ACCELERATED PAPER

Strain dependent effects of sex hormones on hepatocarcinogenesis in mice

Therese M. Poole and Norman R. Drinkwater

McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, 1400 University Avenue, Madison, WI 53706, USA

To whom correspondence should be addressed

In order to study the interaction of genetic and hormonal factors during murine hepatocarcinogenesis, we compared the number of liver tumors induced by treatment of 12-day-old mice with N,N-diethylnitrosamine (DEN) (0.05 μmol/g body wt) in intact mice and animals gonadectomized at 8 weeks of age from the three inbred strains, C3H/HeJ (C3H), C57BL/6J (B6), and C57BR/cdJ (BR). At 50 weeks of age, the mean liver tumor multiplicity in intact BR females was 28 ± 13, while that for intact female C3H and B6 mice was 1.4 ± 4.7 and 0.5 ± 1.0, respectively. In ovariectomized mice, the yield of liver tumors was ~8-fold higher than in intact C3H (10.3 ± 7.5) and B6 (4.1 ± 6.6) females. Only a slight increase (35 ± 14) was seen in ovariectomized BR females compared to intact BR females. Castration resulted in lower mean tumor multiplicities at 32 weeks of age in the males of all three strains. Intact male C3H, B6, and BR mice had mean liver tumor multiplicities of 61 ± 34, 7.4 ± 13, and 26 ± 18, respectively, while the mean tumor multiplicities in castrated C3H, B6, and BR mice were 24 ± 14, 0.5 ± 0.9, and 6.1 ± 10 tumors per mouse, respectively. The apparent rate of growth of glucose-6-phosphatase-deficient, preneoplastic foci in DEN-treated BR females was significantly higher than in B6 females. The growth rates of hepatic foci in BR and B6 males were similar but foci in BR males were 5-fold more numerous than in B6 males. The high sensitivity of BR females may be due, at least in part, to the failure of ovarian hormones to inhibit the growth of preneoplastic foci and the subsequent development of liver tumors. Since BR males had a larger number of hepatic foci, it is likely that androgens increase the rate of focus formation in BR males.

Introduction

The hormonal environment of the host affects the development of many types of tumors. For example, in humans and rodents, the growth of mammary cancer is greatly increased by the presence of estrogen and prolactin (1–6) and the growth of prostatic cancer is promoted by androgens (7,8). Although the liver is not a reproductive organ, the sensitivity of inbred mice to hepatocarcinogenesis is also affected by the hormonal status of the host (9–17). In addition to being markedly more sensitive to spontaneous liver tumor induction, male mice are significantly more susceptible than females to the development of liver tumors induced by perinatal treatment with carcinogens (9–12). This difference in sensitivity has been shown to be independent of the type of carcinogen used and occurs over a wide range of doses (10–14).

The sexual dimorphism in murine hepatocarcinogenesis results from the contrasting effects of androgens and ovarian hormones on liver tumor induction (9,11). Thus, the castration of male mice results in a decrease in mean liver tumor multiplicity (9,12,13,15) while the yield of tumors increases following the ovariectomy of females (9,15–17). Previous studies have shown that the removal of circulating sex hormones by gonadectomy of B6C3F1 mice resulted in a similar tumor incidence for males and females (9).

We demonstrated previously that promotion of liver tumor induction by testosterone in mice requires the presence of a functional androgen receptor by comparing hepatocarcinogenesis in Testicular feminization (Tfm) mice, which are mutant for the X-linked androgen receptor gene, to that in wild type C57BL/6J (B6+) male mice (18,19). The analysis of androgen receptor expression in tumors from Tfm/+ mosaic female mice treated with testosterone revealed that the androgen receptor need not be present in the target cell in order for a tumor to develop (19). The inhibition of hepatocarcinogenesis in female mice is likely to be the result of direct or indirect effects of estrogen on the development of liver tumors. Lee et al. (20) demonstrated that treatment of C3H, B6, and BALB/c male mice with high doses of estrogen inhibited liver tumor induction. Studies from Yamamoto and coworkers (17) provide more direct evidence; chronic treatment of ovariectomized female mice with estradiol, but not progesterone, decreased the yield of tumors to that observed for intact females.

There is a broad range of susceptibility to hepatocarcinogenesis among the males of different strains of mice (21). When treated with a single perinatal dose of a carcinogen, sensitive C3H/HeJ (C3H) male mice are 20–50 times more susceptible to liver tumor induction than resistant B6 males (22). In contrast, the inhibitory effect of ovarian hormones results in a narrow range of sensitivity among the females of the same strains; C3H females are only 3 times as susceptible as B6 females and are just 3% as sensitive as C3H males (22). The C57BR/cdJ (BR) mouse strain is unique because, compared to males of other strains of mice, BR males are intermediate in susceptibility to hepatocarcinogenesis, while BR females are extremely susceptible, with a 20- to 40-fold higher mean tumor multiplicity than the females of any other strain (21). Although the BR females are much more sensitive than the females of other strains, they are still relatively resistant when compared to the BR males. The basis for the extreme sensitivity of the BR females is unknown and was unexpected given the intermediate sensitivity of the males. Determining the biological basis for the unusual tumor sensitivity phenotype of the BR females will help gain further insight into the mechanism(s) by which the growth of liver tumors is influenced by sex hormones in inbred mice.

In order to determine the strain-dependent effects of sex hormones on liver tumor induction, we compared tumor

*Abbreviations: B6, C57BL/6J; BR, C57BR/cdJ; C3H, C3H/HeJ; DEN, N,N-diethylnitrosamine; Tₜₑ, apparent doubling time.
multiplicity in intact and gonadectomized BR, B6, and C3H male and female mice treated with DEN. Since histochemically-altered liver foci are thought to be precursors of liver tumors (23), we also studied the growth of glucose-6-phosphatase (G6Pase)-deficient foci in order to evaluate the effects of sex hormones on the promotion phase of hepatocarcinogenesis. In the experiments reported here, we demonstrate that ovarian hormones inhibited hepatocarcinogenesis in C3H and B6 female mice but not in BR females. In contrast, liver tumor induction was promoted by androgens in all three strains. We observed a difference in the rate of growth of hepatic foci between the BR and B6 females that could account for the increased sensitivity of the BR females to hepatocarcinogenesis. BR males are more susceptible to hepatocarcinogenesis than B6 males despite exhibiting similar growth rates in hepatic foci. This difference in sensitivity may result from a higher rate of focus formation in the livers of the BR males. Unexpectedly, the growth rates of preneoplastic lesions were similar in intact BR males and females indicating that androgens may not influence the growth rate of preneoplastic foci, but rather serve to increase the rate of formation of preneoplastic foci in BR mice.

Materials and methods

Mice

Mice were bred in our laboratory from stocks of C57BL/6J, C57BR/cdJ and C3H/HeJ mice purchased from the Jackson Laboratory (Bar Harbor, ME). The mice were housed in plastic cages on corn cob bedding (Bed O’Cobs, Anderson Cobb Division, Maumee, OH) and fed Wayne Breeder Blox (Continental Grain Co., Chicago, IL). Food and acidified tap water were available ad libitum. Mice were inspected daily and weighed monthly.

Tumor induction study

Twelve-day-old male and female C57BL/6J, C57BR/cdJ and C3H/HeJ mice were injected i.p. with DEN (0.05 μmol/g body wt) (Eastman Kodak Co., Rochester, NY) dissolved in sterile trioctanoin (0.01 ml/g) (Pfaltz and Bauer, Inc., Stamford, CT). One group of mice from each of the three strains was gonadectomized, as described below, at 8 weeks of age. All intact and castrated males were sacrificed at 32 weeks and all intact and ovariecetomized females were sacrificed at 50 weeks of age. Mice were euthanized by CO2 asphyxiation and the livers were removed and weighed. All tumors >2 mm on the surface of the liver were counted. A random sample of tumors from each group were excised, fixed in phosphate buffered formalin and later examined microscopically in thin sections stained with hematoxylin and eosin.

Gonadectomy

Gonadectomies were performed as described previously (24). Sterile gloves and instruments were used throughout the procedure. Eight-week-old mice were anesthetized using a mixture of Ketaset (0.04 mg/g body wt) (Pfaltz and Bauer, Inc., Stamford, CT) and rinsed with 70% ethanol. A single incision was made in the skin, and a small incision was made in the peritoneum close to the gonad. In females, both ovaries and part of the uterine horn were tied off with 5-0 silk suture (Medix, Madison, WI) and PromAce (0.025 mg/g body wt) (Aveco, New York, NY). The skin was shaved, washed with Betadine (Purdue Frederick, Norwalk, CT) and rinsed with 70% ethanol. Mice were injected i.p. with DEN (0.05 μmol/g body wt) (Eastman Kodak Co., Rochester, NY) dissolved in sterile trioctanoin (0.01 ml/g) (Pfaltz and Bauer, Inc., Stamford, CT) and rinsed with 70% ethanol. A single incision was made in the skin, and a small incision was made in the peritoneum close to the gonad. In females, both ovaries and part of the uterine horn were tied off with 5-0 silk suture (Medix, Madison, WI) and PromAce (0.025 mg/g body wt) (Aveco, New York, NY). The skin was shaved, washed with Betadine (Purdue Frederick, Norwalk, CT) and rinsed with 70% ethanol. A single incision was made in the skin, and a small incision was made in the peritoneum close to the gonad. In females, both ovaries and part of the uterine horn were tied off with 5-0 silk suture (Medix, Madison, WI) and PromAce (0.025 mg/g body wt) (Aveco, New York, NY). The skin was shaved, washed with Betadine (Purdue Frederick, Norwalk, CT) and rinsed with 70% ethanol.

Materials and Methods Table

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of Mice</th>
<th>Mean Liver Tumor Mult.</th>
<th>Gonadectomized Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/HeJ</td>
<td>28</td>
<td>1.4 (4.7)</td>
<td>29 (10 (8)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>26</td>
<td>0.5 (1.0)</td>
<td>29 (4.1 (6.6)</td>
</tr>
<tr>
<td>C57BR/cdJ</td>
<td>33</td>
<td>28 (13)</td>
<td>30 (35 (14)</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>28</td>
<td>66 (34)</td>
<td>39 (24 (14)</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>29</td>
<td>7.4 (13)</td>
<td>32 (0.5 (0.9)</td>
</tr>
<tr>
<td>C57BR/cdJ</td>
<td>32</td>
<td>26 (18)</td>
<td>32 (6.1 (10)</td>
</tr>
</tbody>
</table>

* Values are means (SD).

Statistical analyses

The yield of tumors from each group was compared to the others using the Wilcoxon rank sum test (26). The same test was used for comparison of the treatment groups for the number and average size of G6Pase-deficient hepatic foci. The frequencies of hepatic adenomas and carcinomas among the groups were compared statistically with Fisher’s exact test (27).

Results

Effects of gonadectomy on liver tumor induction

The mean tumor multiplicity at 50 weeks of age in intact BR females (28 ± 13) was 20 and 56 times greater than that in intact C3H (1.4 ± 4.7) and B6 females (0.5 ± 1.0), respectively (Table I). Ovariectomy resulted in a large increase in the mean liver tumor multiplicity in C3H and B6 females but not in the BR females (Table I). An ~8-fold increase in mean tumor multiplicity was observed in both B6 and C3H ovariectomized female mice as compared to the intact females of the same strain (P < 4 × 10^-6; two-sided Wilcoxon rank sum test). The mean tumor multiplicity in BR females after ovariectomy was only 25% higher than that in intact BR females (P < 0.02). These observations indicate that BR females are less responsive to the inhibitory effects of ovarian hormones on hepatocarcinogenesis than the other two strains.

Intact 32-week-old C3H male mice had a mean tumor multiplicity of 66 ± 34 which was ~9-fold higher than that in intact B6 male mice (7.4 ± 13) (P < 6 × 10^-6) and ~2.5 times greater than that in the intact BR males (26 ± 18) (P < 2 × 10^-6) (Table I). A decrease in the mean tumor multiplicity was observed following castration of the male mice of all three strains, although the magnitude of the decrease varied among the strains. The mean tumor multiplicity of castrated C3H males decreased to ~33% of the mean value for the intact C3H males while the mean tumor multiplicity for B6 and BR castrated males decreased to 6.7% and 25% of mean for the intact males, respectively.

We sacrificed female mice at an older age than males in the above experiment because of the delayed development of liver tumors in females (28). At 32 weeks of age (see experiment in Table II), the mean numbers of liver tumors observed in intact female B6, C3H, and BR mice were 0.19 ± 0.39 (16 mice), 0.13 ± 0.33 (15 mice), and 0.53 ± 1.1 (15 mice),
The number, size, and volume fraction of G6Pase-deficient foci in the livers of male and female BR and B6 mice treated with DEN are shown in Table II. G6Pase-deficient foci were two-fold more numerous in the BR females (190 ± 180 at 24 weeks) than in the B6 females (99 ± 120) at all comparable times except 32 weeks. We observed a 3-fold increase in focus diameter over time in both BR and B6 female mice from 16 to 40 weeks of age. However, at each time point, the hepatic foci were significantly larger (P < 0.02) in the BR females than in the B6 females. Although the percent liver volume containing G6Pase-deficient foci increased over time in both strains, the volume fraction occupied by foci was significantly greater (P < 0.002) in BR females than in B6 females at all timepoints. At 16 weeks of age the percentage of liver volume occupied by G6Pase-deficient foci in BR females was 20-fold greater than that in B6 female livers and by 40 weeks the value was 75-fold higher in BR females (15 ± 11%) compared to B6 females (0.2 ± 0.3%). Analysis of the change in the number of cells per focus over time revealed that the growth rate of hepatic foci in the BR females was higher than that in B6 females (Figure 1). The apparent doubling time, Td, for foci was significantly shorter in the BR females (4.6 ± 2.1 weeks) than in the B6 females (10.2 ± 2.3 weeks).

The number, size, and volume fraction of G6Pase-deficient foci in the livers of the male mice are also presented in Table II. Hepatic foci could not be quantitated at the 40 week timepoint in the BR males because nearly 100% of the liver volume contained G6Pase-deficient foci. The number of hepatic foci analyzed for glucose-6-phosphatase activity at 16, 24, 32, and 40 weeks of age.

**Fig. 1.** Growth rate of hepatic foci in B6 females (filled circles), BR females (filled squares), B6 males (open circles), and BR males (open squares). The solid and dashed lines represent the regression curves for the focus growth in females and males, respectively. The vertical bars represent the standard deviation of the value. As noted in the text, mice were treated with N,N-diethylnitrosamine at 12 days of age and hepatic foci were analyzed for glucose-6-phosphatase activity at 16, 24, 32, and 40 weeks of age.
was not significant ($P < 0.39$). The percentage of the liver occupied by foci increased in both strains over time, but the percentage in BR males was significantly higher than that in the B6 males at all timepoints ($P < 10^{-5}$).

By determining the change in the number of cells per focus over time in the livers of the males, we observed that the growth rate of lesions was similar in BR and B6 males (Figure 1). The apparent doubling time, $T_d$, for focal growth in BR males was $3.3 \pm 0.8$ weeks and in B6 males it was $3.5 \pm 0.5$ weeks. Strikingly, the rate of growth of hepatic foci in the BR males was similar to that in the BR females.

**Discussion**

Although we observed a 8-fold increase in tumor multiplicity in both ovariectomized C3H and B6 females compared to their intact counterparts, the average number of liver tumors in ovariectomized BR females increased only 25% over that in the intact BR females. Thus, ovarian hormones strongly inhibited hepatocarcinogenesis in C3H and B6 female mice, consistent with previous studies in B6C3F$_1$ females (9,12), while only weakly suppressing the development of liver tumors in BR females. Therefore, BR mice must carry sensitivity genes that overcome the inhibitory effect of ovarian hormones.

Interestingly, the tumor multiplicity in ovariectomized C3H and B6 females was still much lower than that in intact or ovariectomized BR females. If the genetic background were the sole determinant of the difference in sensitivity among the strains, one would expect that C3H would be the most sensitive of the ovariectomized animals since that is what is observed in both castrated and intact males. However, the gonadectomies were not performed until 8 weeks of age and inhibitory effects of ovarian hormones prior to that time could account for the low tumor yield in ovariectomized C3H and B6 females. Previous studies support this conclusion; when groups of B6C3F$_1$ females were ovariectomized at various ages and the tumor multiplicity for each group determined at the same endpoint, a differential inhibition of hepatocellular adenomas was observed. Ovariectomy of females at later timepoints resulted in fewer tumors than in those mice in which the surgery was performed earlier (28), indicating that the degree to which tumor formation was suppressed correlates with the length of time that the ovarian hormones were present in the body.

Our studies do not address the mechanisms by which ovarian hormones inhibit hepatocarcinogenesis in females of typical inbred mouse strains. It is likely that the relevant ovarian hormone is estrogen, based on the observation that this hormone inhibits liver tumor induction when administered chronically to male (20) or ovariectomized female (17) mice. The inhibition of liver tumor development could result from the direct effects of estrogen on the target hepatocyte or from an indirect mechanism involving the estrogen-dependent regulation of a hormone or growth factor produced by another tissue. We have shown that the amounts and affinities of estrogen receptor in the livers of B6 and BR female mice are similar (29). Yamamoto et al. (17) have argued that the effects of estrogen on liver tumor induction are indirect, based on the observation that subcutaneous, but not intrasplenic, implantation of slow-release estrogen pellets inhibited tumor development in ovariectomized female mice. This question could be addressed more straightforwardly by an approach analogous to that used to demonstrate that the promotion of hepatocarcinogenesis by androgens is not cell-autonomous (19). Mice mosaic for the expression of functional estrogen receptor could be constructed by preparing chimeric embryos from wild type mice and mice carrying an insertional mutation in the estrogen receptor locus (30).

We observed a significantly higher net growth rate of G6Pase-deficient foci in the BR females compared to the B6 females indicating that the apparent rate of growth of preneoplastic foci is higher in BR females. We have previously shown that hepatic foci grow at similar low rates in C3H and B6 females (25). The difference in the rate of growth of foci between BR and B6 females may reflect the effect of the ovarian hormones since tumor multiplicity increases to the same degree in B6 and C3H females following ovariectomy. Thus, BR females are deficient in their ability to effectively suppress the growth of preneoplastic lesions, leading to enhanced tumor development. One test of this model would be to compare the growth rate and number of G6Pase-deficient foci in intact and ovariectomized B6, C3H, and BR females. If the hypothesis is correct, then one would expect to see an increased growth rate of hepatic foci in ovariectomized B6 and C3H females, but not in BR females, compared to the intact mice. Goldfarb and Pugh have shown previously that ovariectomy accelerated the growth of preneoplastic and neoplastic hepatocellular lesions in B6C3F$_1$ mice (16).

Tumor multiplicity was lower in castrated male C3H, B6 and BR mice compared to the intact males, indicating that androgens promoted the development of liver tumors in all three strains. The difference in sensitivity to hepatocarcinogenesis between BR and B6 males cannot be explained simply by a difference in the net growth rate of hepatic foci because the growth rate of preneoplastic foci was found to be similar in BR and B6 males. The number of hepatic foci at each timepoint was significantly higher in BR males than in B6 males suggesting that the difference in sensitivity between the strains lies in the process of focus formation.

The disparity in the number of observed preneoplastic foci in the livers of the males of the BR and B6 strains could be caused by a difference in the level of initiation. If the level of DNA damage following carcinogen treatment, or the efficiency with which such damage yielded fixed mutations, were higher in BR males, then a greater number of cells could become preneoplastic. However, DNA damage following DEN treatment has been shown to be similar in C3H and B6 mice, yet the livers from DEN-treated C3H male mice contained many more foci than did the livers from DEN-treated B6 males (22). In addition, BR males are susceptible to both spontaneous (31) and N-ethyl-N-nitrosourea-induced hepatocarcinogenesis (21), which argues against a carcinogen-dependent sensitivity.

Although the multistage model for carcinogenesis, which divides the process into initiation, promotion, and progression phases, was first developed to describe tumor induction in mouse skin (32), this model has also been shown to apply broadly to hepatocarcinogenesis (23). Boutwell (32) has proposed that the promotion stage of skin carcinogenesis can be subdivided further into a conversion step that provides the initiated cell with the capacity to proliferate rapidly and respond to promoting agents and a propagation stage, which is characterized by the clonal proliferation of the preneoplastic cell. The conversion-propagation model for promotion may also be applied to hepatocarcinogenesis. Thus, even though the rate of growth of preneoplastic foci after DEN treatment is similar in B6 and BR males, a difference in the ability to become responsive to promotional stimuli (conversion) could
account for the difference in the number of hepatic foci. Furthermore, androgens may affect this conversion step, since, despite the similar growth rate of preneoplastic lesions in BR males and females, the livers of BR males contain many more foci than those of BR females. Our previous observation that a partial hepatectomy acted as a promoter of liver tumor induction in B6 males but not in C3H males provides evidence consistent with this model (33). Spontaneous conversion could occur more readily in C3H males than in B6 males, while an exogenous stimulus may be required for tumor formation in B6 mice. The observation in the present study that the number of foci increased in an age-dependent manner in BR male mice is also consistent with this model.

Previously, we proposed that androgens strongly interacted with the Hepatocarcinogen sensitivity (Hcs) gene identified as the major determinant of the difference in sensitivity between C3H and B6 male mice (22). Therefore, it was expected that tumor multiplicity in castrated C3H and B6 male mice would be more similar, as had been observed for the female mice. In this study, however, we demonstrated that castrated C3H male mice still were significantly more sensitive to liver tumor induction than castrated B6 male mice. Furthermore, B6 mice which carry resistance alleles at the Hcs locus are still susceptible to the promoting effects of androgens. The genetic effects on the sensitivity of C3H are complex and several other Hcs genes have now been identified (34, 35); it is likely that each Hcs gene will affect a distinct stage of the carcinogenic process.

It was somewhat surprising that the BR mice are sensitive to tumor induction since these mice are closely related to B6. The BR and B6 strains were generated from two siblings in a single cross (36) so 25% of their genes are identical by descent (37). In genetic mapping experiments, we have demonstrated that two loci are the major genetic factors contributing to the sensitivity of both the BR females and males (Poole and Drinkwater, submitted for publication). These gene mapping studies strongly suggest that the same underlying pathway is responsible for the sensitivity of both BR males and females to hepatocarcinogenesis. It is possible that the products of these genes affect the growth rate of hepatic tumors since the rate of growth of preneoplastic foci is nearly identical in BR males and females. In addition, ovariectomized and intact BR female mice and castrated BR male mice, sacrificed at the same timepoint had similar mean tumor multiplicities (data not shown), which supports the hypothesis that a similar tumor induction pathway is followed in the absence of sex hormones.

It is unclear whether our studies of murine hepatocarcinogenesis reflect the mechanisms of liver tumor induction that occur in humans. Analysis of the factors influencing hepatocarcinogenesis in humans is complicated by the association of Hepatitis B Virus (HBV) infection with the development of hepatocellular carcinomas (38). Men develop liver cancer two to five times more often than women, but the higher incidence of this cancer in males may result from the fact that more men are chronically infected with HBV than women (39). Several reports provide evidence that both anabolic steroids and exogenous estrogens in the form of oral contraceptives are associated with a high risk for the development of hepatocellular adenomas (40–43).

The molecular basis for the modulation of liver tumor development by ovarian hormones is unknown. The BR strain provides a unique system in which to identify pathways which overcome the inhibitory effect of ovarian hormones on liver tumor development. Determining the genetic basis for the sensitivity of the BR females will provide a foundation for understanding the pathway(s) responsible for the resistance to the suppressing effects of ovarian hormones in BR mice and may give clues as to how ovarian hormones inhibit tumor development in other strains.

Acknowledgements

The authors would like to thank Mary Winkler and Donald Kehler for assistance with the animal work, Paul Merline and Jane Weeks for preparation of the tissue sections, Dr. Gang-Hong Lee for the histological analysis, Teresa Chiaverotti for helpful discussions, and Susan Schadewald and Ray Carabeo for critical comments on the manuscript. This work was supported by Public Health Service Grants CA22484, CA07175, CA09135.

References


Hormonal regulation of hepatocarcinogenesis

195
diethylnitrosamine-initiated two-stage hepatocarcinogenesis in C3H,
C57BL, and BALB mice promoted by various hepatopromoters.
Carcinogenesis, 10, 2227–2230.
susceptibility, plasma testosterone levels, and androgen receptor binding
22. Drinkwater, N.R. and Ginsler, J.J. (1986) Genetic control of hepato-
carcinogenesis in C57BL/6J and C3H/HeJ inbred mice. Carcinogenesis,
7, 1701–1707.
growth of preneoplastic lesions in hepatocarcinogen-sensitive C3H/HeJ
male mice relative to C57BL/6J male mice. Carcinogenesis, 9, 885–890.
sensitive carcinogenesis model and the role of the sex hormonal
environment in tumor development. In Stevenson, D.E., Popp, J.A.,
Ward, J.M., McClain, R.M., Slaga, T.J. and Pitot, H.C. (eds), Mouse Liver
Carcinogenesis: Mechanisms and Species Comparisons. Wiley-Liss New
York, pp. 53–68.
in murine hepatocarcinogenesis. In McClain, R.M., Slaga, T.J., LeBoeuf, R.
and Pitot, H. (eds), Growth Factors and Tumor Promotion. Wiley-Liss,
30. Lubahn, D.B., Moyer, J.S., Goldberg, T.S., Couse, J.F., Korach, K.S., and
Smithies, O. (1993) Alteration of reproductive function but not prenatal
sexual development after insertional disruption of the mouse estrogen
207–250.
hepatectomy is a promoter of hepatocarcinogenesis in C57BL/6J male
34. Gariboldi, M., Manenti, G., Canzian, F., Falvella, F.S., Pierotti, M.A., Della
Porta, G., Binelli, G. and Dragani, T. (1993) Chromosome mapping of
35. Manenti, G., Binelli, G., Gariboldi, M., Canzian, F., De Gregorio, L.,
genetic predisposition to hepatocarcinogenesis in mice. Genomics, 23,
118–124.
University Press, New York.
appears rapid. Science, 228, 1169–1175.
association between hepatomas and oral contraceptives. Lancet, 2, 926–929.
Lancet, 2, 1200.
Regression of liver cell adenomas associated with oral contraceptives. Ann
Internal Med., 86, 180–182.

Received on October 20, 1995; revised on November 15, 1995; accepted on