N-Ethyl-N-nitrosourea Treatment of Multiple Intestinal Neoplasia (Min) Mice: Age-related Effects on the Formation of Intestinal Adenomas, Cystic Crypts, and Epidermoid Cysts

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ABSTRACT

The timing of intestinal tumor initiation in B6-Min/+ mice has been examined by treating mice at 5–35 days of age with a single i.p. injection of the direct-acting alkylating agent N-ethyl-N-nitrosourea (ENU). Treatment of Min/+ mice at 5–14 days of age resulted in a 3.8-fold increase in intestinal tumor multiplicity over untreated mice. Mice treated at 20–35 days of age showed only a 1.6-fold increase in tumor number. These results, in conjunction with examination of tumor multiplicities of untreated Min/+ mice as a function of age, suggest that the majority of intestinal tumors in Min/+ mice are initiated relatively early in life. Min/+ mice treated with ENU also showed an increase in the number of cystic intestinal crypts. However, the relationship between age at ENU treatment and cystic crypt multiplicity was distinct from that seen for intestinal adenomas. Mice treated at 5–9 days of age showed only a 1.9-fold increase in cystic crypts over untreated animals. By contrast, the increase in average cystic crypt multiplicity for mice treated at 10–35 days of age was 4.5-fold. In addition, 60% of Min/+ mice treated with ENU before 25 days of age developed epidermoid cysts, an extracolonic manifestation commonly associated with familial adenomatous polyposis in humans.

INTRODUCTION

The ability of many carcinogens to induce tumors in mice is strongly influenced by various factors including the sex (1), strain (1–3), and treatment age of the animal (1, 3, 4). Perinatal treatment of animals with alkylating agents such as ENU often results in the formation of tumors in the lungs (1, 3, 5, 6), liver (1, 5, 6), lymphoid organs (1, 5, 6), and nervous system (1, 7). ENU does not commonly induce intestinal tumors in most strains of mice. However, Oomen et al. (5) have shown that H-2 haplotype can strongly influence intestinal tumor incidence in mice treated with ENU at 15 days of age.

We have been investigating intestinal neoplasia in mice that are heterozygous for ApcMin (Min), a mutant allele of the mouse homologue of the human APC gene (8). On the C57BL/6J (B6) background, Min is a fully penetrant, autosomal dominant mutation that predisposes mice to develop multiple intestinal adenomas (9). B6-Min/+ mice develop approximately 50 adenomas throughout the intestinal tract and rarely live beyond 150 days of age (9). Analysis of longer-lived hybrid mice carrying the Min mutation has shown that the majority of intestinal adenomas are established by 100 days of age (10).

In this report, we have more rigorously examined the timing of intestinal adenoma initiation in Min/+ mice. Specifically, we have examined whether there is a correlation between age at treatment with ENU and intestinal tumor multiplicity in Min/+ mice. The results presented here suggest that intestinal adenomas in Min/+ mice are most likely to be initiated during the first several weeks of life. In addition, we have examined the relationship between age at ENU treatment and the multiplicity of a distinct type of intestinal lesion, the cystic crypt. We have also found a relationship between age at ENU treatment of Min/+ mice and the development of epidermoid cysts of the skin.

MATERIALS AND METHODS

Mice. B6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or bred at the McArthur Laboratory for Cancer Research. The B6-Min pedigree is maintained by crossing B6-++ females with B6-Min/+ males (9). All B6-Min mice used in these experiments were from the twenty-sixth to thirty-first backcross generations to B6. Animals were genotyped using DNA isolated from blood samples collected from retroorbital sinus shortly after weaning (approximately 40 days of age) or from spleen tissue collected at the time of sacrifice (11). Min/+ animals were identified using an allele-specific PCR assay described previously (11).

ENU Treatments. Mice from entire B6-++ X B6-Min/+ litters were given single i.p. injections of the alkylating agent ENU (Sigma Chemical Co., St. Louis, MO) at a dose of 50 µg/gm body weight. All injections were performed as described previously (12). All animals were 5 to 35 days of age at the time of ENU treatment. To analyze the results more effectively, the mice were grouped into sets of 5–9, 10–14, 15–19, 20–25, and 30–35 days of age, based on the age at the time of treatment. The weighted average treatment ages for these groups were: 6.7, 11.7, 16.4, 22.6, and 32.6 days, respectively. Litters from at least four of these five age groups were included in each round of treatments to control for any ENU variability. Each age group included 16–25 Min/+ mice. Wild-type littermates served as +/+ treated controls. Six ENU-treated mice, one female and five males, died before the end of the experiment; therefore, they could not be analyzed. Three of the males were determined to be Min/+; the other three animals were not genotyped before death. These six animals came from all treatment age groups except for the 5–9-day group.

Tumor Scoring. Animals were killed when moribund or 65 days after injection. Untreated animals that corresponded in age to the final ages of treated animals were also examined. Approximately one-third of the small intestine from each mouse was scored for tumors by examining 4-cm sections from the proximal, middle, and distal small intestine. The entire large intestine was also scored for tumors (9). For these experiments, tumors were counted under a dissecting microscope at ×10 after the intestine had been spread out flat on bifolius paper (Baxter Scientific Products, McGraw Park, IL), fixed overnight in 10% buffered formalin, and subsequently washed in 70% ethanol. Cystic crypts were quantitated concurrently from the same intestinal regions at ×10–40. All tumor and cystic crypt counts were performed by a single observer (A. R. S.). Tumors and cystic crypts from ENU-treated Min/+ and +/+ animals were analyzed histologically as described previously (9).

Statistical Analyses. The Wilcoxon rank sum test was used for all comparisons of total intestinal tumor or cystic crypt multiplicity of Min/+ mice (13). P < 0.05 was considered to indicate a significant difference between sample populations. This nonparametric statistical test was used for these analyses because it requires few assumptions about the distribution of the tumor or cystic crypt multiplicity data (13). Comparisons of the regional distribution of tumors and/or cystic crypts along the length of the intestinal

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4 The abbreviations used are: ENU, N-ethyl-N-nitrosourea; Min, multiple intestinal neoplasia; APC, adenomatous polyposis coli.
tract between two sample populations were performed using the \( \chi^2 \) test based on 4 x 2 contingency tables constructed by calculating the percentage of total tumor or cystic crypt multiplicity in the proximal, middle, and distal small intestine and the large intestine (14). \( P < 0.05 \) was used to reject the null hypothesis that the regional distribution of tumors and/or cystic crypts along the length of the intestine between two sample populations was not different. This test was used because it permitted simultaneous comparisons of all four intestinal regions between sample populations (14). The intestinal tumor incidence of \(+/-\) mice treated with ENU at different ages was compared using Student's \( t \) test (14). The \( t \) test was also used to compare the incidence of epidermoid cysts in Min/+ mice treated with ENU at different ages. \( P < 0.05 \) was considered to indicate a significant difference in tumor incidence between sample populations.

RESULTS

Intestinal Tumors in ENU-treated Min/+ Mice

To determine if there is a relationship between age at ENU treatment and intestinal adenoma multiplicity, tumor numbers were compared between groups of mice treated with ENU at 5 to 35 days of age. All mice were examined for intestinal tumors at approximately 65 days after ENU treatment. The intestinal tumor multiplicities for Min/+ mice treated with a single injection of ENU at various ages are shown in Fig. 1A.

Intestinal tumor counts were also done on 68 untreated Min/+ mice that ranged from 67 to 97 days of age. Statistical analysis revealed no difference in tumor multiplicities for mice within this age range (Wilcoxon rank sum test; \( P > 0.07 \) for comparisons between groups of untreated animals that were 67–74, 75–80, 81–84, 85–90, or 95–97 days of age). Therefore, the untreated animals were pooled for comparisons to the treated animals (Fig. 1A).

All mice treated with ENU had significantly higher average tumor numbers in comparison to untreated animals (\( P \leq 1 \times 10^{-6} \) for each comparison of the ENU-treated groups to the untreated control animals). However, tumor multiplicity was also dramatically affected by the age at time of treatment. The average tumor multiplicity for Min/+ mice treated at 5 to 9 days of age (104.1) was not different from that for animals treated at 10 to 14 days of age (116.2; \( P = 0.24 \); Fig. 1A). However, animals treated at 15 to 19 days of age had an average of 74.1 tumors. This was significantly different from the tumor multiplicity for mice treated before 15 days of age (\( P < 8 \times 10^{-9} \)). Tumor multiplicity continued to decline for mice treated at older ages. Mice treated at 20–25 days of age averaged 54.3 intestinal tumors, while the average for treatment at 30 to 35 days of age was 41.4 tumors (Fig. 1A). Each of these decreases represents a highly significant change from the average tumor multiplicity for animals treated at the next youngest age group (\( P \leq 0.01 \) for each comparison between successive ages of treatment).

In summary, treatment of Min/+ mice at 5–14 days of age resulted in a 3.8-fold increase in average tumor multiplicity. After 14 days of age, each increase of 5 days in treatment age resulted in a significant decrease in tumor multiplicity. For mice treated at 30–35 days of age, the average tumor multiplicity was only 1.4-fold higher than the average tumor number for untreated animals.

The regional distribution of tumors along the length of the intestinal tract was examined by calculating the percentage of total tumor multiplicity in each of the four counted regions of the intestine for untreated mice and each of the ENU-treated groups of Min/+ mice (Fig. 2). The regional distribution of tumors in treated mice closely paralleled that seen in untreated mice (Fig. 2). Comparisons of the tumor distribution in untreated Min/+ animals to each of the five ENU treatment groups revealed no difference in regional tumor distribution (Fig. 2; \( \chi^2 \) test; \( P > 0.10 \)). ENU treatment at different ages also did not affect greatly the regional tumor distribution (Fig. 2). The only possibly significant difference in regional tumor distribution between age groups was for treatment at 5–9 days of age versus treatment at 20–25 days of age (Fig. 2; \( P < 0.03 \)). The weighted percentage of total tumor multiplicity (based on the number of ani-
likely to induce intestinal tumors than treatment at 15 to 35 days of age (Student's t test, P < 0.005). The total number of tumors for ENU-treated +/+ animals ranged from one to three tumors with a mean of 1.5. One of the tumors was in the large intestine, while the rest were in the proximal part of the small intestine. Excluding the single tumors seen in the 15–19 and 30–35-day age groups, tumors from each age group of +/+ mice were examined histologically. These tumors resembled typical intestinal adenomas in Min/+ mice (data not shown). No intestinal tumors were found in any untreated +/+ mice (Table 1).

Cystic Lesions in ENU-treated Min/+ Mice

Cystic Crypts. Cystic crypts were counted at the same time and in the same intestinal regions as were intestinal tumors. These lesions are morphologically and histologically distinct from intestinal adenomas (Fig. 3). Serial sectioning of intestinal samples revealed these structures to be crypts that appear to be sealed off and lined with abnormal cells that are often anaplastic (Fig. 3b). The cysts were typically covered by a layer of histologically normal epithelium. Cystic crypts were primarily located in the upper one-third of the small intestine of both untreated and ENU-treated Min/+ mice. No cystic crypts were identified in the large intestine of any mice. The crypts ranged in diameter from ~0.1 mm to approximately the size of a small adenoma (~0.50 mm).

There was no difference in cystic crypt multiplicity for untreated Min/+ mice that ranged in age from 67 to 97 days of age (Wilcoxon rank sum test; P = 0.24 for comparisons between groups of untreated animals that were 67–74, 75–80, 81–84, 85–90, and 95–97 days of age). For this reason, cystic crypt counts for all untreated animals were pooled for comparisons to the treated animals (Fig. 1b). Of 68 untreated Min/+ animals that were 67–97 days of age, 65 (96%) had cystic crypts. The average number of cysts for these 68 animals was 6.4 (Fig. 1b).

The relationship between age at ENU treatment and the number of intestinal cystic crypts is presented in Fig. 1b. Treatment with ENU at all ages led to significant increases in the number of cystic crypts compared to untreated Min/+ mice (P ≤ 3.2 × 10−5 for each comparison of ENU-treated groups to the untreated animals). Mice treated with ENU at 5 to 9 days of age averaged 12.5 cystic crypts. Treatment at all other ages resulted in significant increases in the number of cystic crypts relative to the 5–9-day group (P ≤ 5.7 × 10−5 for comparisons of the 10–14, 15–19, 20–25, and 30–35-day treatment groups to the 5–9-day treatment group). However, there was no difference in average cystic crypt multiplicity for mice treated at 10–14, 15–19, 20–25, or 30–35 days of age (P = 0.45 for comparisons between these age groups). The average number of cysts for all Min/+ mice treated with ENU at 10 to 35 days of age was 29.4 ± 17.0.

As with the comparisons of regional intestinal tumor distribution,

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<th>Table 1</th>
<th>Intestinal tumors in ENU-treated +/+ mice</th>
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<td>Age at ENU treatment (days)</td>
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<td>5–9</td>
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The number of +/+ animals with intestinal tumors approximately 65 days after treatment with ENU at the indicated ages is shown. Seventeen % of animals treated at 5 to 14 days of age developed tumors in comparison to only 4% of mice treated at 15 to 35 days of age. The total number of intestinal tumors for ENU-treated +/+ mice ranged from 1–3, with an average of 1.5 tumors per animal.

Intestinal Tumors in ENU-treated +/+ Mice

ENU-treated animals that typed as +/+ for the Apc locus were also scored for intestinal tumors. Mice that had tumors in any of the four regions of the intestine described above were subsequently examined for additional tumors in the remaining portions of the intestine. Seventeen percent (8 of 48) of the +/+ mice injected with ENU at 5 to 14 days of age developed tumors by 65 days after treatment (Table 1). In comparison, only 4% (3 of 75) of the +/+ mice treated at 15 to 35 days of age developed intestinal tumors (Table 1). ENU treatment of +/+ mice at 5 to 14 days of age was, therefore, significantly more
the regional distribution of cystic crypts along the length of the intestinal tract was examined by calculating the percentage of total cystic crypt multiplicity in each of the four counted regions of the intestine for untreated groups and each of the ENU-treated groups of Min/+ mice (Fig. 2). ENU treatment did not alter the regional distribution of cystic crypts along the length of the intestinal tract (Fig. 2; χ² test, P > 0.50 for each comparison of untreated animals to the ENU-treated groups). There was also no difference in the regional distribution of cystic crypts between groups of animals treated with ENU at different ages (P > 0.10). The weighted percentage of total cystic crypt multiplicity (based on the number of animals in each group) in each of the four intestinal regions for combined untreated and ENU-treated mice was as follows: proximal small intestine, 58.7%; middle small intestine, 31.1%; distal small intestine, 10.2%; and large intestine, 0% (Fig. 2). No cystic crypts were seen in either untreated or ENU-treated +/+ mice.

The regional distribution of cystic crypts along the length of the intestinal tract was clearly distinct from that seen for intestinal tumors. Comparisons of regional cystic crypt distribution in each of the untreated or ENU-treated groups with the regional tumor distribution in each of these groups revealed a significant difference in each case (P < 0.005; Fig. 2).

Epidermoid Cysts. Epidermoid cysts were first noted in the third set of animals to be treated with ENU. For this reason, mice from the first two rounds of mutagenesis are not included in these analyses. All 126 animals (83 Min/+ from subsequent rounds of ENU treatment were scored for the presence or absence of these lesions at the time of sacrifice.

Min/+ mice treated with ENU had a high incidence of epidermoid cyst development. These pigmented lesions were primarily located in the skin of the back and were often found in association with hair follicles in the subdermal layer of the skin (Fig. 4). Epidermoid cysts were found in mice of both sexes and at all treatment ages. ENU treatment on or before 25 days of age resulted in 60% (40 of 67) of Min/+ mice developing epidermoid cysts. Only 6% (1 of 16) of Min/+ mice treated at 30–35 days of age developed these lesions. Min/+ mice were, therefore, significantly more likely to develop epidermoid cysts when treated with ENU on or before 25 days of age (Student's t test, P < 0.005). The total number of visible cysts per animal varied from one to several dozen. No epidermoid cysts were seen in 36 untreated Min/+ mice that were 70–97 days of age. Of the 43 +/+ mice treated with ENU at 5 to 35 days of age, one mouse treated at 25 days of age developed a single, visible epidermoid cyst. This cyst was histologically similar to lesions seen in Min/+ mice (data not shown).

DISCUSSION

We have demonstrated previously that there is no difference in average intestinal tumor multiplicity for (AKR/J × B6)F, Min/+ mice ranging from 100–300 days of age (10). This result led us to examine the timing of intestinal tumor initiation in Min/+ mice. Here we have described experiments that determined the age-related sensitivity of intestinal tumor initiation by the chemical carcinogen ethylnitrosourea. ENU is a direct-acting, short-lived alkylating agent with a half-life of less than 1 h under the conditions used in these experiments (15). Treatment of Min/+ mice with a single i.p. injection of this carcinogen at 5 to 14 days of age increased intestinal tumor multiplicity 3.8-fold relative to untreated mice (Fig. 1A). ENU treatment at 20 to 35 days of age resulted in only a 1.6-fold increase in intestinal tumors. The short life span of Min/+ mice on the B6 background precluded examination of the effect of treating mice with ENU at older ages. These results demonstrate that ENU-induced
intestinal tumors in Min/+ mice are most likely to be initiated during the first 2 weeks of life. In addition, the tumor multiplicity of untreated Min/+ mice remained unchanged in the interval from 67 to 97 days of age. Mice that did not carry the Min mutation were also more susceptible to intestinal tumor development when treated at 5–14 days of age (Table 1). These results suggest that tumors arising spontaneously in Min/+ mice are also initiated relatively early in life and that this period of enhanced tumor initiation susceptibility is not unique to mice carrying the Min mutation. Our findings are in agreement with those of Laird et al. (16), which suggest that inhibition of polyP initiation in Min/+ mice by treatment with the DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine is most effective when administered before 50 days of age.

The results presented here must be interpreted in the context of what is known about postnatal development of the intestine in the mouse. The intestinal epithelium of both mice and humans consists of a single layer of cells organized structurally into crypts, which are surrounded by villi in the small intestine. It is believed that intestinal tumors originate from stem cells located near the base of each crypt (17). Ponder et al. (18) have shown that, in mice, individual adult crypts are clonal structures. However, intestinal crypts in the mouse actually begin development as polyclonal structures, and clonality is not established until approximately day 14 of postnatal development through a process known as crypt purification (19). Therefore, during the first 2 weeks of life, there may be a larger population of target cells/crypt that could acquire a tumor-initiating mutation.

In addition, the number of intestinal crypts increases by as much as 77% during the third week of life (20–22). The mechanism for this increase seems to involve a process of crypt fission whereby a preexisting crypt splits into two (22–23). A mutation acquired before crypt fission could, therefore, be inherited by multiple crypts.

Developmental changes in stem cell mitotic index and/or cell cycle time could influence the ability of ENU-induced mutations to become fixed. O’Connor (24) has shown that mitotic activity in small intestinal crypts of White Swiss mice from days 13 to 19 after birth is 25% lower than between days 5 and 12. This decrease in mitotic activity could be one explanation for the decline in tumor multiplicity seen in Min/+ mice treated at 15 to 19 days of age relative to mice treated at 5 to 14 days of age. However, mitotic activity at all times during the first 3 weeks of life is lower than that from day 21 to adulthood (20, 24). There is also a gradual decline in the apparent cell cycle time in the small intestine from birth to adulthood (20, 24). These observations suggest that changes in the intestinal mitotic index and/or cell cycle time are not likely explanations for elevated susceptibility of young Min/+ mice to tumor induction by ENU. However, it must be noted that these measurements of mitotic index and cell cycle time in intestinal crypts include both the stem cells and their transitory daughter cells. Direct measurement of these kinetic parameters in the stem cells alone during intestinal development will be required to determine if these factors might influence the timing of intestinal tumor initiation.

Another possible explanation for differences in susceptibility to ENU-induced tumors is an age-related difference in DNA repair systems. Nakatsuru et al. (25) have shown that O6-methylguanine DNA methyltransferase activity in the liver of 3-week-old B6 mice is lower than in 30-week-old mice. Whether there is a correlation between age and ability to repair DNA damage in the intestinal stem cell is not known.

Other factors, including hormonal influences, changes in intestinal microflora, and immunological status, may also be important in the enhanced susceptibility of young mice to intestinal tumor induction. It has been postulated that the immune system might play a role in inhibiting tumor formation by eliminating cells that express tumor-specific antigens (26). Interestingly, very low levels of immunocytes have been reported in the intestine of C57BL/6J mice during the first 2 weeks of life (27). The immunosurveillance capacity of the intestine might, therefore, be partially compromised during this developmental period in the mouse.

Whichever biological factor(s) contribute to the age-related susceptibility to intestinal tumor initiation, it seems clear that the proportion of tumors arising in each region of the intestinal tract is independent of ENU treatment. The increased total tumor number seen in ENU-treated mice was distributed along the length of the intestinal tract in the same proportions as observed in untreated Min/+ mice (Fig. 2). Except for the difference noted for mice treated at 5 to 9 versus 20 to 25 days of age, the distribution of tumor number throughout the intestine in ENU-treated mice was also not affected by the age at treatment (Fig. 2).

ENU is believed to exert its carcinogenic effect by causing point mutations and, to a lesser degree, small deletions (28–29). There are numerous gene targets upon which ENU could be acting to cause an increase in the number of intestinal adenomas in Min/+ mice. One of the earliest somatic genetic events involved in intestinal tumor formation for both humans with familial adenomatous polyposis and Min/+ mice is mutation or loss of the wild-type APC/Apc allele (30–32). In humans, both germline and somatic APC mutations tend to be clustered in the first one-half of the gene, and almost invariably cause truncation of the gene product (33). Intestinal tumors from untreated Min/+ mice all show loss of the chromosome 18 homologue, which carries the wild-type Apc allele (30, 31). In addition to Apc, murine chromosome 18 carries Mcc (mutated in colorectal cancer) and Dcc (deleted in colorectal carcinomas), two other genes for
which loss of function has been implicated in human intestinal cancer (32, 34). Other potential targets for ENU activity include the K-ras proto-oncogene and p53 tumor suppressor gene, which have been shown to be mutated in human intestinal tumors (32), and the Mam-1 (modifier of min) locus, which is known to strongly influence tumor multiplicity in Min-/+ mice (11). Whether ENU treatment results in intragenic mutation of the wild-type Apc allele and/or causes mutations in other loci known to be involved in intestinal tumor formation is currently under investigation.

The relationship between age at ENU treatment and cystic crypt multiplicity was clearly distinct from that seen for intestinal tumors (Fig. 1B). The smallest increase in the number of cystic crypts observed 65 days after ENU treatment occurred in mice treated at 5 to 9 days of age. The average number of cysts was significantly higher for mice treated at all other ages. The relatively small increase in the number of cystic crypts and large increase in the number of tumors for mice treated at 5–9 days of age is consistent with a hypothesis of cystic crypts being converted into neoplastic lesions. However, mice treated at 10–14 days of age had high numbers of both cystic crypts and tumors (Fig. 1). In addition, mice treated at 30–35 days of age continued to develop a high number of cystic crypts, yet tumor multiplicity had declined significantly relative to mice treated at younger ages (Fig. 1). These results argue against a neoplastic fate for intestinal cystic crypts. The distribution of cystic crypts along the length of the intestinal tract was also shown to be significantly different from that seen for intestinal adenomas (Fig. 2).

Oshima et al. (35) have recently reported intestinal lesions, which they term microadenomas, in mice heterozygous for a targeted truncation mutation in the codon 716 of the Apc gene (Apc<sup>716+</sup>). These microadenomas are strikingly similar to cystic crypts in histological appearance; they were also reported to arise predominantly in the small intestine of the Apc<sup>716+</sup> mice. It is noteworthy that this group also reports that microadenomas show loss of the wild-type Apc allele.

Recent studies of small, benign human colorectal lesions revealed two distinct molecular classes. Apc and K-RAS mutations were associated with dysplastic lesions, whereas mutation of K-RAS alone was common in nondysplastic lesions, which seemed to have limited neoplastic potential (36). It will be important to learn if cystic crypts in Min-/+ mice have acquired Apc and/or K-Ras mutations and what the potential fate of these lesions might be.

Min-/+ mice treated before 25 days of age also had a high incidence of epidermoid cysts. Terracini and Testa (43) described similar lesions in (C57BL × C3H)F<sub>1</sub> mice that were treated with methyl nitrosourea at birth, but these lesions were not seen in mice treated at 35 days of age. The high incidence of these lesions in Min-/+ mice treated with ENU before 25 days of age is particularly noteworthy since epidermoid cysts are a common extracranial manifestation in humans with familial adenomatous polyposis (37). Although not considered a health risk, these lesions are often important in the diagnosis of this disease (37). The induction of epidermoid cysts by treatment of Min-/+ mice with ENU suggests that somatic events are required for the development of these lesions. It will be interesting to learn if these somatic event(s) include loss or mutation of the wild-type allele of Apc.

In summary, we have demonstrated that early postnatal life is a time of enhanced susceptibility to intestinal adenoma induction by ENU in both Min-/+ and +/+ mice. ENU treatment of Min-/+ mice also increased the numbers of cystic intestinal crypts, but susceptibility to these lesions remained high up to 35 days of age and their distribution along the length of the intestinal tract was quite different from that seen for intestinal tumors. A high percentage of Min-/+ mice treated with ENU before 25 days of age also developed epidermoid cysts.

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**REFERENCES**


