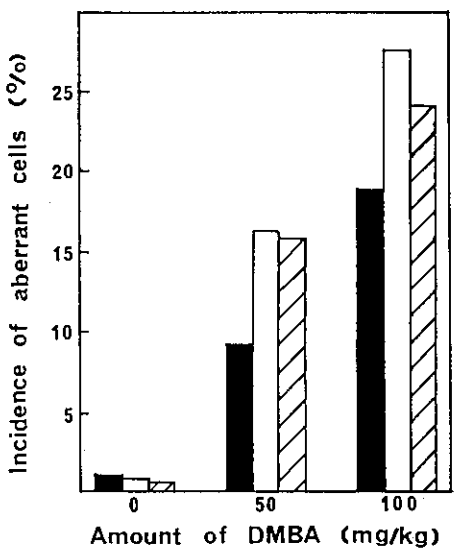


Fig. 1. Relationship between DMBA dose and the incidence of aberrant cells in rat bone marrow cells 24 h after the DMBA injection. Each value represents the mean for 6 male rats. ■, LEC; □, LEA; ▨, Wistar rats.

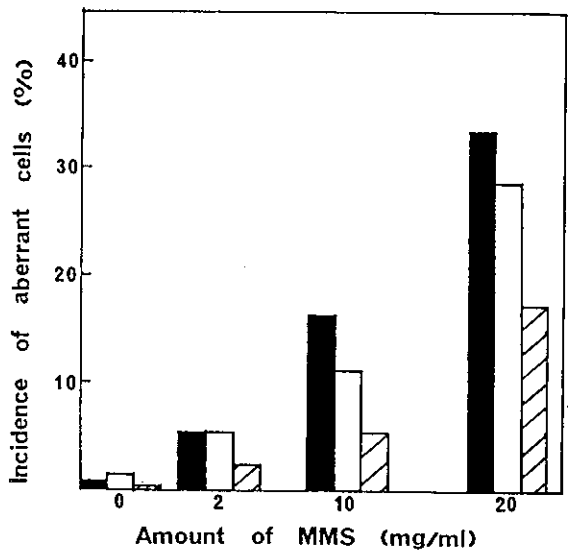


Fig. 2. Relationship between MMS dose and the incidence of aberrant cells in rat bone marrow cells treated with MMS for 3 h *in vitro*. Each value represents the mean for the bone marrow cells collected from 4 male rats. ■, LEC; □, LEA; ▨, Wistar rats.

Table III. Chromosome Aberrations Induced by MMS *in vitro*[a]

| Strain | MMS (mg/ml) | Percentage of cells with[b] | | | | Number of aberrations per cell | Incidence of aberrant cells (%) |
|---|---|---|---|---|---|---|---|
| | | Gap | Break | Ex. | Multi. | | |
| LEA | 0 | 0.5±1.0 | 1.5±1.0 | 0±0 | 0±0 | 0.02±0.01 | 1.5±1.0 |
| | 2 | 2.0±2.8 | 5.5±2.5 | 0±0 | 0±0 | 0.07±0.03 | 5.5±2.5 |
| | 10 | 1.0±1.2 | 8.0±1.6 | 0.5±1.0 | 3.0±1.2 | 0.42±0.13[e] | 11.5±1.0[d] |
| | 20 | 0.5±1.0 | 18.0±6.3 | 1.5±1.9 | 9.5±4.1 | 1.32±0.31[e] | 29.0±2.6[d] |
| LEC | 0 | 0.5±1.0 | 1.0±1.2 | 0±0 | 0±0 | 0.01±0.01 | 1.0±1.2 |
| | 2 | 1.5±3.0 | 5.5±1.9 | 0±0 | 0±0 | 0.06±0.02 | 5.5±1.9 |
| | 10 | 0.5±1.0 | 12.0±2.3 | 0±0 | 4.5±1.0 | 0.66±0.15[d] | 16.5±3.0[d] |
| | 20 | 1.5±1.0 | 8.0±3.3 | 3.5±1.9 | 22.5±4.4 | 2.26±0.27[c,d] | 34.0±5.9[e] |
| Wistar | 0 | 0±0 | 0.5±1.0 | 0±0 | 0±0 | 0.01±0.01 | 0.5±1.0 |
| | 2 | 2.0±1.6 | 2.5±1.0 | 0±0 | 0±0 | 0.03±0.02 | 2.5±1.0 |
| | 10 | 2.0±1.6 | 5.5±1.0 | 0±0 | 0±0 | 0.09±0.03 | 5.5±1.0 |
| | 20 | 2.0±1.6 | 13.5±2.5 | 0±0 | 4.0±1.6 | 0.61±0.12 | 17.5±1.9 |

*a*) Cells were treated with MMS for 3 h.
*b*) Ex., exchange; Multi., multiple CA (cells having more than 10 aberrations).
*c*) Statistically significant compared with LEA rats at the same dose ($P<0.05$).
*d*) Statistically significant compared with Wistar rats at the same dose ($P<0.01$).
*e*) Statistically significant compared with Wistar rats at the same dose ($P<0.05$).

**MMS-induced CA in bone marrow cells of various rat strains exposed to MMS *in vitro*** The CA frequency induced by MMS *in vitro* was significantly higher in both LEA and LEC than in Wistar rat bone marrow cells. It was also higher in LEC than in LEA rat bone marrow cells, although the difference was statistically significant only for the number of aberrations per cell (Table III). Dose-related increase of aberrant cells induced by MMS *in vitro* was also observed in LEA, LEC and Wistar rats (Fig. 2). The number of aberrations per cell also increased in proportion to the MMS dose.

## DISCUSSION

In the present study, we compared the clastogenic responses to direct- and indirect-acting carcinogens in bone marrow cells of LEA, LEC, Wistar and SD rats. The CA frequency induced by BNU or MMS, which does not need matebolic activation, was significantly higher in both LEA and LEC rats than in Wistar or SD rats. The bone marrow cells of each rat strain exposed to MMS *in vitro* also showed the same tendency in the CA frequency. Therefore, the high sensitivity of both LEA and LEC to the clastogenic effects *in vivo* of direct-acting carcinogens seems to result from the sensitivity of the bone marrow cells themselves. However, we do not have other evidence to rule out the possibility that the difference in the clastogenic responses *in vivo* may be due to differences in the amounts of chemical reaching the bone marrow cells arising from differences of absorption, metabolism, and so on between different rat strains.

On the other hand, the CA frequency induced by DMBA or $AFB_1$, which requires metabolic activation, was lower in LEC rats than in the other 3 strains. Contrary to our expectations, the sensitivity of LEA rats was not so high, compared with that of Wistar or SD rats. The reason is not clear at present. The lower sensitivity of LEC rats to the clastogenic effects of indirect-acting chemicals may be explained by the lower level of cytochrome P-450 in LEC rat liver. Masuda *et al.* reported[13, 14] that LEC rats showed a lower frequency of CA and sister chromatid exchanges in bone marrow cells than LEA rats when exposed to cyclophosphamide, a promutagen and/or procarcinogen requiring P-450-dependent metabolic activation, though such a strain difference was not apparent in CA induced by direct-acting mitomycin C. These results, which are consistent with our results, was explained by the lower level of cytochrome P-450 in LEC rat liver.[13, 14]

The CA frequency induced by DMBA or $AFB_1$ in Cu-free LEC rats was higher than that in LEC rats given normal diet and lower than that in LEA rats, although a statistically significant difference was observed only between Cu-free LEC rats and LEC rats treated with DMBA. The values of copper concentration in the liver of LEA, Cu-free LEC and LEC rats were $5.0\pm0.4$, $33\pm7.7$ and $106\pm3.4$ $\mu$g/g wet weight, respectively. Furthermore, there was no difference between the cytochrome P-450 content in Cu-free LEC rats and that in LEC rats (data not shown). Therefore, the lower sensitivity of LEC rats to the clastogenic effects of indirect-acting carcinogens seems to be due to the effect of the large amount of copper accumulated in LEC rat liver. Yamane and Sasaki reported[15, 16] the effect of copper on the metabolism of 4-dimethylaminoazobenzene in the liver of Wistar rats fed copper. According to their results, N-demethylation activity was somewhat lower, and both ring hydroxylation activity and azo-reduction activity were higher in the rat group given copper than in the control rat group. Elevation of azo-reduction activity, which is concerned in the detoxication of 4-dimethylaminoazobenzene, was observed with increase of copper content in rat liver. Therefore, the lower sensitivity of LEC rats to the clastogenic action of indirect-acting chemicals may be explicable as follows. A great deal of copper accumulated in LEC rat liver elevates the detoxication enzyme activity or inhibits the enzyme activity of cytochrome P-450, and in consequence the dose of active carcinogens reaching the bone marrow cells may be decreased. However, further studies are required to clarify the mechanisms of susceptibility of LEA and LEC rats to genotoxic effects.

## ACKNOWLEDGMENTS

We thank Dr. N. Takeichi, Hokkaido University, for his kindness in providing LEA and LEC rats and Ms. M. Nishi for her technical assistance.

*(Received July 27, 1993/Accepted September 21, 1993)*

## REFERENCES

1) Sasaki, M., Yoshida, M. C., Kagami, K., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. Spontaneous hepatitis in an inbred strain of Long-Evans rats. *Rat News Lett.*, **14**, 4–6 (1985).
2) Yoshida, M. C., Masuda, R., Sasaki, M., Takeichi, N., Kobayashi, H., Dempo, K. and Mori, M. New mutation causing hereditary hepatitis in the laboratory rat. *J. Hered.*, **78**, 361–365 (1987).
3) Li, Y., Togashi, Y., Sato, S., Emoto, T., Kang, J.-H., Takeichi, N., Kobayashi, H., Kojima, Y., Une, Y. and

Uchino, J. Abnormal copper acumulation in non-cancerous and cancerous liver tissues of LEC rats developing hereditary hepatitis and spontaneous hepatoma. *Jpn. J. Cancer Res.*, **82**, 490–492 (1991).
4) Li, Y., Togashi, Y., Sato, S., Emoto, T., Kang, J.-H., Takeichi, N., Kobayashi, H., Kojima, Y., Une, Y. and Uchino, J. Spontaneous hepatic copper accumulation in Long-Evans cinnamon rats with hereditary hepatitis: a model of Wilson's desease. *J. Clin. Invest.*, **87**, 1851–1861 (1991).
5) Sugiyama, T. Specific vulnerability of the largest telocentric chromosome of rat bone marrow cells to 7,12-dimethylbenz(a)anthracene. *J. Natl. Cancer Inst.*, **47**, 1267–1275 (1971).
6) Sugiyama, T. and Brillantes, F. P. Cytogenetic studies of leukemia induced by 6,8,12- and 7,8,12-trimethylbenz(a)-anthracene. *J. Exp. Med.*, **131**, 331–341 (1970).
7) Sugiyama, T., Kurita, Y. and Nishizuka, Y. Chromosome abnormality in rat leukemia induced by 7,12-dimethylbenz-(a)anthracene. *Science*, **158**, 1058–1059 (1967).
8) Ito, Y., Maeda, S., Fujihara, T., Ueda, N. and Sugiyama, T. Suppression of 7,12-dimethylbenz(a)anthracene-induced chromosome aberrations in rat bone marrow cells after treatment with Sudan III and related azo dyes. *J. Natl. Cancer Inst.*, **69**, 1343–1346 (1982).
9) Ueda, N., Fujie, K., Goto-Mimura, K., Chattopadhyay, S. C. and Sugiyama, T. Acute cytogenetic effect of sterigmatocystin on rat bone marrow cells *in vivo*. *Mutat. Res.*, **139**, 203–206 (1984).
10) Fujie, K., Shimazu, H., Maeda, S. and Sugiyama, T. Acute cytogenetic effects of potassium bromate on rat bone marrow cells *in vivo*. *Mutat. Res.*, **206**, 455–458 (1988).
11) Fujie, K., Ito, Y. and Maeda, S. Acute cytogenetic effect of benzene on rat bone marrow cells *in vivo* and the effect of inducers or inhibitors of drug-metabolizing enzymes. *Mutat. Res.*, **298**, 81–90 (1992).
12) Sugiyama, T., Goto, K. and Uenaka, H. Acute cytogenetic effect of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2, a food preservative) on rat bone marrow cells *in vivo*. *Mutat. Res.*, **31**, 241–246 (1975).
13) Masuda, R., Abe, S., Yoshida, M. C., Sasaki, M., Sugiyama, T. and Taniguchi, N. Drug-metabolizing ability and inducibility of chromosomal aberrations and sister chromatid exchanges in LEC rats exposed to cyclophosphamide. *In* "The LEC Rat. A New Model for Hepatitis and Liver Cancer," ed. M. Mori, M. C. Yoshida, N. Takeichi and N. Taniguchi, pp. 261–272 (1991). Springer-Verlag, Tokyo.
14) Masuda, R., Abe, S., Yoshida, M. C., Sasaki, M., Sugiyama, T. and Taniguchi, N. Cytochrome P-450 and chromosome damage by cyclophosphamide in LEC strain rats predisposed to hereditary hepatitis and liver cancer. *Mutat. Res.*, **244**, 309–316 (1990).
15) Yamane, Y. and Sasaki, K. Effect of basic cupric acetate on biochemical changes in the liver of the rat fed carcinogenic aminoazo dye. III. Effect of copper compared with some other metals, phenobarbital and 3-methylcholanthrene on the metabolism of 4-dimethylaminoazobenzene. *Chem. Pharm. Bull.*, **22**, 1126–1132 (1974).
16) Yamane, Y. and Sasaki, K. Studies on the induction of DAB metabolizing enzyme activity and other microsomal enzyme activity in the liver of rats fed copper. *Chem. Pharm. Bull.*, **23**, 1440–1445 (1975).