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Spontaneous Tumors in SENCAR Mice

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A total of 305 female and 36 male SENCAR mice in six different groups were observed for 540 to 875 days for tumors as well as nonneoplastic lesions. Three of these groups were observed for their lifespans. The 123 females and 36 males, observed for their lifespans (800 to 875 days) showed median survival times ranging from 730 to 745 days. The average peak body weights were 42.4 g for females and 50.0 g for males. In the animals observed for their lifespans, the total tumor incidence was 65.6% for females and 69.4% for males. Both sexes showed a high incidence of papillary tumors of the lung, 16 to 39%, which was independent of aging. Females showed a 5.1 to 21.0% incidence of mammary tumors, which increased with age. Males showed a high incidence of hyperplastic nodules of the liver, i.e., 12 of 36 animals. Other frequently occurring tumors were leukemia and papillomas of the forestomach.

Introduction

In a companion report from this laboratory (*1*), we described the use of female SENCAR mice in the bioassay of chemicals for cocarcinogenic and tumor-promoting activity and, in some instances, compared the skin tumor responses in these animals with those observed in our earlier work with female ICR/Ha mice (*2*).

During the course of the above work, and including earlier trial experiments with SENCAR mice (*3*), we have accumulated some background information on the incidence of spontaneous tumors (benign and malignant) and nonneoplastic lesions in SENCAR mice. In these no-treatment control groups, 305 females and 36 males were observed in six different experiments for periods ranging from 540 to 875 days.

Because of the current interest in the use of these mice for skin carcinogenesis assays, which was the main topic of the conference at which the above paper (*1*) was presented, it was considered useful to present our findings on the incidence of spontaneous lesions in SENCAR mice.

The findings reported here were accumulated during the course of mouse skin cocarcinogenesis research. Hence, we do not have the detailed results that would have been produced from a protocol designed for the purpose of studying spontaneous tumors in SENCAR mice.

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Table 1. Groups of mice observed for spontaneous lesions.

Group number and sex	Number of mice: At start	Number of mice: Necropsied	Median survival time, days	Duration of observation, days
1 (female)[a]	60	59	>540	540
2 (female)[a]	65	63	>600	600
3 (female)[a]	57	56	>605	605
4 (female)[b]	80	78	745	800
5 (male)[b]	36	36	750	815
6 (female)[b]	43	41	730	875

[a] Mice were sacrificed at end of experiment. Mice were 4 to 7 weeks old at the beginning of experiment and were sacrificed 540 to 605 days after the beginning of experiment (see last column).
[b] Mice were kept to end of lifespan.

Methods

All mice were obtained from Oak Ridge National Laboratories (Oak Ridge, TN). They were used as no-treatment control groups for concurrently running skin cocarcinogenesis and two-stage experiments, some of which have been reported earlier (*1,3*). The six groups of mice represent animals used over a period of 7 years in this laboratory. They were 4 to 7 weeks of age at time of arrival in the laboratory. Animals were allocated randomly and numbered by toe-clipping during the week of arrival. After one week, they were assigned to experimental groups as no-treatment controls. Mice were housed, five per stainless steel cage, on heat-treated hardwood chip bedding. Males and females were housed in separate animal rooms, and males showing aggressive behavior were separated and housed individually. Rooms were maintained at 68 to 72°F and 50% relative humidity. Fluorescent lighting used was on a

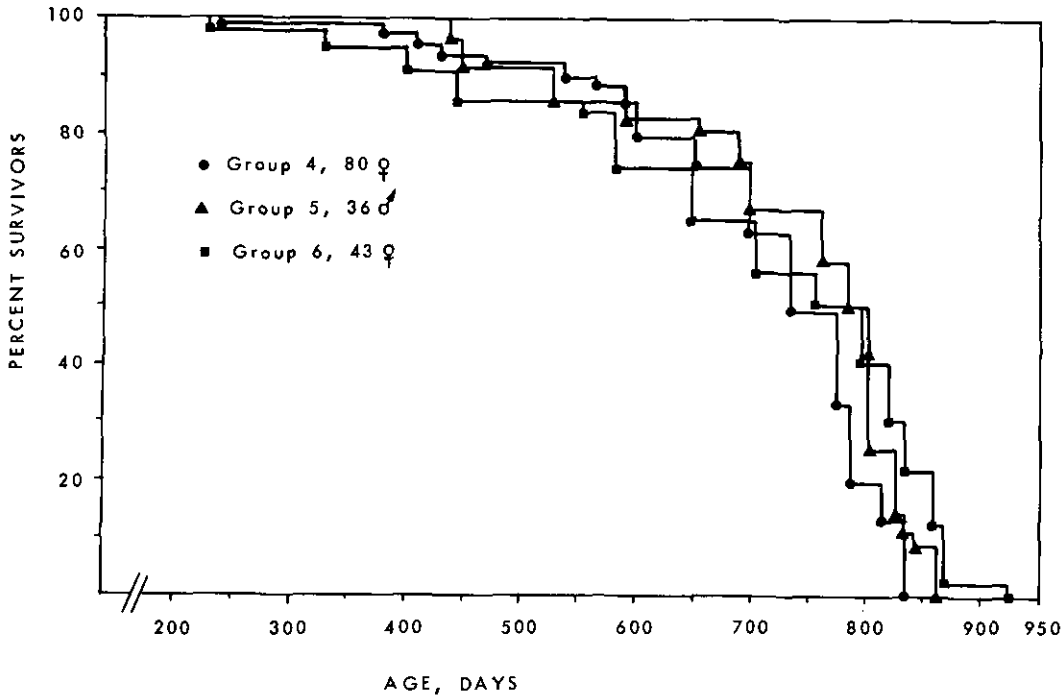

FIGURE 1. Survival curves for SENCAR mice groups 4 to 6.

12-hr on-off cycle, and fresh air was supplied at the rate of 12 to 15 changes per hour. The animals had free access to Purina Rodent Chow (Number 5001) and tap water. The six groups and relevant information are given in Table 1.

Groups 1 to 3 were matched with skin application groups and hence were killed at the termination of the experiment. Groups 4 to 6 were kept to the end of their lifespans.

Animals in moribund condition were killed. Necropsies did not include the cranium, and routine sections of tissues were not taken for groups 1 to 3. Only those tissues which appeared abnormal by gross examination were taken for histopathology. In groups 4 to 6, routine sections of the following clinically normal tissues were taken in addition to abnormal tissues: lung, liver, kidney, stomach, spleen, and skin. Tissues were fixed in formalin and stained with hematoxylin and eosin; special stains were not used.

Results

Survival

The survival curves for groups 4, 5, and 6, which were allowed to live out their lifespans, are shown in Figure 1. The median survival times for these three groups are shown in Table 1.

Body Weight

Body weights of the six groups of animals are shown in Figure 2. The average peak body weight was 42.4 g for females, and peak body weight for the one group of males was 50.0 g.

Tumors

Types of tumors observed are presented in Table 2, along with the number and percentage of mice in each group with each type of tumor. The mammary tumors were defined by several criteria. Tumors were classified as fibroadenomas if proliferation of both the epithelium and stroma was present and no necrosis was observed. If necrosis was present, then the tumors were designated as adenocarcinomas. If the tumor could not be classified as either a fibroadenoma or an adenocarcinoma, the generic term mammary tumor was used. These latter tumors are designated as unclassified tumors in Table 2.

Nonneoplastic Lesions

Nonneoplastic lesions were seen in all six groups of SENCAR mice examined and are listed in Table 3. With the few exceptions noted below, these lesions were all clinically apparent at necropsy and thus represent a conservative number. In the males, group 5, cystic dilatation of the seminal vesicles was probably an age-related lesion. The changes seen in the male skin and some of the inflammatory changes seen in the kidneys may have been attributable to amyloidosis. We did not use special stains for amyloid, since nonneoplastic lesions were not of prime interest in our study.

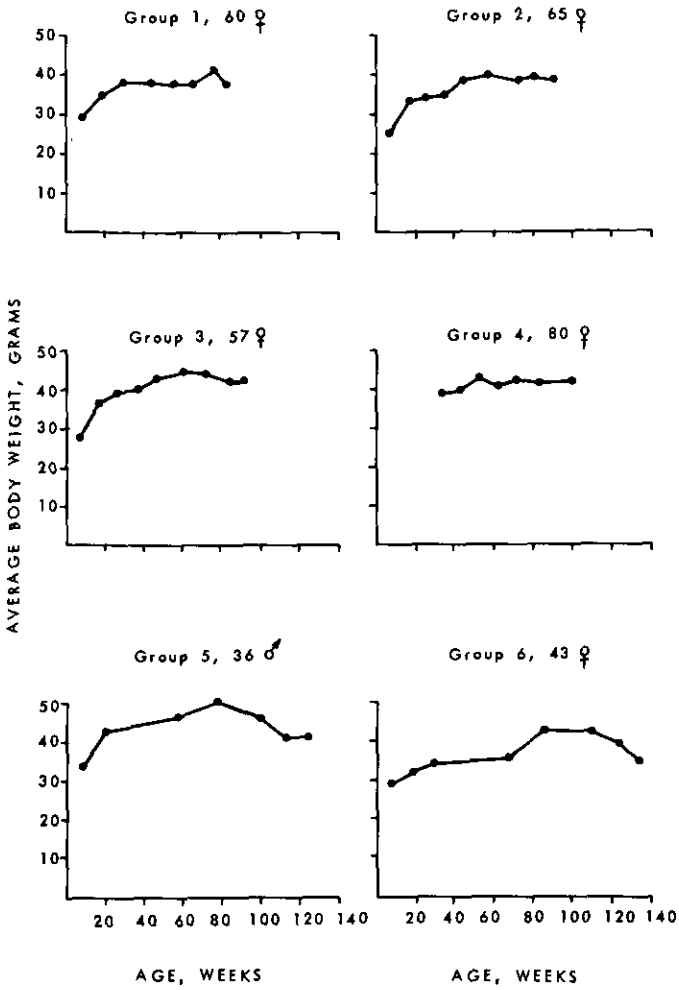

FIGURE 2. Average body weights of SENCAR mice groups 1 to 6.

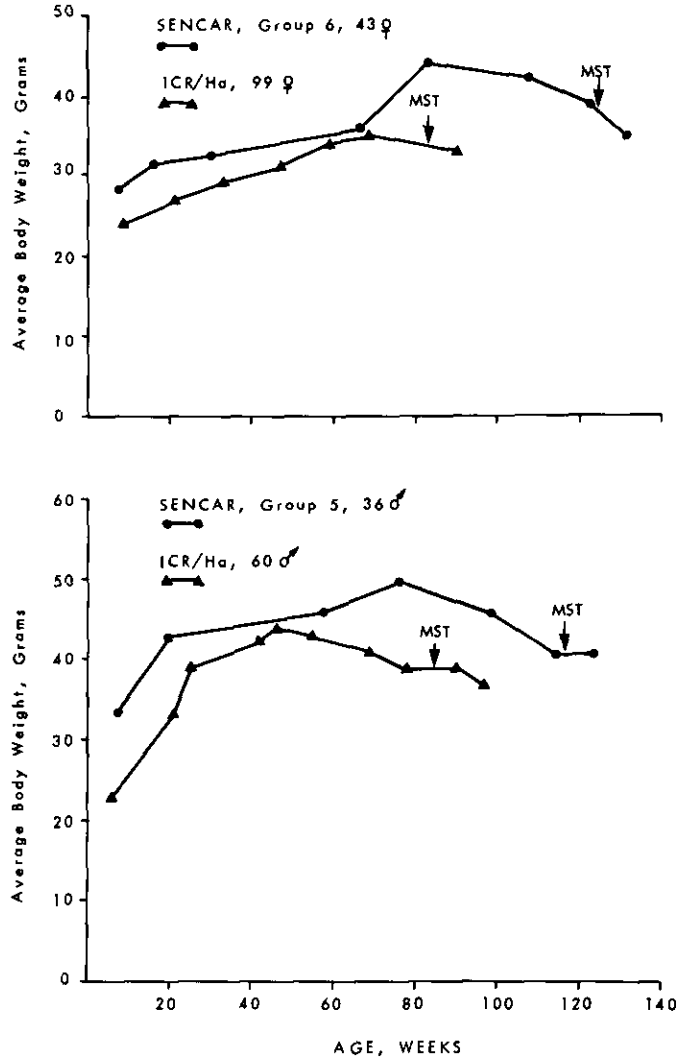

FIGURE 3. Comparison of body weights and median survival times (MST) between SENCAR and ICR/Ha mice.

Routine Sections

The numbers of routine sections of various organs taken from groups 4 to 6, and the corresponding diagnoses are shown in Table 4. The total number of clinically normal sections from all tissues listed in Table 4 was 143. Of these, only 10, i.e., about 7%, were diagnosed as tumors or nonneoplastic lesions. Thus, these routine sections did not markedly alter the overall tumor and nonneoplastic lesions incidences shown in Tables 2 and 3.

Discussion

An examination of the tumor incidences in SENCAR mice used in this laboratory, listed in Table 2, revealed that five tumor types occur most frequently. These tumor types and the frequency of their occurrence are summarized in Table 5. These tumors occurred in one or more of the six groups with a frequency of above 10%. With the exception of group 3, the overall tumor inidences were greater than 50%. The most commonly occurring lung tumors were benign papillary tumors, and the frequency of incidence ranged between 16 and 39% in the six groups. The incidence of lung tumors does not appear to be age related, e.g., group 1, which was observed for 540 days, had essentially the same lung tumor incidence as that of groups 5 and 6, which were observed for 815 and 875 days, respectively. In another study (*4*), the most frequently observed tumors were those of the lung in CD-1 mice of both sexes.

Male mice showed a high incidence of hyperplastic nodules of the liver (group 5), 33.3%, as compared to females, which showed a range of 0 to 2.4%. This difference between sexes has also been observed in B6C3F1 hybrids and other mouse strains (*5*).

The mammary tumor incidences summarized in Table 5 showed a distinct increase with age, from 5.1% in group 1 (540 days) to 22% in group 6 (875 days).

It was of interest to compare median survival times and body weights of SENCAR mice from some of the

Table 2. Tumor incidences in SENCAR mice.

Site	Tumor type	Number of mice with tumors (%)					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Lung	Papillary tumor	19 (32.2)	18 (28.6)	9 (16.1)	20 (25.6)	12 (33.3)	15 (39.0)
	Papillary tumor and secondary carcinoma	—	—	—	—	—	1 (2.4)
	Secondary mammary carcinoma	—	—	—	1 (1.3)	—	—
Liver	Hyperplastic nodule	1 (1.7)	1 (1.6)	—	—	12 (33.3)	1 (2.4)
	Hemangioma	—	—	—	1 (1.3)	4 (11.1)	1 (2.4)
Mammary gland	Unclassified	3 (5.1)	8 (12.7)	6 (10.7)	16 (20.5)	—	6 (14.6)
	Fibroadenoma	—	1 (1.6)	—	—	—	—
	Carcinoma	—	—	1 (1.8)	2 (2.6)	—	2 (4.9)
	Adenocarcinoma	—	—	1 (1.8)	—	—	
	Squamous cell carcinoma	—	—	—	—	—	1 (2.4)
Forestomach	Papilloma	5 (8.5)	4 (6.3)	3 (5.4)	5 (6.4)	6 (16.7)	6 (14.6)
Glandular stomach	Undifferentiated malignant tumor	—	—	—	—	—	1 (2.4)
Duodenum	Adenoma	—	—	—	—	1 (2.8)	—
Kidney	Carcinoma	—	—	—	1 (1.3)	—	—
Spleen	Hemangioma	—	—	—	—	—	2 (4.9)
Skin	Papilloma	—	—	1 (1.8)	—	1 (2.8)	—
	Sarcoma	—	—	—	—	1 (2.8)	1 (2.4)
Uterus	Leiomyoma	—	—	2 (3.6)	—	—	—
	Sarcoma	—	—	—	1 (1.3)	—	—
Ovary	Adenoma	—	—	—	1 (1.3)	—	—
	Carcinoma	—	—	1 (1.8)	1 (1.3)	—	—
	Brenner tumor	—	—	—	2 (2.6)	—	—
	Hemangioma	—	—	—	—	—	1 (2.4)
	Interstitial cell tumor	—	—	—	—	—	1 (2.4)
	Benign epithelial tumor	—	—	—	—	—	1 (2.4)
Adrenal gland	Secondary carcinoma	—	—	—	—	—	1 (2.4)
Bone	Sarcoma	—	—	—	1 (1.3)	—	—
Blood vessel	Hemangioma	—	1 (1.6)	—	—	—	—
Ear duct gland	Squamous cell carcinoma	—	1 (1.6)	—	—	—	—
Paraspinal cord	Squamous cell carcinoma (undetermined origin)	—	1 (1.6)	—	—	—	—

Table 2. Continued.

Site	Tumor type	Number of mice with tumors (%)					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Prostate gland	Adenoma	—	—	—	—	1 (2.8)	—
Multiple	Leukemia[a]	11 (18.6)	8 (12.7)	6 (10.7)	20 (25.6)	3 (8.3)	10 (24.4)
Total number of mice with tumors		33 (55.9)	35 (55.5)	25 (44.6)	51 (65.4)	25 (69.4)	27 (65.9)

[a] Include lymphomas and leukemias at other sites.

Table 3. Nonneoplastic lesions in SENCAR mice.

Site	Lesion type	Number of mice with lesions					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Skin	Focal hyperplasia	1	–	–	–	–	–
	Chronic inflammation and hyperplasia	–	–	–	–	1	–
	Chronic inflammation, ulcer	–	–	–	–	1	–
	Chronic inflammation, fibrosis	–	–	–	–	1	–
	Fibrous polyp	–	–	–	–	1	–
Thymus	Hyperplasia	1	–	–	–	–	–
Trachea	Necrosis	–	–	–	1	–	–
Heart	Chronic inflammation	–	–	–	1	–	–
Ear duct gland	Ulcer, granulation tissue	–	1	–	–	–	–
Lung	Chronic inflammation	–	1	–	–	–	–
	Pneumonia	–	–	1	–	–	–
	Chronic inflammation, fibrosis	–	–	–	–	–	1
Lymph node	Hyperplasia	1	–	–	1	1	–
	Abscess	–	–	–	–	1	–
Spleen	Hyperplasia	–	1	–	–	–	–
Liver	Focal necrosis	–	–	1	–	–	–
Kidney	Chronic inflammation	–	2	2	3	3	1
	Scar	–	–	–	1	–	–
	Dilated tubules	–	–	–	1	–	–
	Chronic inflammation with cystic dilatation of tubules	–	–	–	–	1	–
	Focal lymphocytic infiltrate	–	–	–	–	–	2
Forestomach	Hyperplasia of mucosa	–	1	–	–	–	–
	Hyperkeratosis	–	–	–	1	–	–
	Keratosis	–	–	–	–	–	1
Glandular stomach	Chronic inflammation	1	–	–	–	–	–
	Glandular hyperplasia	1	–	–	–	2	2
Duodenum	Focal hyperplasia of mucosa	–	–	–	2	–	–
	Glandular hyperplasia and atypia	–	1	–	–	–	–
Anus	Hyperplastic polyp	–	–	–	–	1	–
Mammary gland	Hyperplasia	–	–	–	4	–	2
	Dilated acini	–	–	–	–	–	1
	Dilated duct with squamous metaplasia	–	1	–	–	–	–
Uterus	Endometrial polyp	2	–	1	2	–	–
	Cystic hyperplasia	–	–	–	2	–	1
	Fibrous polyp	–	–	–	–	–	1
	Chronic inflammation with squamous metaplasia	–	–	–	1	–	–
	Squamous metaplasia of endometrium	–	–	–	–	–	1
Ovary	Hemmorrhagic cyst	2	4	3	15	–	8
Prostate gland	Acute inflammation, necrosis	–	–	–	–	1	–
	Chronic inflammation, squamous metaplasia and dilatation of ducts	–	–	–	–	1	–
	Multiple fibrous-lined cysts	–	–	–	–	1	–
Seminal vesicle	Cyst	–	–	–	–	1	–
	Cystic dilatation	–	–	–	–	2	–
Testes	Atrophy and focal calcification	–	–	–	–	1	–
Total number of lesions		9	12	8	35	20	21

Table 4. Pathology of clinically normal sections (groups 4–6).

Tissue	Number of sections			Number of positive diagnoses		
	Group 4	Group 5	Group 6	Group 4	Group 5	Group 6
Lung	3	2	1	0	0	0
Liver	8	15	23	0	1[a]	4[d]
Kidney	1	20	20	0	0	1[d],2[e]
Stomach	0	20	12	–	1[b]	0
Skin	4	7	5	0	1[c]	0
Spleen	2	0	0	0	–	–

[a] Hyperplastic nodule.
[b] Papilloma.
[c] Chronic inflammation and hyperplasia.
[d] Leukemia.
[e] Focal lymphocytic infiltrate.

control groups discussed here with ICR/Ha mice used in most of our earlier studies on promoters, cocarcinogens, and whole carcinogens. Groups 5 and 6 from the present report were compared with ICR/Ha mice of both sexes used as control groups in an earlier study (*6*). This comparison is illustrated in Figure 3. The markedly higher weight and increased median survival times of SENCAR mice compared to ICR/Ha mice of both sexes are noteworthy.

In the course of the preparation of this report and another one for the symposium (*1*), the differences in results obtained in different laboratories with the same strain of mice became obvious. The fact that these differences are apparent stressed to us again the importance of standardizing laboratory animal care and handling procedures. So many variables, often overlooked and thought of as having minor relevance, are in fact important for comparing chronic animal bioassay results among different laboratories. The importance of all these variables has been discussed and reviewed (*7*).

Table 5. Summary of frequently occurring tumors.

Tumors	% Tumors					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Total tumor-bearing animals	55.9	55.5	44.6	65.4	69.4	65.9
Lung, papillary tumors	32.2	28.6	16.1	25.6	33.3	39.0
Mammary tumors	5.1	14.3	14.3	24.4	0	22.0
Liver hyperplastic nodules	1.7	1.8	0	0	33.3	2.4
Leukemia	18.6	12.7	10.7	25.6	8.3	24.4
Forestomach papillomas	8.5	6.3	5.4	6.4	16.7	14.6
Number of mice necropsied	59	63	56	78	36	41
Duration of observation (days)	540	600	605	800	815	875

From the papers presented at this symposium, it was clear that the SENCAR mouse will be valuable for mouse skin tumorigenesis studies, particularly in two-stage, i.e., initiation-promotion experiments. In this regard, the utility of SENCAR mice for carcinogenesis studies relating to tissues and organs other than skin merits further attention. This point is important because *in vitro* models, while most valuable, must continue to be regarded as adjuncts to, rather than replacements for *in vivo* animal bioassays. The latter view is shared by other researchers in toxicology (*8,9*).

Our findings from the present work and from that presented elsewhere in this volume (*1*) indicate the need for further work on the utility of the SENCAR mouse in toxicology research.

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