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Comparison of the Tumorigenic Response of SENCAR and C57BL/6 Mice to Benzo(a)pyrene and the Interexperimental Variability Over a Three-Year Period

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SENCAR and C57BL/6 mice were compared for their ability to produce tumors after benzo(a)pyrene [B(a)P] initiation and 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion. SENCAR mice initiated with 101 μg/mouse B(a)P and promoted with TPA (2 μg/mouse, twice weekly) produced large numbers of papillomas, whereas C57BL/6 mice produced none after 26 weeks of promotion. Continued treatment of the B(a)P-initiated C57BL/6 mice with TPA up to 52 weeks did not induce any papillomas nor did higher doses of B(a)P. Application of increased doses of TPA (10 μg/mouse, twice weekly) to B(a)P-initiated C57BL/6 mice (404 μg/mouse) for 50 weeks produced few papillomas. Substantial papilloma formation in C57BL/6 mice was observed after weekly treatment with B(a)P (101 μg/mouse), with maximal production occurring at weeks 39 to 41 of treatment. In contrast, SENCAR mice treated according to the same protocol produced an equivalent response with maximal papilloma formation occurring 12 to 13 weeks earlier. Therefore, C57BL/6 mice exposed to B(a)P are capable of producing papillomas under certain experimental conditions.

The inter-experimental variability of B(a)P-induced (50.5 μg/mouse) papilloma formation after 30 weeks of TPA promotion (2 μg/mouse, twice weekly) was examined in SENCAR mice over a 37-month period. Low statistical variation was observed in papilloma multiplicity, papilloma incidence, or papilloma latency. Male and female SENCAR mice produced equal values in the three parameters: 4.4 ± 1.6 papillomas/mouse, 87% ± 10% of the mice bearing papillomas, and 9.6 ± 1.3 weeks (time at which 10% of the mice bore papillomas). The numbers of papillomas per mouse did not follow a Poisson distribution.

These data can serve as a reference source for future comparisons of mouse skin tumor initiation results between SENCAR and other strains or stocks of mice and with other chemicals.

Introduction

The SENCAR mouse was originally developed by Boutwell (*1*) by outbreeding Rockland STS mice with Charles River CD-1 mice against a selective pressure of 7,12-dimethylbenz(a)anthracene (DMBA) initation and 12-O-tetradecanoylphorbol-13-acetate (TPA) promotion for eight generations (*2*). Because of its demonstrated increased sensitivity to the tumor-initiating effects of DMBA and benzo(a)pyrene [B(a)P] (*3,4*), this mouse has been the subject of intensive investigations using both complex mixtures and pure chemicals. We have reported in a series of publications the tumor-initiating, tumor-promoting, tumor-coinitiating, and complete carcinogenic properties of a wide variety of complex mixtures, including gasoline exhausts, diesel exhausts, coke oven and roofing tar emissions, and cigarette smoke condensate (*5–8*). In terms of pure chemicals, many polycyclic aromatic hydrocarbons and their derivatives have been examined using the SENCAR mouse bioassay system (*2–11*). Also evaluated have been respiratory carcinogenic metal salts (*8*), as well as a series of known bladder, liver, and lung carcinogens (*12*).

In our studies we had the opportunity to compare the tumor-initiating and complete carcinogenic effects of B(a)P in both SENCAR and C57BL/6 mice. In previous papers we have reported that C57BL/6 mice are insensitive to two-stage initiation-promotion with B(a)P and TPA when the TPA was administered in 4-μg doses, twice weekly (*13*). In the studies reported here, we have reconfirmed this phenomenon and have examined the response of C57BL/6 mice initiated with B(a)P and pro-

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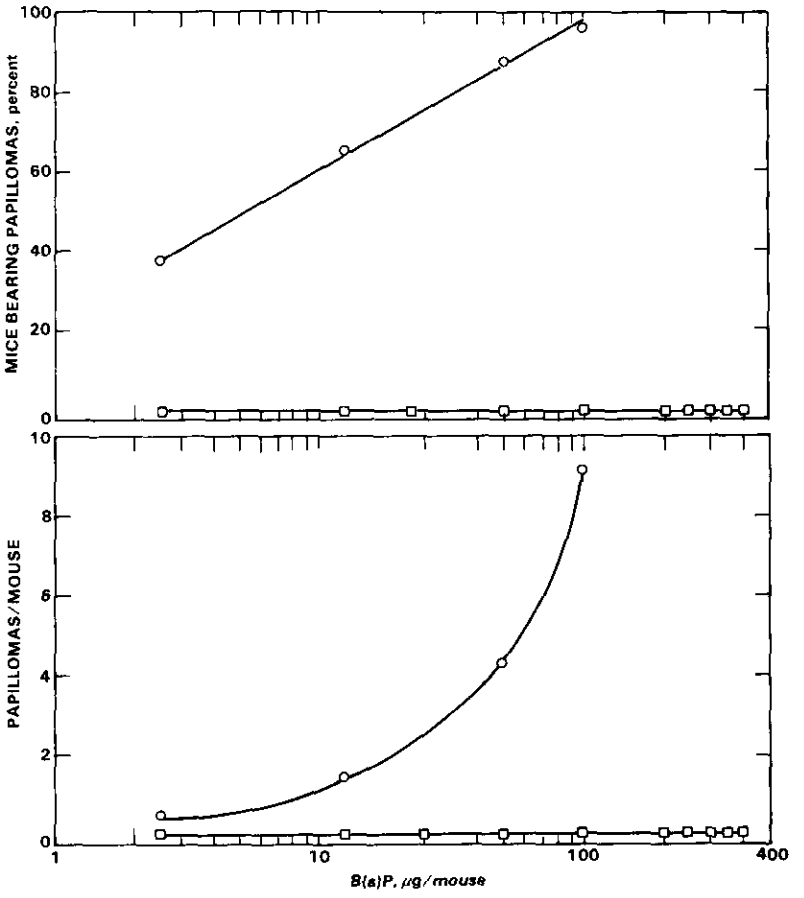

FIGURE 1. Comparison of mouse skin tumor initiation induced by B(a)P in male and female SENCAR and C57BL/6 mice: (○) SENCAR; (□) C57BL/6. Mice were initiated with B(a)P and promoted twice weekly with 2 μg of TPA and scored at week 26.

moted with an increased dose of TPA. Also examined in C57BL/6 mice is the papillomagenic response of B(a)P administered as a complete carcinogen.

We have also examined the interexperimental variation of papilloma response in SENCAR mice over a 37-month period. The results of these experiments are presented and discussed, and these results may provide a reference source for future comparisons with results from other SENCAR mice experiments or for comparisons of responses with other mouse strains or stocks.

Materials and Methods

Seven- to nine-week-old SENCAR mice bred at Oak Ridge National Laboratory or C57BL/6 mice (Charles River Breeding Laboratory) were used. There were 80 animals (40 of each sex) per treatment group. Animals were housed in plastic cages (10 per cage) under yellow light with hardwood chip bedding, fed Purina chow and water *ad libitum*, and maintained at 22–23°C with 10 changes of air per hour. All mice were shaved with surgical clippers 2 days before the initial treatment, and only those mice in the resting phase of the hair cycle were used.

Under the tumor-initiation protocol, B(a)P (Aldrich Chemical Co., Milwaukee, WI) was applied as a single topical treatment in 0.2 mL spectral quality acetone. Beginning one week after treatment, a 2.0-μg dose of TPA (CCR, Eden Prairie, MN) in 0.2 mL acetone was administered topically twice weekly, unless otherwise

Table 1. Mouse skin tumor initiation in C57BL/6 mice by B(a)P using higher doses of TPA.[a]

	Number of mice surviving[b]		Mice bearing papillomas, %[b]		Papillomas/mouse[b]	
Dose, μg/mouse	Male	Female	Male	Female	Male	Female
0	40 (39)	39 (38)	0 (0)	0 (0)	0 (0)	0 (0)
202	39 (37)	38 (35)	3 (22)	5 (11)	0.07 (0.35)	0.05 (0.11)
404	38 (36)	40 (37)	10 (6)	10 (14)	0.10 (0.05)	0.10 (0.13)

[a] Mice promoted with TPA 10 μg, twice weekly, 1 week after initiation.
[b] Mice scored after 26 weeks and after 50 weeks of promotion. Values in parentheses are data after 50 weeks of promotion.

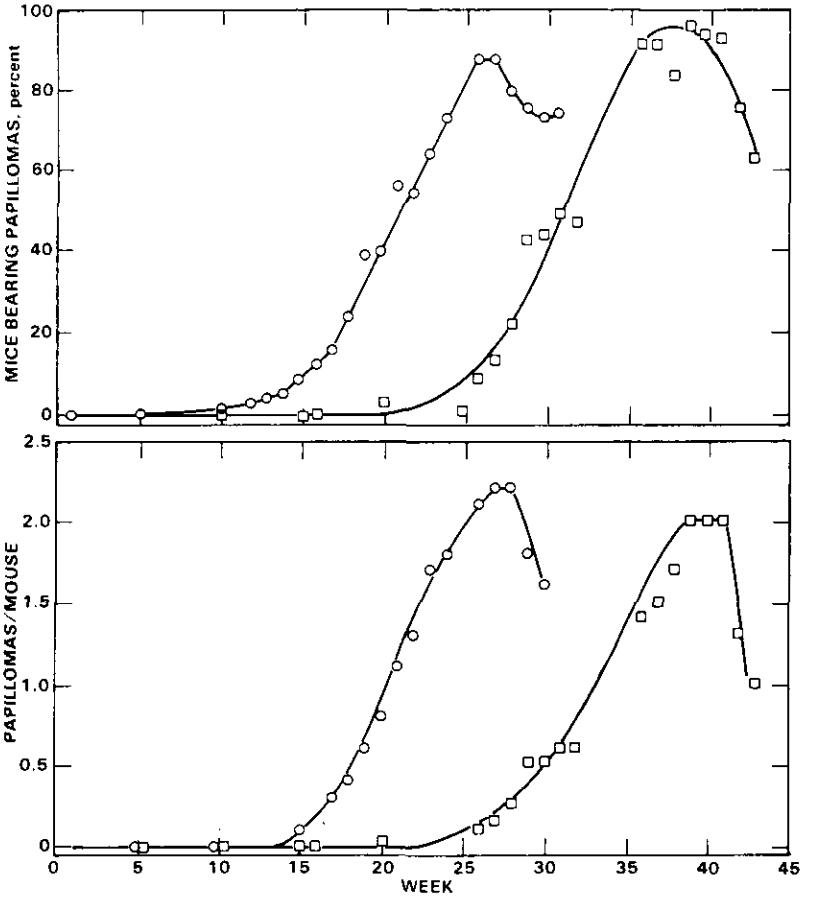

FIGURE 2. Comparison of mouse skin complete carcinogenesis induced by B(a)P (101 μg/mouse administered weekly) in male and female SENCAR and C57BL/6 mice: (○) SENCAR; (□) C57BL/6.

specified. Under the complete carcinogenesis protocol, B(a)P was administered in 0.2 mL acetone weekly for 52 weeks.

Skin tumor formation was recorded weekly, and papillomas greater than 2 mm in diameter and all carcinomas were included in the totals if they persisted for one week or longer. The number of mice with tumors, the number of mice surviving, and the total number of tumors were determined and recorded weekly. At 6 months, the number of papillomas per surviving animal was recorded for statistical purposes. The tumors were histologically verified.

Results and Discussion

Comparison of SENCAR and C57BL/6 Mice

Using tumor initiation as the experimental protocol, mice were initiated with B(a)P over a wide dose range: 0–101 μg/mouse for SENCAR mice and 0–404 μg/mouse for C57BL/6 mice. Survival of both strains/stocks was very high at week 26 with greater than 95% of the mice surviving. Acetone-initiated/TPA-promoted control mice (males and females) gave the following response: SENCAR, 0.07 papillomas/mouse, 7% of mice bearing papillomas; C57BL/6, 0 papillomas/mouse, 0% of mice bearing papillomas.

B(a)P elicited a strong dose-related effect in the production of papillomas in SENCAR mice (Fig. 1), whereas C57BL/6 mice exhibited no papillomas at any dose tested at 26 weeks.

SENCAR mice were extremely sensitive to B(a)P initiation/TPA promotion; 5.4 μg of B(a)P induced tumors in 50% of the mice. The C57BL/6 mice were subjected to additional treatment and were continuously dosed with TPA (2 μg/twice weekly) to week 52. Again, these mice did not exhibit any papillomas (or carcinomas) at any dose, nor did the TPA control group. The minimal survival at 52 weeks for the C57BL/6 mice was 88%. These results reconfirm our earlier findings of the insensitivity of C57BL/6 mice to the tumor-promoting effects of TPA (*13*).

To attempt to induce papillomas in C57BL/6 mice, groups of 40 males and 40 females were initiated with 0, 202, and 404 μg/mouse of B(a)P and promoted with 10 μg of TPA twice weekly for 50 weeks. This dose of TPA was five times that used in the previous experiment (Fig. 1) and 2.5 times the dose reported by Reiners et al. (*13*). Survival was high at both 26 and 50 weeks of scoring with a minimum survival of 88% (Table 1).

Acetone-initiated/TPA-promoted mice again did not

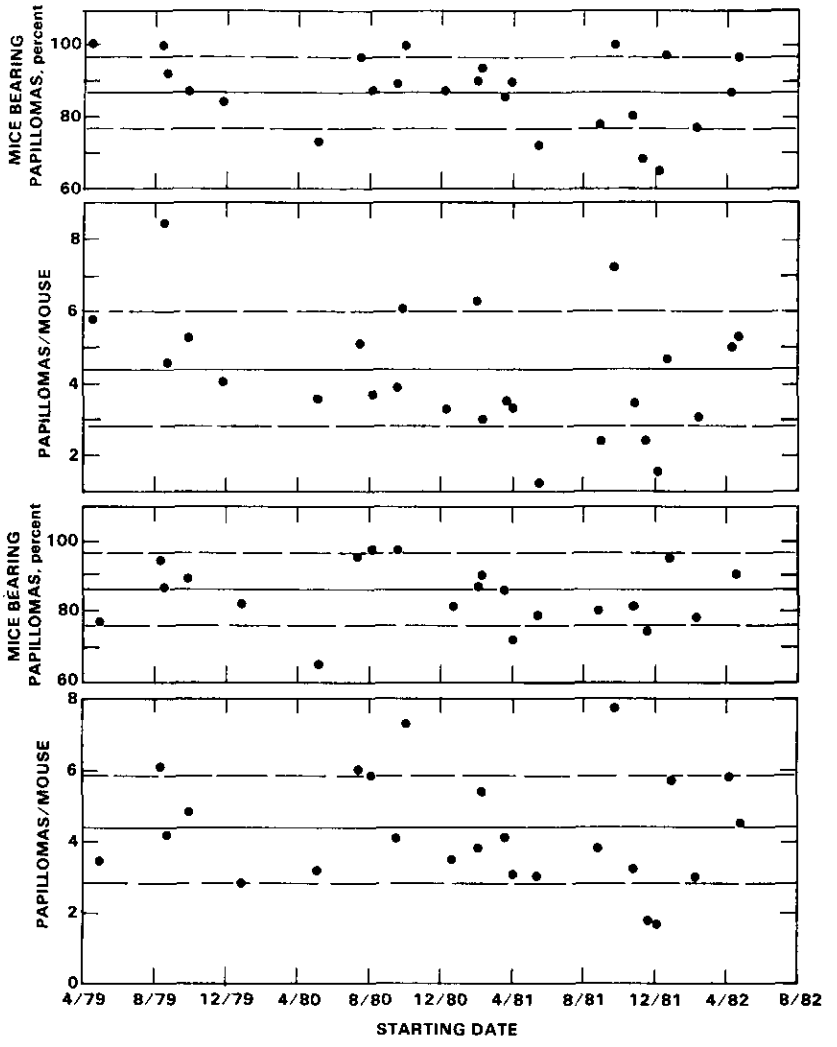

FIGURE 3. Interexperimental variation of skin papilloma incidence and multiplicity in B(a)P-initiated/TPA-promoted SENCAR mice. Male and female mice were initiated with 50.5 μg/mouse of B(a)P and subsequently treated one week later with TPA (2 μg, twice weekly) for 30 weeks. Each data point represents an initial group of 40 mice. Average survival was: male, 95%; female, 93%. Upper panel, male mice; lower panel, female mice. Areas between dashed lines are ± 1 SD.

produce any papillomas or carcinomas. At 26 weeks, a few papillomas were recorded at 404 μg/mouse of B(a)P, and this number did not increase at 50 weeks. The largest number of papillomas was observed in the 202-μg/mouse group at 50 weeks: 0.35 papillomas/mouse, with 22% of the mice bearing papillomas. In previous studies we have demonstrated that C57BL/6 mice initiated with B(a)P are susceptible to the tumor-promoting properties of benzoyl peroxide (*13*). We conclude from the studies reported here and from previous studies that C57BL/6 mice are resistant to the tumor-promoting effects of TPA rather than to the tumor-initiating effects of B(a)P.

Papilloma formation in male and female SENCAR and C57BL/6 mice induced by B(a)P under a complete carcinogenesis protocol was compared (Fig. 2). Mice were treated weekly with 101 μg/mouse of B(a)P. Papillomas were induced to about the same extent in both strains/stocks in multiplicity (~ two papillomas/mouse) and incidence (~ 90% of the mice bearing papillomas). However, the temporal production of tumors was dramatically different. Maximal papilloma formation in SENCAR mice occurred at 27 to 28 weeks, whereas in C57BL/6 mice this peak occurred at 39 to 41 weeks, a shift of 12 to 13 weeks.

In previously reported studies, SENCAR and C57BL/6 mice were topically treated weekly with 12.5, 25.2, and 50.5 μg of B(a)P. Under these experimental conditions, C57BL/6 mice produced few papillomas at any time point, whereas SENCAR mice produced approximately one papilloma/mouse, with 40% of mice bearing papillomas between weeks 30 and 40 of treatment at the highest dose (*13*). The studies reported here extend and augment the published investigations using a two-fold higher dose of B(a)P. Papilloma formation in C57BL/6 mice is clearly evident at the higher dose of

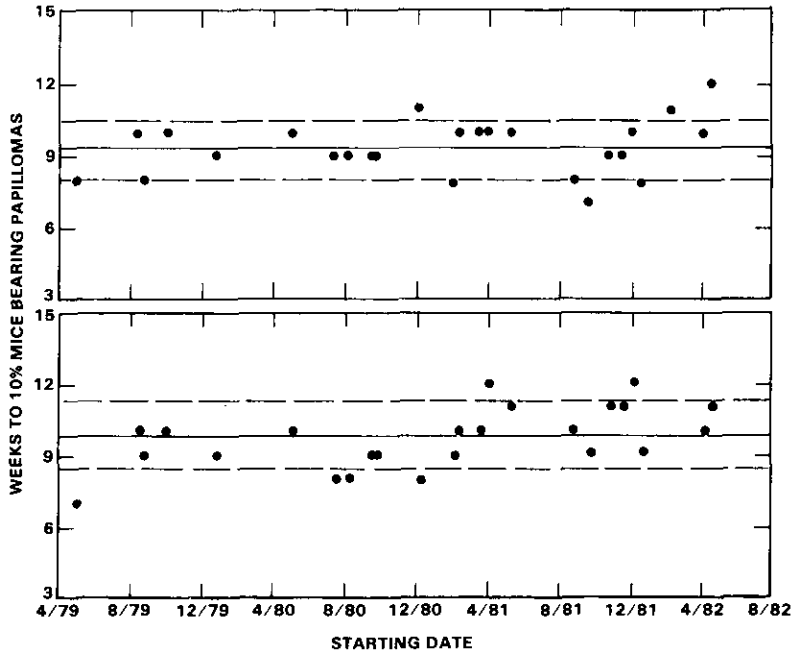

FIGURE 4. Interexperimental variation in skin papilloma latency in B(a)P-initiated/TPA-promoted SENCAR mice: (Upper panel) male mice; (lower panel) female mice. See Fig. 2 legend for experimental details. Areas between dashed lines are ± 1 SD.

B(a)P and is equivalent in both SENCAR and C57BL/6 mice. The reasons for the temporal shift in papilloma formation between SENCAR and C57BL/6 mice are unknown. Several explanations are possible, including increased resistance of C57BL/6 mice to B(a)P-induced papillomagenesis or increased cytotoxic effects of B(a)P on C57BL/6 mouse epidermis with subsequent production of populations of cells resistant to the cytotoxic effects of B(a)P.

Since both SENCAR and C57BL/6 mice produce large numbers of carcinomas after weekly administration of B(a)P (12.5–50.5 μg/mouse/week) in previously reported studies (*14*), the results reported here suggest that production of carcinomas and papillomas may not be directly related in C57BL/6 mice as suggested for other mouse strains.

Interexperimental Variability of B(a)P-Induced Papillomas

A series of 25 experiments with male and female SENCAR mice treated with B(a)P (50.5 μg/mouse) and promoted with TPA (2 μg, twice weekly) were begun over a 37-month period between April 1979 and May 1982. In these experiments, the same suppliers of B(a)P, TPA, and acetone were used, as well as the same barrier conditions and breeding protocols for producing SENCAR mice.

Individual mice were scored at 30 weeks and the results of these studies presented in Figures 3–5. The mean responses (± SD) and ranges for papilloma incidence for male and female mice were 87 ± 10% (65% to 100%) and 86 ± 10% (65 to 100%), respectively (Figs. 3 and 4). The coefficient of variation was the same for both sexes at 12%. Papilloma multiplicity for male and female mice was also equal, 4.4 ± 1.6 papillomas/mouse. The coefficient of variation was 36%. The ranges of papilloma multiplicity for male and female mice were 1.6–8.4 and 1.7–7.7, respectively. In terms of median papilloma incidence scores, male mice exhibited 89% mice bearing papillomas, whereas females exhibited 87%. Median papilloma multiplicity scores were 4.6 and 4.1 papillomas/mouse for male and female mice, respectively.

Skin papilloma latency was determined by measuring the time at which 10% of the mice bore papillomas (Fig. 4). The mean, standard deviation (SD), range, and median values in weeks were: 9.4, 1.1, 7–12, and 10 for male mice and 9.8, 1.4, 7–13, and 10 for female mice. Based on the three parameters—papilloma incidence, papilloma multiplicity, and papilloma latency—equivalent responses and variations were observed for male and female SENCAR mice under these experimental conditions.

Except for a few sporadic points, there seem to be no consistent time-related effects with respect to the production of mouse skin papillomas in SENCAR mice over a 3-year period. The interexperimental variation is relatively small in any of the three parameters measured and probably reflects the consistent breeding scheme used in the production of SENCAR mice.

Skin papilloma distribution in B(a)P-initiated TPA-promoted male and female SENCAR mice was determined from 15 experiments at 30 to 32 weeks of promotion (Fig. 5). A non-Poisson distribution was observed with many mice bearing 0 to 6 papillomas/mouse

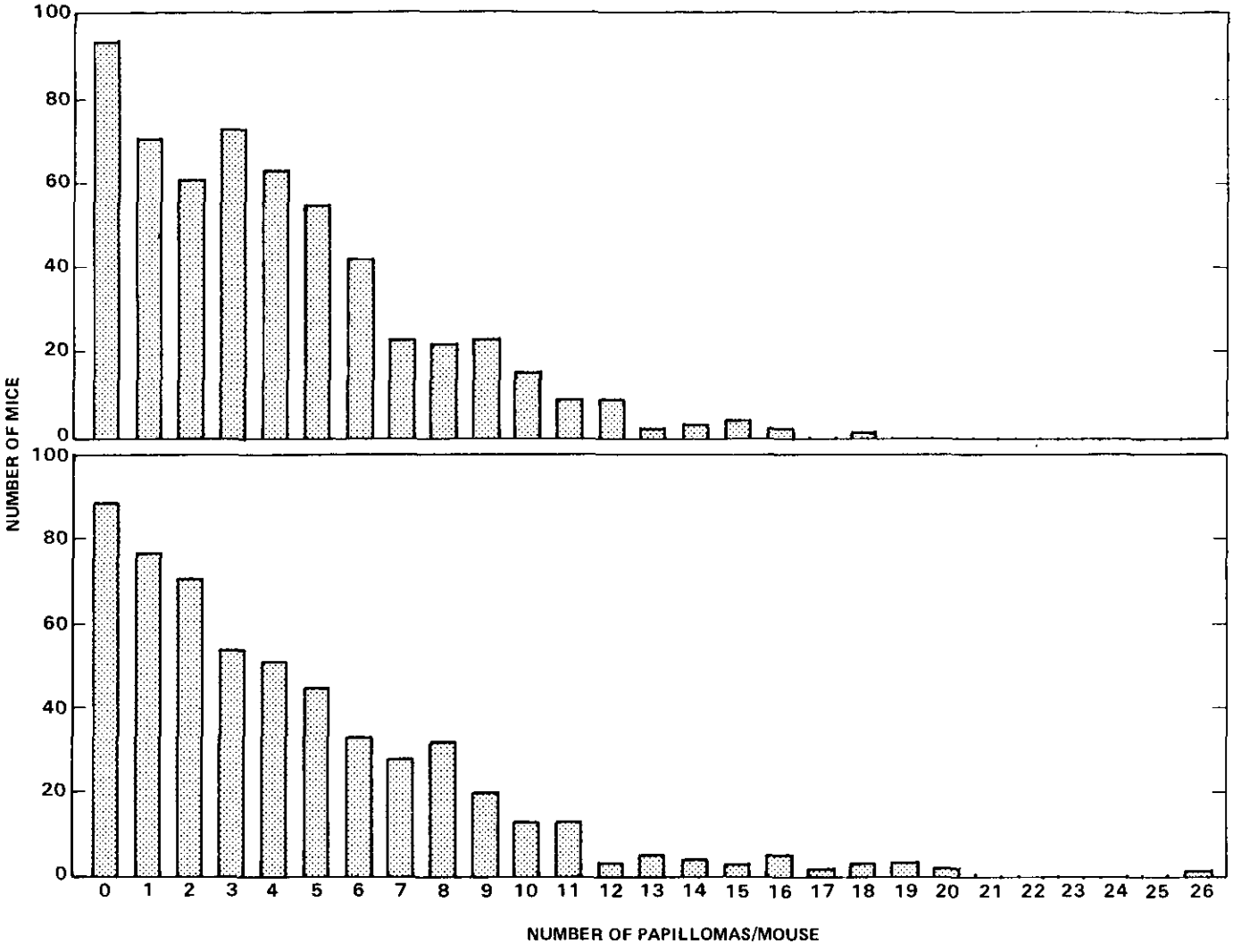

FIGURE 5. Distribution of skin papillomas in B(a)P-initiated/TPA-promoted SENCAR mice: (Upper panel) male mice; (lower panel) female mice. Results presented are combined from 15 experiments with male or female mice in which individual animals were scored at weeks 30 to 32. See Fig. 2 legend for experimental details.

and sporadic occurrences of mice bearing more than 12 papillomas. One mouse had 26 papillomas. Drinkwater and Klotz (*15*) have reported that mouse skin papilloma multiplicity data follow a negative binomial distribution. It is evident from these studies that statistical comparisons using normal or Poisson assumptions cannot be applied to these data. In this regard, we have reported on the use of a log probit analysis with nonzero background (*16*), which can be used to analyze mouse skin papilloma data (*5,6*).

These data provide a useful tool for future comparisons of mouse skin tumor initiation data in SENCAR or other strains or stocks of mice with B(a)P or with other chemicals and can serve as a reference source for this information.

The research described has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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