

Differences in Survival among Germfree Mice Following Transfer to a Conventional Colony (39284)

D. A. COHN AND JAMES B. HAMILTON

Department of Anatomy, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203

Germfree mice are considered to be free of bacteria, bacteriallike organisms, fungi, protozoan and metazoan parasites, within limitations of tests to detect contamination. Following transfer of these mice to a conventional colony and sudden exposure to ordinary pathogens, survival depends upon the development of defenses against microbial attack. Survival of germfree animals after conventionalization, however, has varied in different laboratories (1-6). The possibility exists that sex and/or strain differences may have contributed to the conflicting reports in the literature.

The purpose of the present study was to examine these factors of sex and strain in survival. Accordingly, observations of differences in survival were made on conventionally reared mice and on one outbred and three inbred strains of germfree mice.

Materials and methods. Mice. A total of 272 conventionally reared and germfree male and female retired breeders, approx 7-9 months old, were used for these experiments. Older mice were chosen in preference to younger animals because they reportedly succumb more readily after exposure to pathogens (1, 7). The mice were divided into three groups. (a) Conventionally reared RF/J, C3H/HeJ, and DBA/2J mice, purchased from the Jackson Laboratory, Bar Harbor, Maine. The RF/J and C3H/HeJ were obtained specifically for these experiments. The DBA/2J were transferred into our colony as part of other experiments. (b) Outbred CD-1 germfree mice purchased from the Charles River Breeding Laboratories, Wilmington, Mass. (c) Inbred germfree mice obtained as follows: DBA/2f/Wg (DBA/2) and RFMf/Wg (RFM) were kindly supplied by Dr. H. E. Walburg, Jr., formerly of the Oak Ridge National Laboratory, Oak Ridge, Tenn. The RFM were germfree RF mice originally derived in 1963 by foster-nursing a hyster-

ectomy-derived litter from an inbred RF mother (F55) on a germfree ICR female (8). C3Hf/Wg (C3H) were supplied by Dr. Walburg and Dr. Serrano of Oak Ridge. C3H/HeSchGn (C3H) were purchased from ARS/Sprague-Dawley, Madison, Wis.

Maintenance of the germfree state. All germfree mice were delivered by caesarean section into sterile isolators and either hand-fed with sterile formulae or foster-nursed on germfree lactating mothers. After weaning they were fed sterilized solid food and water, and maintained aseptically with periodic monitoring for viruses and bacteria. Cultures were negative when tested immediately before shipping.

Transfer of mice. Mice of all strains were shipped to our laboratories within a 3-month period and maintained simultaneously. A year later, an additional 60 germfree C3H mice arrived along with 50 conventional RF/J and 50 conventional C3H/HeJ mice. All germfree mice were transported by air express in germfree shipping isolators (Charles River Model 20 or 25). Transit time did not exceed 24 hr. Upon arrival, containers were opened immediately. Animals were examined and transferred in groups of 8-10 to conventional acrylic cages (Keystone Plastic Co., Media, Pa.) which contained bedding of pinewood chips (Pinewood Sawdust, Moonachie, N.J.). Males which had not been weaned together were shipped and caged separately. The cages were placed in rooms housing approximately 600 nongermfree mice and available to students, researchers, and laboratory personnel. Mice were supplied with tap water *ad libitum* and fed Mouse Diet 96W Old Guilford Breeders Chow with wheat germ (Emory Morse Co., Guilford, Conn.). Mice were maintained for either 4 or 7 months after transfer.

Statistics. Records indicated that survival rates of all germfree C3H mice were similar

regardless of their source or time of arrival; therefore, the data for germfree C3H mice were pooled. Differences in the survival rates between outbred males and females and between inbred strains were analyzed by the Friedman two-way analysis of variance. The Wilcoxon matched pair signed rank analysis was used to demonstrate statistical differences in survival between inbred males and females.

Results. Conventional mice. No conventionally reared Rf/J, C3H/HeJ, or DBA/2J mice died during the 7 months following entry into this colony.

Germfree outbred mice. After 2 weeks, the majority of CD-1 mice weighed as much as upon arrival. Four months after transfer, all mice were still alive and now weighed 50% more than their original weight (Table I). CD-1 mice were then exposed to lethal irradiation and died 4 months after transfer.

TABLE I. CHANGES IN MEAN BODY WEIGHT OF CD-1 MICE FOLLOWING CONVENTIONALIZATION.^a

	Body weight (g)		
	0 Days	14 Days	120 Days
15 Males	31.8	32.8	47.3 ^{b, c}
15 Females	26.9 ^{b, c, d}	27.1 ^{c, d}	40.8 ^{b, c, d, e, f}

^a Two-way analysis of variance (ANOVA) with repeated measures. Sex, $F = 13.90^*$; Time, $F = 166.50^*$; Sex - time, NS (not significant). * $P < 0.05$.

^b Significantly different at $P < 0.05$ from males at 0 days (Multiple-Comparisons Tests).

^c Significantly different at $P < 0.05$ from males at 14 days (Multiple-Comparisons Tests).

^d Significantly different at $P < 0.05$ from males at 120 days (Multiple-Comparisons Tests).

^e Significantly different at $P < 0.05$ from females at 0 days (Multiple-Comparisons Tests).

^f Significantly different at $P < 0.05$ from females at 14 days (Multiple-Comparisons Tests).

Germfree inbred mice. When survival rates were analyzed by sex, significant differences were observed (Table II). Females had a higher proportion of survivors at all periods following introduction into a conventional colony with differences in survival most marked at 7 months.

When analyzed by strain (Table III), differences in percentages of survivors were not statistically significant; however, the patterns of survival seemed to vary among the strains. For example, 1 month after transfer, 100% of the DBA/2 were alive compared to 87.5 and 83.3% of the C3H and RFM. Thereafter, the DBA/2 incurred the greatest losses between 1 and 2 months and 4 to 7 months after transfer, leaving 45.5% alive at 7 months. Among the C3H, there was a gradual loss, with 63.5% surviving 7 months. In the RFM, losses were negligible after the first month and 70.8% survived 7 months.

Discussion. All conventionally reared mice survived transfer to our colony. Among germfree mice, the majority withstood the strain of transportation and transfer to a contaminated environment. Every germfree CD-1 mouse survived for 4 months after transfer. While some losses occurred among germfree inbred mice, 73% of the inbred mice were still alive 4 months after transfer and 62% alive 7 months after transfer. In the germfree group, females had a higher proportion of survivors throughout the experimental period with sex differences in survival most prominent 7 months after transfer.

The studies reported were repeated twice over a period of 2 years. At both times, our experiences with conventionalization of germfree inbred mice were considerably

TABLE II. SEX DIFFERENCES IN SURVIVAL OF GERMFREE INBRED* MICE FOLLOWING CONVENTIONALIZATION.^a

Months	Males		Females		Percentage difference
	Number	Percentage	Number	Percentage	
0	73	100.0	69	100.0	0
1	61	83.6	65	94.2	10.6
2	51	69.9	60	87.0	17.1
3	48	65.8	57	82.6	16.8
4	47	64.4	56	81.2	16.8
7	36	49.3	52	75.3	26.0

^a Wilcoxon Matched Pair-Signed Ranks Test. $T = 0$; $P < 0.05$. * DBA/2, C3H, and RFM strains combined.

TABLE III. SURVIVAL OF THREE INBRED STRAINS OF GERMFREE MICE FOLLOWING CONVENTIONALIZATION.^a

Months	DBA/2		C3H		RFM		Total	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
0	22	100.0	96	100.0	24	100.0	142	100.0
1	22	100.0	84	87.5	20	83.3	126	88.7
2	15	68.2	77	80.2	19	79.2	111	78.0
3	15	68.2	71	74.0	19	79.2	105	73.9
4	15	68.2	70	72.9	19	79.2	104	73.2
7	10	45.5	61	63.5	17	70.8	88	61.9

^a Friedman two-way ANOVA (X^2r) not significant.

better than those of other investigators. Reyniers and Sacksteder (3, 4) had reported that germfree C3H mice showed signs of contamination and infection within 30 to 60 min of transfer. Less than 15% were alive after 48 hr, and there were no sex differences in survival. Outzen and Pilgrim (1) had found that 18% of male C3H survived for 15 days after conventionalization and only 9% survived for 35 days, in contrast to a 75% survival of females. Of our inbred females, 87% survived 2 months after transfer in agreement with Outzen and Pilgrim (1) but not with Reyniers and Sacksteder (3, 4). In contrast to the results reported in both of these laboratories, a high proportion of our inbred males also remained alive, with 69.9% surviving 2 months and 48.3% still alive at 7 months.

The high percentage of survivors among our conventionalized germfree mice raises the question of whether these mice were contaminated before transfer. This is unlikely since the animals were derived and maintained in sterile isolators and subjected to periodic microbiological examination in three different laboratories prior to shipping. More likely the germfree mice survived conventionalization because: (a) as a group they have long lifespans and generally outlive their conventional counterparts (2); and (b) despite their sparse lymphatic tissue, germfree animals are immunologically competent (9, 10).

Survival of more than 50% of our inbred males is noteworthy and conflicts with the previously reported poor viability of the male sex (1). Perhaps the differences in resistance reported for males by other workers reflect differences in prior exposure to cross reacting bacterial antigens (11). Species dif-

ferences in survival are also known to exist (2, 5, 6). In this study, there is the additional possibility that stresses in transit prior to transfer may have also influenced survival following later exposure to conventional microorganisms. It has recently been reported that both stress and aging may produce paradoxical effects on the immune response by altering suppressor cell activity (12). Regardless of the mechanisms involved, the present report indicates that germfree mice, including inbred males, can be transferred to a conventional environment and that the majority should survive for several months.

Summary. Sex and strain differences in survival were studied in 7-9 month old germfree mice following transfer to a conventional colony. One outbred and three inbred strains were observed. All outbred CD-1 mice survived transfer and in 4 months increased their weight by 50%. The majority of inbred mice survived 7 months after transfer. Sex differences in survival were evident throughout the experimental period and were most marked 7 months after transfer. An unexpected new finding was the viability of the male sex in germfree mice after transfer. Possible explanations are considered.

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