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Comparison of Two-Stage Epidermal Carcinogenesis Initiated by 7,12-Dimethylbenz(a)anthracene or *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine in Newborn and Adult SENCAR and BALB/c Mice

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ABSTRACT

In order to define factors which determine susceptibility to chemical carcinogenesis, mice sensitive (SENCAR) and resistant (BALB/c) to epidermal carcinogenesis were studied under several treatment conditions for sensitivity to initiation by 7,12-dimethylbenz(a)anthracene or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and promotion by 12-*O*-tetradecanoylphorbol-13-acetate. In newborns of both strains, topical application of initiator was much less effective than in adults. However, initiation by i.p. injection of 7,12-dimethylbenz(a)anthracene is at least as effective in newborns as in adults, which may indicate that topically applied carcinogen is not delivered effectively to target cells in newborns. Thus, newborn epidermis can respond to 7,12-dimethylbenz(a)anthracene as well as adult epidermis when the initiator is appropriately administered. SENCAR mice are much more sensitive than are BALB/c mice to both initiators, which suggests that enhanced metabolic activation of hydrocarbon carcinogens by SENCAR mice is unlikely to account for their sensitivity. Newborn male SENCAR's developed approximately 50% more papillomas than did females in all groups. BALB/c newborn mice developed so few tumors that a meaningful comparison of sensitivity of males and females could not be made. Thus, the increased sensitivity of SENCAR's was apparent regardless of route of administration of initiator or the age or sex of the mice. SENCAR mice also developed a significant number of papillomas and squamous cell carcinomas with 12-*O*-tetradecanoylphorbol-13-acetate promotion in the absence of an exogenous initiator. Therefore, the skin of SENCAR mice may contain an initiated population of cells capable of responding to tumor promoters.

INTRODUCTION

Studies of chemical carcinogenesis in mouse skin have provided major insights into our basic understanding of the processes involved (27). The concepts of multiple stages in neoplasia (initiation-promotion), anticarcinogenesis, and carcinogen metabolism and the involvement of cell proliferation in carcinogenesis have been developed largely from studies utilizing the mouse skin model. Skin carcinogenesis studies have been carried out in several strains of mice; however, direct comparisons of the sensitivity of the skin of different strains to 2-stage chemical carcinogenesis have seldom been made (3). This information is particularly important at present because of the current expanding interest in the process of promotion in mouse skin and other tissues (21) and because conditions of enhanced susceptibility to cancer have been reported for human populations (9).

Little is known regarding the determinants for susceptibility to carcinogenesis in general or to experimental skin carcinogenesis in particular. However, sensitivity appears to reside in the target tissue. The availability of a mouse line (SENCAR) bred specifically for susceptibility to skin tumor initiation and promotion has provided new impetus to study the determinants of susceptibility. SENCAR mice were derived from crossing STS[2] males with Charles River (Charles River Breeding Laboratories, North Wilmington, Mass.) CD-1 females, then breeding those mice which responded maximally to initiation by DMBA and promotion by TPA (7).

In this laboratory, chemical carcinogenesis in the epidermis has been studied by a combined *in vivo-in vitro* approach (27, 29). The major *in vivo* biochemical responses to carcinogens and tumor promoters have been reproduced in epidermal cell cultures (5, 15, 20, 26, 27). Results from studies with epidermal cell cultures prepared from the skin of both newborn and adult SENCAR mice have shown that the growth characteristics of these cells differ from those of BALB/c mice which we have studied extensively (6, 29). There appear to be inherent differences in the susceptible target tissue when separated from the susceptible host.

This study was undertaken to establish the relative sensitivity of SENCAR and BALB/c mice to initiation and promotion by a variety of treatment schemes in newborns and adults. The establishment of this *in vivo* data base allows the design of more meaningful experiments, both *in vivo* and *in vitro*, regarding those factors responsible for susceptibility and resistance to chemical carcinogenesis.

MATERIALS AND METHODS

Animals. SENCAR mice were obtained from Oak Ridge Animal Resources, Oak Ridge, Tenn. BALB/c mice were obtained from NIH Animal Production, Bethesda, Md. For experiments with newborns, pregnant mice were supplied at 14 days of gestation. For experiments with adult mice, females were shipped at 4 to 5 weeks of age.

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[2] The abbreviations used are: STS, skin tumor sensitive; DMBA, 7,12-dimethylbenz(a)anthracene; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; SSV, steroid-suspending vehicle.

Chemicals. MNNG was purchased from Aldrich Chemical Co., Milwaukee, Wis. DMBA was obtained from Eastman Organic Chemicals, Rochester, N. Y. TPA was purchased from Dr. Peter Borchert, Chemical Carcinogenesis, Eden Prairie, Minn. SSV, containing 9 mg NaCl, 5 mg sodium carboxymethylcellulose, 4 μl polysorbate 80, and 9 μl benzyl alcohol per ml solution in water, was provided by Cancer Chemotherapy, National Cancer Institute, NIH, Bethesda, Md.

Tumor Induction Experiments. Groups of approximately 30 male and 30 female newborn mice were treated with DMBA, MNNG, or solvent within 72 hr of birth. Groups of 30 female adult mice were shaved 2 days before treatment with an initiator or solvent at 8 weeks of age. All solutions of initiators were prepared just prior to treatment of the animals. Solutions for topical applications were prepared in acetone with a volume of 20 μl applied to the backs of newborns and 200 μl applied to the shaved backs of adults. Solutions of DMBA for i.p. injection were prepared by dissolving the DMBA in DMSO, then diluting 1–10 with SSV. This solution was administered to newborns in a volume of 25 μl and to adults in 250 μl. Doses of the initiators are indicated in the figures and tables. Promotion with twice weekly applications of 5 μg TPA were begun 7 weeks after initiation in both newborn and adult groups. Because SENCAR mice developed ulcerative skin lesions with this frequency of treatment, TPA application was reduced in all groups to once weekly after 16 weeks of twice weekly applications. Mice were shaved carefully once every 2 weeks during the 45-week promotion phase of the experiment. Papillomas and carcinomas were counted and recorded for individual mice each week throughout the 52 weeks of the experiment. Suspected squamous cell carcinomas were verified by histological examination of the lesions.

Expression of Results. Papilloma data is expressed in the following 2 ways. Percentage with papillomas is the number of mice with one or more papillomas per number of live mice, expressed as a percentage. Papillomas per mouse is the total number of papillomas divided by the number of mice remaining in each group. When expressed in the figures or tables at a particular week, that week was chosen because the papilloma yield was maximal in SENCAR or BALB/c mice. Percentage with carcinomas was calculated from the number of mice which had developed one or more squamous cell carcinomas within 45 weeks of promotion divided by the number of mice alive in each group when the first carcinoma appeared, expressed as a percentage.

RESULTS

Initiation by Topical Application of DMBA. A comparison of the tumor response of SENCAR or BALB/c adult mice to topical application of 4 μg DMBA followed by TPA promotion is shown in Chart 1. The papilloma and carcinoma yields are plotted *versus* weeks of promotion. SENCAR's reached a maximum of 22.6 papillomas/mouse after 11 weeks of promotion, a time at which BALB/c mice had not developed any papillomas. The apparent decrease in papilloma incidence in SENCAR's after 11 weeks appears to be due to difficulty in distinguishing individual papillomas as they coalesce, but papilloma regression cannot be ruled out. In SENCAR's, carcinomas were first seen after 17 weeks of TPA promotion, and by 45 weeks nearly one-half of the animals had developed at least one carcinoma. In BALB/c mice, a maximum papilloma incidence of less than 4/mouse was seen after 45 weeks of promotion. The first carcinoma was noted in BALB/c mice when the experiment was terminated.

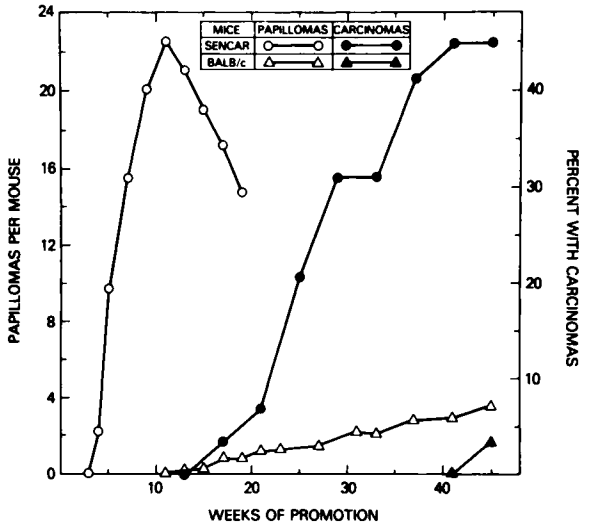

Chart 1. Time course of papilloma and carcinoma development in adults initiated by 4 μg topical DMBA and promoted by TPA. Papillomas per mouse and percentage with carcinomas are plotted *versus* weeks of promotion.

After initiation of adults with a higher dose of DMBA (20 μg) followed by 11 weeks of promotion, the yield of papillomas per mouse was 39.7 in SENCAR's compared to less than one in BALB/c mice. After a further 20 weeks of TPA promotion, 4.8 papillomas/mouse were seen in BALB/c mice. At this dose of DMBA, 47% of the SENCAR's developed carcinomas compared to 13% in BALB/c adults (Table 1).

Initiation of newborn mice with 1 or 5 μg topical DMBA induced many more papillomas in SENCAR than in BALB/c mice (Chart 2). However, the papilloma response after 16 weeks of promotion was considerably lower than that found in adults. With initiation by 1 μg DMBA, 7.8% of the newborn SENCAR's developed carcinomas (Table 1); with 5 μg DMBA, 21.1% with carcinomas were found. No squamous cell carcinomas were observed in BALB/c newborns initiated by topical DMBA (Table 1). SENCAR males developed more papillomas than females (Chart 2), but this difference was not seen with carcinomas (data not shown). The greatly reduced papilloma yield in newborns compared to adults suggests that either the target cells are less responsive in newborns or that the topically applied DMBA may be delivered to the target cells less effectively in the newborns.

Initiation by i.p. Injection of DMBA. In order to verify the results found with topical DMBA initiation, initiation was carried out by DMBA administered i.p. Since the solvent used previously (10) proved to be toxic to newborns, DMBA was suspended in SSV (see "Materials and Methods"). Preliminary toxicity experiments indicated that most newborns were killed by 30 μg DMBA i.p., while all survived an injection of 20 μg DMBA. This dose of DMBA corresonds to 8.7 μg/g body weight of the SENCAR or BALB/c newborn mice which averaged 2.3 g (1.5 to 3.1 g). Adults were initiated by injection of 300 μg DMBA, a nontoxic dose which had been shown previously to be effective as an initiator (10). Since the average weight of the adult SENCAR's was 28.5 g, the DMBA dose was 10.5 μg/g body weight. The BALB/c adults, weighing only 18 g, were

Table 1

Squamous cell carcinoma response in mice initiated with DMBA or MNNG and promoted with TPA

The cumulative yield of carcinomas after 45 weeks of promotion is shown. Since no significant differences in response between male and female newborns were noted, the carcinoma results for males and females are pooled. Carcinoma data for newborns initiated with MNNG are shown in Table 2 and for all solvent controls in Table 3.

Mice	Age	Initiator	Dose (μg)	Route of administration	No. of mice	Mice with carcinomas	% with carcinomas
SENCAR	Adult	DMBA	4	Topical	29	13	45
SENCAR	Adult	DMBA	20	Topical	30	14	47
BALB/c	Adult	DMBA	4	Topical	30	1	3
BALB/c	Adult	DMBA	20	Topical	30	4	13
SENCAR	Newborn	DMBA	1	Topical	51	4	8
SENCAR	Newborn	DMBA	5	Topical	57	12	21
BALB/c	Newborn	DMBA	1	Topical	60	0	0
BALB/c	Newborn	DMBA	5	Topical	58	0	0
SENCAR	Adult	DMBA	300	i.p.	24	10	42
BALB/c	Adult	DMBA	300	i.p.	24	2	8
SENCAR	Newborn	DMBA	20	i.p.	52	11	21
BALB/c	Newborn	DMBA	20	i.p.	53	0	0
SENCAR	Adult	MNNG	120	Topical	30	3	10
SENCAR	Adult	MNNG	600	Topical	26	6	23
BALB/c	Adult	MNNG	120	Topical	30	2	7
BALB/c	Adult	MNNG	600	Topical	29	6	21

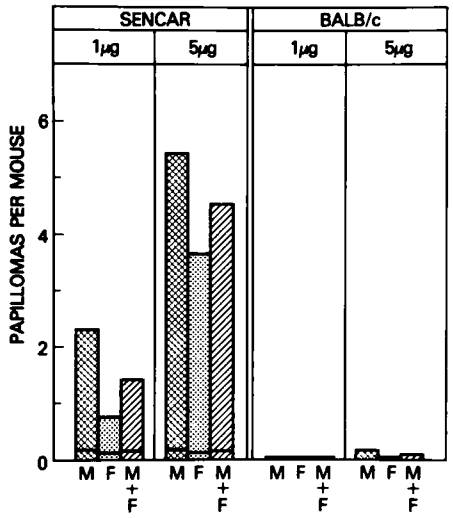

Chart 2. Papilloma incidence in SENCAR or BALB/c mice initiated by 1 or 5 μg topical DMBA as newborns and promoted with TPA. Results are shown in males (*M*), females (*F*), and as an average (*M* + *F*) after 16 weeks of promotion. The *horizontal line* near the baseline on each *column* indicates the papilloma response to TPA in mice treated once with acetone as a solvent control.

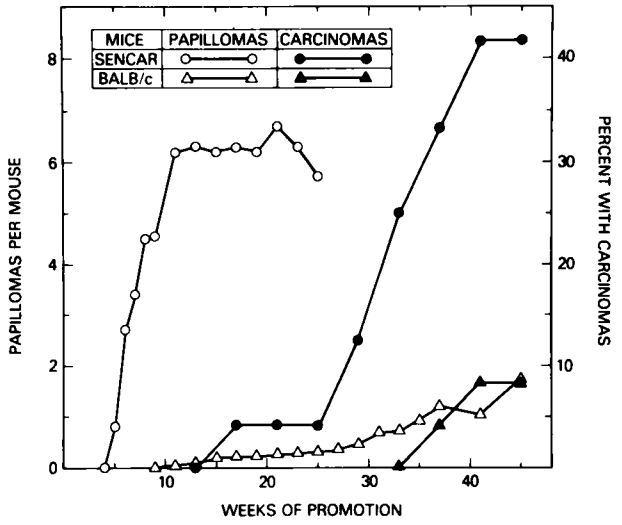

Chart 3. Time course of papilloma and carcinoma development in SENCAR and BALB/c mice initiated as adults by i.p. injection of 300 μg DMBA and promoted by TPA. Papillomas per mouse and percentage with carcinomas are plotted *versus* weeks of promotion.

injected with 16.7 μg DMBA/g body weight. While the DMBA doses were not strictly comparable, rough comparisons can be made between SENCAR's and BALB/c's and between adults and newborns.

In adults, SENCAR's reached a maximum response of 6 to 7 papillomas/mouse after 11 weeks of promotion (Chart 3). At this time, the first papilloma was observed in BALB/c adults. A maximum papilloma yield of 1.5 to 2/mouse was seen in BALB/c adults only after 35 to 45 weeks of promotion. In SENCAR's, the first carcinoma was seen at 17 weeks; 42% of the mice had developed carcinomas when the experiment was terminated. The first carcinoma developed in BALB/c mice after 37 weeks, with carcinomas in 8% of the mice after 45 weeks of promotion.

Initiation of SENCAR newborns with DMBA i.p. resulted in 13.8 papillomas/mouse in males and 8.2 in females after 16 weeks of promotion (Chart 4). Combining the data for both sexes, 54 of 55 mice developed at least one papilloma, with an average of 11.1 papillomas/mouse. In the SENCAR newborns, 11 mice (21.2%) developed squamous cell carcinomas by 45 weeks (Table 1). In this group only, 9 suspected carcinomas had not invaded the muscle layer and thus were scored as "papillomas" since invasiveness remained within the dermis. These lesions probably represent early squamous cell carcinomas, which would increase the carcinoma yield to nearly 40%. Carcinoma development in these mice initiated by DMBA i.p. as newborns appears to be delayed when compared to mice initiated by DMBA i.p. as adults. DMBA initiation i.p. of

BALB/c newborns produced one or more papillomas in only 26% of the mice or 0.5 papilloma/mouse. No carcinomas were seen in these BALB/c newborns (Table 1).

With initiation by DMBA i.p., SENCAR adults and newborns are much more sensitive than BALB/c's, as we had found with initiation by topical DMBA. In contrast to the results with topical DMBA, i.p. initiation was at least as effective in newborns as in adults. Thus, cells of the newborn epidermis can be initiated as readily as adult epidermis if the initiator is delivered in an appropriate way.

Initiation by Topical Application of MNNG. We had demonstrated previously that MNNG, a carcinogen not requiring metabolism, could accomplish initiation in mouse skin (4) and that the proliferative rate of the epidermis could alter the efficacy of MNNG (4, 12). Therefore, MNNG was chosen for a comparison of its initiating ability in newborn and adult SENCAR and BALB/c mice.

SENCAR adults initiated by 120 μg MNNG and promoted for 13 weeks developed 1.9 papillomas/mouse (Chart 5); with 600 μg MNNG, the papilloma incidence was 2.8. Papillomas in BALB/c adults arose considerably slower than in SENCAR's, but by 35 weeks BALB/c mice treated with 120 or 600 μg MNNG had developed 0.53 or 1.2 papillomas/mouse, respectively (Chart 5). Surprisingly, the carcinoma incidence after the higher MNNG dose (Table 1) was nearly as high in BALB/c adults (21%) as in SENCAR's (23%).

Newborn mice were relatively insensitive to initiation by MNNG (Table 2). MNNG doses of 30, 150, or 600 μg were completely ineffective in BALB/c newborns. In SENCAR newborns, only with the highest MNNG dose was the tumor yield increased relative to solvent controls.

Tumor Induction by TPA Promotion in Noninitiated Mice. Although no mice were treated with TPA only, it is likely that mice treated once with the initiator solvent (either SSV or acetone) followed by TPA promotion developed tumors solely as a result of the repeated TPA treatment. As shown in Table 3, only 4 of 262 TPA-treated BALB/c mice developed papil-

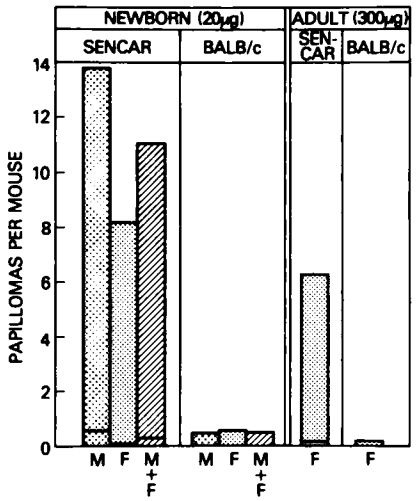

Chart 4. Papilloma response to initiation by i.p. injection of DMBA in newborn or adult SENCAR or BALB/c mice followed by TPA promotion. Papillomas per mouse after 16 weeks of promotion are plotted separately for males (*M*) and females (*F*); the results are averaged for both sexes (*M* + *F*). The *horizontal line* near the baseline indicates the papilloma response to TPA in mice injected i.p. with the solvent SSV. The papilloma yield in mice initiated as adults is shown for comparison to newborns.

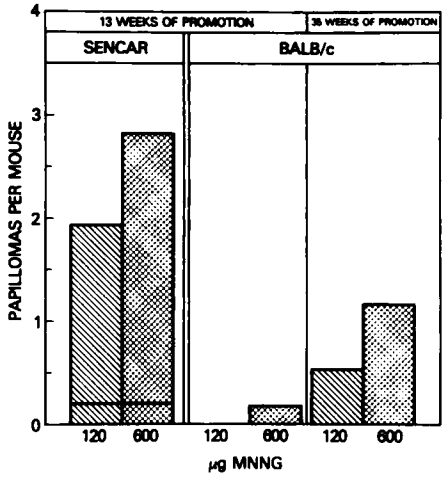

Chart 5. Papilloma response in adult SENCAR and BALB/c mice after initiation with 120 or 600 μg MNNG followed by TPA promotion. The papilloma yield is shown after 13 weeks of promotion, the time of maximal response in SENCAR's, and after 35 weeks, the time of peak response in BALB/c mice. The *horizontal line* near the baseline in SENCAR's indicates the papilloma response to TPA in mice treated once with acetone as a control for MNNG application.

Table 2

Tumor response in newborns initiated with MNNG and promoted with TPA

Groups of 48 to 61 newborn SENCAR or BALB/c mice were treated once with acetone or one of the 3 indicated doses of MNNG. Papilloma and carcinoma data are presented for 28 and 45 weeks of promotion, respectively.

Mice	Topical treatment	No. of mice	Mice with papillomas	% with papillomas	No. of papillomas	Papillomas/mouse	% with carcinomas
SENCAR	Acetone	51	13	26	22	0.43	0
SENCAR	MNNG (30 μg)	51	12	24	13	0.25	0
SENCAR	MNNG (150 μg)	54	15	28	17	0.31	2
SENCAR	MNNG (600 μg)	48	20	42	41	0.85	4
BALB/c	Acetone	55	2	4	2	0.04	0
BALB/c	MNNG (30 μg)	57	0	0	0	0	0
BALB/c	MNNG (150 μg)	54	2	4	2	0.04	0
BALB/c	MNNG (600 μg)	61	1	2	1	0.02	0

Table 3

Papilloma and carcinoma response to TPA promotion in noninitiated solvent-treated SENCAR or BALB/c mice

The maximum number of papillomas observed at any time during the 45 weeks of promotion is shown.

Mice	Age when solvent treated	Sex	No. of mice	Mice with papillomas (maximum)	% with papillomas	% with carcinomas
SENCAR	Newborn	M	74	21	28	4
	Newborn	F	85	15	18	4
	Adult	F	90	21	23	2
			249	57	22	3
BALB/c	Newborn	M	82	1	1	0
	Newborn	F	90	2	2	0
	Adult	F	90	1	1	0
			262	4	2	0

lomas, with none developing carcinomas. In contrast, papillomas were observed in nearly 25% of the TPA-treated SENCAR's, with 8 carcinomas developing in 249 mice.

DISCUSSION

In the early 1960's, Boutwell (3) carried out a selective breeding program based on skin tumor response to a 2-stage tumor induction regimen. Initiation by a single topical application of DMBA was followed by croton oil or TPA promotion, and mice were selected for breeding based on the rapid induction of large numbers of skin tumors. Selection for several generations led to the STS line of mice (3). The breeding of STS males with Charles River CD-1 females, followed by frequent challenges and selections for sensitivity, has led to the DMBA-TPA-sensitive line of mice now called SENCAR (7). The SENCAR's respond to topical DMBA initiation and TPA promotion (7, 16) with a much higher tumor yield than the STS mice (1, 3, 10) and Charles River CD-1 mice from which they were derived (7). In our experiments (Chart 1), topical initiation of SENCAR's by 4 μg DMBA followed by TPA promotion induced over 22 papillomas/mouse; with 20 μg DMBA, nearly 40 papillomas/mouse were found. Baird and Boutwell (1) reported 11 papillomas/STS mouse with an initiating dose of 51.2 μg DMBA.

The resistance of BALB/c mice to 2-stage skin carcinogenesis was first reported by Walters and Roe (24). To test DMBA as an initiator in BALB/c mice, Walters and Roe injected newborns s.c. with 5, 15, or 45 μg DMBA, injected 8-week-old mice with a total of 180 μg DMBA, or painted 12-week-old mice with 150 μg DMBA. After 40 weeks of promotion with croton oil, fewer than 20% of the mice in any of the groups had developed papillomas. In contrast, Chester Beatty stock mice were clearly initiated by 45 μg DMBA injected in newborns or topical application of 150 μg DMBA to adults.

We have shown here that BALB/c newborns are not initiated by topical application of MNNG (Table 2) and developed only 5 tumors in 58 animals treated topically with 5 μg DMBA (Chart 2). Injection i.p. of DMBA into newborn and adult BALB/c's induced a low but finite number of papillomas (Chart 4) when followed by TPA promotion. Topical application of DMBA (4 or 20 μg) to BALB/c adults was most effective as an initiator, producing tumors in more than 90% of the animals, with a maximum of nearly 5 papillomas/mouse at the higher DMBA dose. Repeated treatment for 45 weeks with TPA alone induced papillomas in only 1.5% of BALB/mice (Table 3). The use of TPA instead of croton oil for promotion could explain the higher tumor incidence in our studies compared to those of Walters and Roe (24).

Although BALB/c mice were less sensitive than SENCAR's to the initiation-promotion regimen used here, they are not generally resistant to skin tumor induction. Bibby and Smith (2) induced squamous cell carcinomas in 12 of 30 BALB/c males after 20 weeks of repeated 3-methylcholanthrene applications. Kripke (14) has shown that skin tumors induced by repeated UV irradiation developed much more rapidly in BALB/c than in C3H or C57BL/6 mice. Thus, the low sensitivity of BALB/c mice to 2-stage carcinogenesis may be related to their resistance to promotion by TPA. However, tumor induction experiments with repeated MNNG or DMBA treatments (19) would be required to exclude the possibility that BALB/c mice are resistant to initiation by MNNG or DMBA.

The unusual sensitivity of adult SENCAR's to initiation by topical DMBA has not yet been explained experimentally. It is clear, however, that an alteration in oxidative metabolism of DMBA (8) is not involved. High-pressure liquid chromatography profiles of DMBA metabolites were qualitatively and quantitatively similar when DMBA was incubated with epidermal homogenates from SENCAR, CD-1 (intermediate sensitivity), and RECAR (resistant) mice.

The early work of Suntzeff *et al.* (23) demonstrated that newborn mice were insensitive to carcinogenesis by a single painting with a 3-methyl-cholanthrene solution which produced squamous cell carcinomas in 10- to 12-week-old adults. Thus, our result that topically applied DMBA was considerably less effective as an initiator in newborn SENCAR's than in adults (Chart 2) was not unexpected. The nearly complete refractoriness of BALB/c newborns demonstrates the value of a sensitive strain such as SENCAR in assessing the initiating ability of a low dose of DMBA or other carcinogens.

Based only on the results with topical initiation by DMBA, one would infer that the epidermal cells of newborn mice are less susceptible to the initiating change induced by DMBA. However, the thymidine labeling index of newborn rodent epidermis is 2 to 3 times that of adults (20), and a large body of evidence (4, 10, 12, 17) suggests that initiation is enhanced under conditions of increased cellular proliferation. Thus, newborn epidermis would be expected to be more sensitive. The structure of newborn skin with a greatly thickened epidermis with few hair follicles and sebaceous glands (23) could prevent the topically applied DMBA from reaching the potential initiated cells. Therefore, administration of DMBA by another route might be necessary to accurately assess the sensitivity of newborn epidermal cells.

Injection i.p. of 300 μg DMBA was chosen because of our earlier experience with this route of administration (10). A correspondingly lower dose of DMBA (20 μg) based on the weight of the newborn mice was utilized for comparison of newborns with adults. As shown in Chart 4, newborn female SENCAR's developed 8.2 papillomas/mouse, compared to 6.3 in adult females. Thus, the epidermis of newborns is at least as sensitive to DMBA initiation as adult epidermis.

In order to determine whether sensitivity of SENCAR mice was evident with agents other than polycyclic hydrocarbons, MNNG, an initiator chemically unrelated to DMBA, was tested. MNNG was 2 to 3 times more effective in adult SENCAR (Chart 5) than in BALB/c or in Charles River CD-1 mice (4). Papillomas developed rapidly, with a near maximum papilloma incidence after only 12 weeks of promotion. However, the maximum yield of less than 3 papillomas/mouse after 600 μg MNNG demonstrated that the mice were not exceptionally susceptible to papilloma formation after initiation by MNNG. Of interest, however, was the observation that both BALB/c and SENCAR mice developed significant numbers of carcinomas when MNNG was the initiator (Table 1). Newborns were quite resistant to MNNG initiation for both papilloma and carcinoma induction, although SENCAR's developed a few malignant lesions (Table 2). These results suggest that the biology of carcinogenesis with MNNG as initiator may differ from that with hydrocarbons as initiators. Alternatively, the exceptional sensitivity of SENCAR mice to papilloma formation may be limited to specific classes of initiators.

Of 249 SENCAR's treated repeatedly with TPA but no initiator, 57 developed at least one papilloma (Table 3). In these SENCAR's, 3.3% developed carcinomas. Papillomas were seen on only 4 of 262 BALB/c mice treated with TPA. No carcinomas were observed in TPA-treated BALB/c mice. The tumors developing after TPA alone (13) may indicate the weak complete carcinogenic action of TPA in SENCAR's. Alternatively, TPA may be acting as a pure promoter and indicate a high incidence of "spontaneous" initiation in SENCAR's.

The striking difference between SENCAR and BALB/c mice in susceptibility to DMBA-initiated TPA-promoted skin carcinogenesis suggests the importance of a detailed comparison of cultured epidermal cells from these mice in order to separate host factors from target tissue susceptibility. Preliminary experiments comparing morphology and growth characteristics of SENCAR and BALB/c epidermal cells in culture have indicated no major differences between the cells, although [^{3}H]thymidine incorporation and the thymidine labeling index is somewhat higher in SENCAR's.[3] In transformation experiments, a higher level of morphologically transformed colonies capable of being subcultured was consistently found in SENCAR control and MNNG-treated cultures (6) compared to BALB/c cultures. A similar result was obtained in an assay for DMBA- or MNNG-induced variants after induction of terminal differentiation by calcium[4] (11, 29). The presence of a preexisting population of TPA-responsive cells in adult SENCAR (but not in BALB/c) epidermis has been suggested by the continued growth in TPA-containing medium of adult epidermal cells cultured after treatment with TPA for 4 weeks *in vivo* (29).

The above findings in epidermal cell culture suggest that the basis for susceptibility of SENCAR mice resides in the target tissue rather than in the host. However, the recent finding of renal changes in aging SENCAR's which are characteristic of lupus erythematosis (25) indicates that this strain develops an alteration in their immune system. Such an alteration could affect their susceptibility to carcinogenesis (18). SENCAR mice appear to be more responsive to both the initiation and promotion phases of carcinogenesis. They are much more sensitive than BALB/c mice to initiation by either DMBA or MNNG followed by promotion with TPA (Charts 1 to 5; Tables 1 and 2) and to TPA promotion alone (Table 3). Selective breeding for sensitivity to initiation and to promotion separately[5] could yield other potentially useful lines of mice. Thus, combinations of *in vivo* and *in vitro* approaches in genetically susceptible and resistant strains of mice can be applied to understanding the determinants of sensitivity or resistance to chemical carcinogenesis. Such approaches should lead to new insight into the cellular basis for susceptibility and ultimately to the mechanisms involved in carcinogenesis.

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