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COMMENTARY

Lifespan and Lesions in Genetically Heterogeneous (Four-way Cross) Mice: A New Model for Aging Research

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Abstract. Genetically heterogeneous animal models provide many advantages for research on aging but have been used infrequently. We present here lifespan and lesion data from a study of mice bred as a cross between (AKR/J × DBA/2J)F_1 females and (C57BL/6J × SJL/J)F_1 males. In such a four-way cross population, each mouse is genetically unique, but replicate populations of essentially similar genetic structure can be generated quickly, at low cost, and of arbitrary size from commercially available, genetically stable hybrid parents. We employed a protocol in which mice judged to be severely ill were euthanatized to obtain tissue in optimal condition for necropsy, and we were able to infer a likely cause of illness in 42 of 44 animals. Malignant lymphoma, including at least four histopathologically distinct subtypes, was the most common cause and was also a frequent incidental finding in mice dying of other causes. Neoplastic disease, benign or malignant, was the sole or a contributing cause of illness in 90% of the mice for which a cause could plausibly be assigned. A wide range of lethal and nonlethal degenerative lesions was also noted. The coefficient of variation for lifespan in these genetically heterogeneous mice was 26%, similar to that seen in analyses of recombinant inbred mouse lines. Baseline lifespan and pathology data on four-way cross mice is a useful prelude to the exploitation of this rodent model in tests of genetic and mechanistic hypotheses about aging.

Key words: Aging; genetics; longevity; lymphoma; mice; neoplasia.

The overwhelming majority of modern laboratory studies on aging in mice use genetically homogeneous stocks, either inbred mice or F_1 hybrids bred as the progeny of different inbred parents. Although inbred and F_1 rodents are easily obtained commercially and can be obtained at various ages from colonies subsidized by the National Institute on Aging (NIA), their routine use presents major disadvantages for experimental gerontology. The central problem with inbred models is that of reproducibility and robustness. Each inbred and each F_1 hybrid mouse strain includes only a single genotype. Thus, it is impossible without further work to guess whether a particular experimental finding will or will not apply to conspecific animals of different genotype. Few investigators are eager to replicate their work on multiple inbred or F_1 strains, and the literature is filled with reports whose general applicability is uncertain.

The focus of many gerontologic studies on old animals compounds this difficulty, because many old mice of inbred or F_1 strains exhibit strain-specific diseases potentially able to affect the experimental variables of interest. Many such examples are already well known, including the preponderance of pulmonary adenomas in A/J mice, of hepatomas in C3H/HeJ mice, of Leydig cell tumors and glomerulonephritis in inbred F344 rats, of lymphomas in BALB/c and C57BL/mice, and of reticulum cell sarcomas in SJL/J mice.[1] In this context it seems highly plausible that mice of different inbred strains may also differ in a number of strain-specific physiological traits too subtle to be diagnosed easily by conventional histopathologic methods yet significant enough to alter the biochemical and physiologic outcomes that are typically the focus of experimental analyses. A compelling case against overreliance on a particular inbred rodent model, the F344 rat, has been made previously,[31] and similar arguments can be made against other specific strains and against the routine use of genetically homogeneous rodents as an exclusive focus for experimental gerontology.

As long ago as 1976, a committee chartered by the NIA recommended the broader use of genetically het-

erogeneous rodents in aging research. Among their specific recommendations[5] was the development of experimental models that utilized a four-way cross (4WC) breeding scheme. This breeding method involves the mating of two different F_1 strains, each generated by a cross between different inbred parents. Thus if A, B, C, and D represent four unrelated inbred mouse lines, the 4WC progeny could be produced by a mating between (AB)F_1 and (CD)F_1 parents. Each of the 4WC mice is genetically unique, each bearing a different set of alleles from the four inbred grandparents. Although no individual mouse bred by a 4WC is replicable, the genetic characteristics of the population of 4WC mice can be replicated at any desired population size and in any laboratory with access to the original inbred grandparents. Because the genetic characteristics of many inbred lines are extremely stable and maintainable by periodic rederivation from frozen embryo stocks, it is both in principle and in practice feasible to reproduce a specific 4WC population in any laboratory at any time.

Greater use of 4WC mice for experimental aging research will depend on the acquisition of background information about the gerontologically relevant characteristics of mice produced in this way, in particular information about the life span and lesions of 4WC mice. We have now completed a pilot study on 44 4WC mice bred as the progeny of two commercially available F_1 hybrid mice, (AKR/J × DBA/2J)F_1 females crossed with (C57BL/6J × SJL/J)F_1 males. Because one of our goals was to obtain a high yield of useful necropsy data, we examined each mouse at least once each day, and we used a "euthanatize when moribund" strategy to further reduce the number of necropsies impaired by advanced postmortem autolysis. By focusing on terminal lesions, we avoided the systematic bias, characteristic of the far more common cross-sectional design, against diseases, like many lymphomas, that lead rapidly to death. Our data provide evidence for both diversity and regularity in this genetically heterogeneous mouse population: although neoplasia, and particularly various forms of lymphoma, contributed to critical illness in most of the animals, we found many cases of neoplastic and serious degenerative lesions that are infrequently reported in individual inbred or F_1 hybrid strains.

Materials and Methods

Mice

The experimental animals for this aging study were the progeny of (AKR/J × DBA/2J)F_1 females crossed with (C57BL/6J × SJL/J)F_1 males. This 4WC breeding scheme produces a population of genetically heterogeneous mice, no two of which are genetically identical but whose average characteristics can be stably reproduced in populations of arbitrary size and whose genetic constitution is unlikely to show the genetic drift and founder effects characteristic of commercially available outbred stocks. The mice were bred at the University of Michigan's Core Facility for Aging Rodents. They were housed from the time of weaning in polycarbonate cages with metal lids containing Bed-O-Cobs as absorbent in a monitored specific-pathogen-free colony as mated pairs, with one male and one female per cage. Mice were given access to rodent chow (Purina Rodent Diet 5001) and water ad libitum. Litters were weaned at 3 weeks of age and used for another study. Peripheral blood samples were taken by tail venipuncture from each mouse at 6 and 11 months of age for the measurement of immune markers. Sentinel mice were housed in the same room and blood samples were checked quarterly for antiviral antibodies. The serologic results were negative until the population reached 14 months of age, at which point the sentinels were found to have positive titers for mouse coronavirus (mouse hepatitis virus, MHV). Breeding within the colony was stopped at this point to eliminate the coronavirus from the colony.[32] Since that time, sentinel mice have been repeatedly negative for murine pathogens.

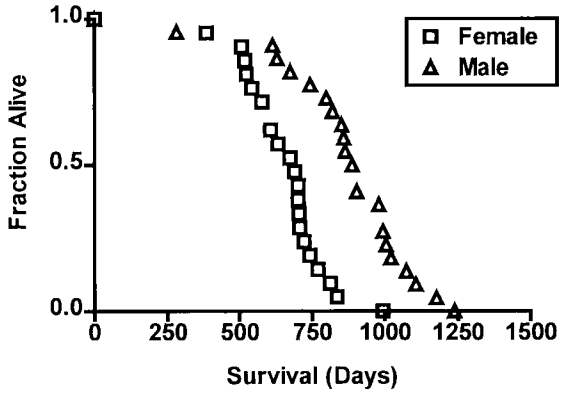
The initial study group consisted of 20 males and 20 females. In the early phases of the study, animals that died were replaced by new age-matched mates, which were also followed until they died or became moribund; thus the population available for necropsy consisted of 22 males and 21 females. In addition, one female was euthanized in error at 18 months of age; this animal was not included in survival curves and summary tables.

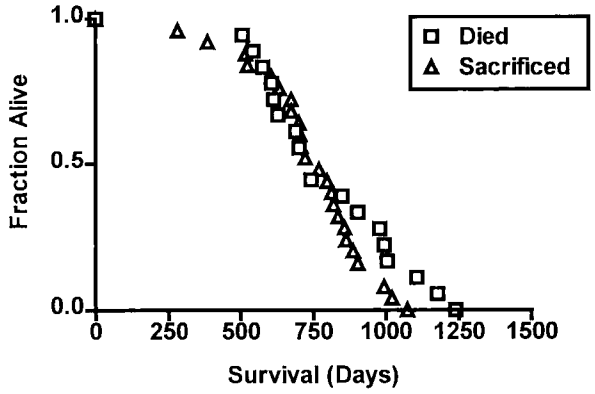
Cage monitoring strategy

Each mouse was inspected at least once daily, including weekends, by an experienced animal caretaker. Mice were weighed weekly from 18 months of age. Mice that were judged to be near death, by a combination of clinical signs including weight loss, poor grooming, rapid growth of visible or palpable tumors, or lethargy, were designated moribund, transported while alive to the pathology suite, and euthanized for necropsy. Animals found dead were also submitted for necropsy.

Pathology

Gross lesions were described at necropsy, and the following tissues were fixed in buffered formalin and submitted for histology: salivary glands, mandibular lymph nodes, Harderian glands, trachea, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, liver mesenteric lymph nodes, spleen, pancreas, uterus, ovaries, vagina, urinary bladder, heart, lung, thymus, kidneys, adrenals, thyroids, pituitary, testicles, prostate, epididymis, parathyroids, brain, middle ears, knee joint including distal femur and proximal tibia, cross section of nasal cavity, skeletal muscle, cross section of lumbar spinal cord and lumbar vertebra, skin and eyes. In addition, representative sections of gross lesions were submitted for histologic evaluation. Processed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Lymphomas were characterized morphologically using criteria previously described.[20]





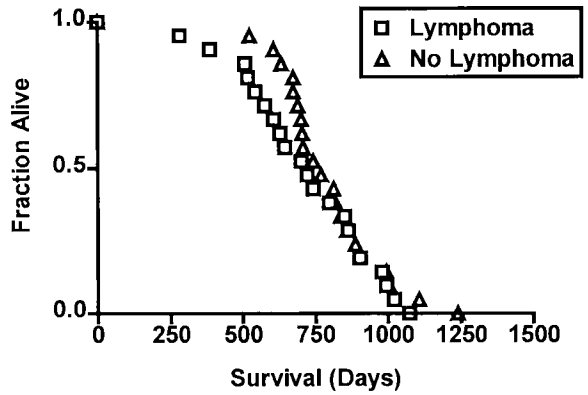


Fig. 1. Survival curves for subsets of four-way cross mice. Each point represents a single mouse. Life span is given in days. Forty-three mice were alive at the start of the study.

Statistical analysis

The log-rank test was used to compare survival between groups of mice; two-tailed criteria were used to assess significance.

Results

Does a "euthanatize when moribund" strategy bias estimates of life span?

Two central goals of our study of 4WC mice were to generate a life table and to compile a record of terminal lesions. A plan in which animals judged to be moribund are euthanatized is likely to decrease the fraction of mice for which interpretable histopathologic data are lost to postmortem autolysis but could in principle lead to systematic underestimation of mean and maximal life span if animals are euthanatized long before they would have succumbed to natural processes. Studies (D. Harrison, unpublished) have shown that a protocol in which moribund mice are euthanatized need not lead to a significant underestimation of mean or maximal longevity when the animals' health is assessed by skilled and experienced caretakers (D. Harrison, personal communication). Data from our current study of 4WC mice provide further support for the idea that a moribund necropsy strategy can identify mice with severe illness. Of the 44 mice originally entered into the study, 26 were euthanatized when moribund (59%). Of these 26 mice, 25 were found at necropsy to have one or more advanced diseases, such as widespread malignancy, that were judged likely to have been incompatible with extended long-term survival. The 96% incidence of severe lesions in the euthanatized mice is also consistent with the idea that a policy of euthanatizing animals that appear to be severely ill does not seriously bias life span estimates.

Survival

Figure 1 shows the cumulative mortality curves for both male and female 4WC mice and the mortality curves for the animals that were euthanatized when moribund and those that were found dead. Table 1 shows the mean, median, and range of life span for these groups of mice. Survival of males was significantly greater than that of females (log-rank test, $P < 0.001$). There is, however, no significant difference between survival of mice that died spontaneously and those that were euthanatized when moribund (log-rank test, $P = 0.26$).

Causes of death

Although it is notoriously difficult to prove that a specific lesion is the sole cause of death, it is in practice frequently possible to find one serious lesion or a combination of lesions that seems very likely to have led

Table 1. Survival curve statistics for four-way cross mice.

Group	*n*	Life Span (months)			
		Median	Mean	SD	Range
All mice	43	25	26	7	9–41
Sex					
Females	21	23	22	4	13–33
Males	22	30	29	7	9–41
Death					
Euthanatized	25	25	25	6	9–36
Not euthanatized	18	25	27	8	17–41
Disease					
Lymphoma	21	24	24	7	9–36
No lymphoma	22	26	27	6	17–41

to spontaneous death or to the development of severe, life-threatening clinical illness. In this sense, we inferred the cause of death for 42 of the 43 mice in this study. These diagnoses are listed in Table 2. In a few individuals, more than one disease may have contributed to morbidity/mortality, and it was not possible to attribute morbidity/mortality to a single cause; in these cases, multiple diseases are listed together as the cause of death. One mouse was too badly autolyzed to allow pathologic assessment.

Malignant lymphomas, considered as a group, were the most common cause of death in both males and females (Table 2). Advanced lymphoma was found in 55% of male mice and in 43% of female mice. The median age at death due to lymphoma (in two mice due to lymphoma plus other lesions) was 19 months for females and 29 months for males. There was a significant difference ($P = 0.002$) between the sexes in age at death from lymphoma. Age at death from lymphoma, however, did not differ significantly from age at death from other causes (log-rank test, $P = 0.22$). Lymphoma-induced death was seen in mice as young as 9 months of age and as old as 36 months. Figure 1 shows the survival curves for mice whose deaths were attributed to lymphoma and for mice whose deaths were due to other causes. The curves are largely overlapping, suggesting that lymphoma is not predominantly a cause of early death in these 4WC mice. Small nodules of lymphoma, not likely to have been clinically symptomatic at the time of death, were also found in two male and five female mice (see Table 3).

There was considerable heterogeneity within the group of lymphomas. Using established criteria,[20] we were able to classify the lymphomas into four histologically distinct groups: follicular center cell (30%), small lymphocytic (20%), lymphoblastic (37%), and immunoblastic (10%); one other lymphoma could not be assigned to a subtype because of tissue autolysis.

Table 2. Causes of death (actual or inferred) in four-way cross mice.

Cause of death	No. Mice Affected			
	Male	Female	Total	Median Age (months)
Enamel organ dysplasia (central incisor teeth)	1		1	39
Endometrial stromal polyp		1	1	23
Glomerular amyloidosis	1	1	2	24
Granulocytic leukemia	1	1	2	24
Hemangiosarcoma (liver)		1	1	17
Hemangiosarcoma (mesenteric lymph node)		1	1	33
Hepatocellular carcinoma	1		1	33
Hepatocellular carcinoma and chronic pancreatitis	1		1	28
Kupffer cell sarcoma	1		1	33
Malignant lymphoma	11	8	19	24
Malignant lymphoma and glomerular amyloidosis	1		1	33
Malignant lymphoma and membranoproliferative glomerulonephritis		1	1	23
Malignant mast cell tumor (heart) and pituitary adenoma		1	1	23
Mammary adenocarcinoma		2	2	25
Neurofibrosarcoma (brachial plexus)	1		1	22
Pituitary adenoma		1	1	23
Pituitary adenoma and foreign body inhalation		1	1	24
Pituitary adenoma and right atrial thrombus		1	1	28
Pulmonary adenocarcinoma	1		1	28
Rhabdomyosarcoma (muscle)	1		1	41
Thoracic cavity aneurysm		1	1	20
Undetermined	1		1	36
Totals	22	21	43	24

Table 4 shows the distribution of these subtypes in males and females and the median age at death.

Other forms of neoplasia were also frequent causes of death in this group of mice. Pituitary tumors caused or contributed to death in four females (19%; Table 2) and were seen as an incidental lesion in one other female (Table 3) but were not observed in males. Two females died of mammary adenocarcinoma, and a similar tumor was seen as an incidental lesion in a third female. Neoplasia of a variety of types accounted for most of the remaining deaths. Nonneoplastic lesions either contributed to death or were the sole cause in only 9 of the 43 mice (21%), including three fatal examples of glomerular amyloidosis (two of them in male mice), membranoproliferative glomerulonephritis, chronic pancreatitis, foreign body inhalation, bilateral

Table 3. Nonlethal neoplasms in four-way cross mice.

Specific Neoplasms	No. Mice Affected			
	Male	Female	Total	Median Age (months)
Hepatocellular adenomas (multiple)	1		1	41
Islet cell adenoma		1	1	24
Malignant lymphoma	2	5	7	24
Malignant pheochromocytoma		1	1	23
Mammary adenocarcinoma		1	1	23
Osteoma of temporal bone	1		1	22
Pituitary adenoma		1	1	24
Pulmonary adenocarcinoma	2		2	37
Pulmonary adenoma	3		3	33
Thyroid adenoma	1		1	26
Total no. neoplasms*	10	10	20	

* Some mice had multiple neoplasms.

enamel organ dysplasia of the maxillary incisor teeth leading to malocclusion, ruptured aneurysm, and atrial thrombus.

Nonlethal neoplastic and degenerative diseases are shown in Tables 3 and 5, respectively. Again, malignant lymphoma was the most common incidental neoplasm. The age-associated degenerative lesions seen in this study were much more common in males, perhaps because of the longer average survival of the males in the cohort. Dysplasia of the enamel organs of the maxillary incisor teeth was the most common nonneoplastic lesion and was also much more common in males. This lesion led to severe malocclusion, inanition, and death in one mouse (Table 2).

Discussion

Our data are relevant to three interrelated topics: 1) optimal methods for longitudinal analysis of aging rodents, 2) genetic control of aging and disease, and 3) animal models of aging.

Table 4. Subtypes of lymphoma in four-way cross mice.

Subtype*	Males		Females	
	No. Affected	Median Age (months)	No. Affected	Median Age (months)
Follicular center cell	2	31	7	22
Small lymphocytic	2	26	4	20
Lymphoblastic	7	29	4	20
Immunoblastic	2	29	1	23
Untypable (autolysis)	1	36		
Total	14	30	16	21

* Includes both lethal and nonlethal lmyphomas. Some individuals had more than one subtype in different locations.

Table 5. Nonlethal diseases in four-way cross mice.

Specific Diseases	No. Mice Affected			
	Male	Female	Total	Median Age (months)
Glomerulonephritis	1	1	2	29
Unilateral hydronephrosis	1		1	31
Endometrial hyperplasia		1	1	31
Testicular atrophy	2		2	32
Prostatic hyperplasia	1		1	31
Seminal vesiculitis	2		2	36
Adrenal capsular hyperplasia	1		1	33
Thyroid C-cell hyperplasia	1		1	41
Arteriosclerosis	1		1	38
Myocardial degeneration	1	1	2	35
Aging cataracts	6		6	34
Erosive arthritis (knee joint)	4		4	30
Enamel organ dysplasia (central incisor)	11	4	14	29
Hepatic cysts (multiple)		1	1	27
Ileal amyloidosis	1		1	29
Gastric ulcer	1		1	41
Total no. diseases*	32	8	40	

* Some mice had multiple diseases.

Methodological implications

Most of the published reports of lesions in mice have employed a cross-sectional design, in which randomly selected mice are euthanatized for necropsy at specific predetermined ages. In many instances, the tabulated data include (sometimes but not always as an explicit subgroup) mice that died spontaneously during the course of the study. The mice analyzed are often controls in a study of drug or radiation effects and are euthanatized at an age at which the effects can best be evaluated, often well before the mean or maximal life span of the population. A recent review[2] provides both an excellent example of such a cross-sectional study and a detailed bibliography of the earlier literature.

Studies of this type systematically underestimate lifetime incidence rates of all clinically significant illnesses and particularly rates of those diseases that, like lymphoma, typically lead rapidly to death. Thus, a design in which animals are euthanatized for study at the ages of 12, 18, and 24 months will greatly underestimate the incidence rate of a disease that leads to death within 2 months after the time at which it would have been histologically detectable. Animals destined to develop a particular lesion at any point after the 24-month end point would also, of course, be missed in the cross-sectional design. This point was dramatically illustrated by a study[33] in which tumor incidence was evaluated in (Af × B6)F_1 mice that died spontaneously at 30–41 months of age or were euthanatized at these

ages. Among the animals dying spontaneously, 62% (31/50) had malignant lymphoma. Among the animals euthanatized at similar ages, the lymphoma incidence was only 28% (5/18).

For these reasons, studies that include necropsies of animals that die spontaneously provide a much more accurate assessment of the lifetime incidence of major lesions than do cross-sectional studies. The yield of useful necropsy data, however, is typically much higher in the cross-sectional designs because postmortem autolysis too often interferes with histologic and sometimes even gross analysis of animals found dead. In studies that employ postmortem necropsy, for example, useful necropsy results are typically obtained from only 15–32% of the mice in the study.[33,34] Even when cages are inspected daily or several times each day, necropsy yields can be as low as 21%.[12]

Our data suggest that a strategy that combines assiduous inspection (including weekends) with a protocol for euthanatizing mice when they appear moribund can greatly increase the yield of useful necropsy data. Of 44 mice entered into the study, only two did not produce evaluable histopathology: one because of autolysis, and one because analysis after euthanasia did not produce evidence of disease. The high proportion of euthanatized animals that had signs of advanced disease suggests that experienced animal caretakers can indeed identify by inspection mice whose health has been severely compromised. The cost of providing increased surveillance is relatively low compared with the cost of rearing three to five old mice for each successful necropsy obtained.

Aging and disease in genetically heterogeneous mice

This is the first report of the terminal lesions of mice generated by a 4WC among inbred grandparental strains. The most common cause of death in the present study was malignant lymphoma. The pathogenesis of this lymphoma in mice is complex and involves the interaction of many factors, including both endogenous and exogenous murine leukemia viruses, genetic factors, and environmental factors including virologic status.[11,16,25] Two of the grandparental strains used in our cross, i.e., AKR[23] and SJL,[27] typically exhibit a high incidence of malignant lymphoma at relatively early ages. C57BL/6 mice have a high incidence of late life lymphoma,[22,24] but lymphoma is relatively rare in DBA/2J mice.[24] The relatively high incidence of lymphoma in our 4WC population may reflect, in part, the genetic contribution from the two high-lymphoma grandparents, and life span studies are now in progress with other 4WC populations to address this issue. However, both the incidence and the latency of mouse lymphomas are under complex control of multiple nondominant genes, including alleles of *H-2, Fv-1* and *Fv-2, RCS-1,* and other less well-characterized loci.[16,25] The reticulum cell sarcoma that appears in 90% of SJL mice, for example, appears in only 9% of F_1 hybrid mice.[3] Studies of F_1, F_2, F_3, and backcross mice generated between AKR and a low leukemia strain (RF) suggested that the incidence of AKR-type lymphoma was roughly proportional to the logarithm of the proportion of alleles contributed by the AKR strain.[16] Other genes, including *H-2,* modify the time of onset although not the incidence of AKR-type lymphoma in congenic strains,[14] perhaps by alteration in immune defenses. Thus, it is not generally possible to predict the expected spectrum or timing of lesions in a 4WC population by inspection of the lesions of the grandparental strains. Analysis of the phenotypic[18] or genetic characteristics of the individual members of such a population may provide insights into the regulation and pathogenesis of the major disease syndromes.

Although many of the 4WC mice were found to have some form of lymphoma at death and although in many cases the lymphoma had clearly been a major factor as a cause of death, we noted at least four histopathologically distinguishable subtypes of lymphoma, and it is likely that special methods, including the use of antibodies that distinguish T from B cells, would have provided evidence of additional diversity. At least four subtypes of lymphoma with characteristic natural histories and histopathologies—lymphoblastic, small lymphocytic, immunoblastic, and follicular center cell lymphomas—have been identified,[10,19,20] and examples of each type were noted in this study. Additional work will be needed to determine whether the incidence and latency of these lymphoma subtypes is under common genetic and pathophysiologic control. The lymphoma of SJL mice, one of the grandparents of our 4WC mice, was originally classified as reticulum cell sarcoma,[7] but some of these tumors have more recently been classified as follicular center cell lymphomas.[20] There is evidence that some of these SJL lymphomas may be derived from B cells[15,20,28] or from histiocytes or macrophages.[9] In the present series, none of the lymphomas fitted the description of histiocytic lymphoma except for one unusual tumor that appeared to arise in Kupffer cells (Table 3).

With the possible exception of the neurofibrosarcoma of the brachial plexus, none of the lesions seen in these hybrid mice were unique, and all have been seen, although often at very low incidence, in previous pathologic studies of aging mice.[2,4,26,29] Enamel organ dysplasia, noted in most of the older mice, was previously recognized[22] as a lesion that could lead to severe malformation of the teeth and subsequent death by inanition in old mice, but this lesion has been commonly overlooked in previous studies.

Males in our study had a significantly greater life span than females. Shorter life spans in females has

been reported for several inbred mouse strains and their F_1 hybrids.[24] Virgin female mice typically survive longer than parous females,[23] and frequent pregnancy may have been an important factor in the relatively low survival of our female mice. Certain fatal lesions, particularly pituitary and mammary tumors, were seen only in females; if incidence or progression of these two lesions were particularly likely to lead to early death, then preferential occurrence in females may have contributed to the sex-specific difference in average longevity. However, there was also a significant difference between the sexes in death from lymphoma, suggesting that other sex-specific factors, besides disease spectrum per se, are also likely to have contributed to differential longevity.

Choice of animal models of aging

Experienced research gerontologists have long recognized that haphazard selection of animal models can greatly compromise the usefulness and generalizability of conclusions drawn from studies of aging rodents.[5] The routine use of inbred rodents, often chosen because of easy availability or unquestioning adherence to tradition, has been recently subject to well-justified criticism.[17,31] Several authors have stressed the problems that can arise in interpreting results based upon studies of rodents whose environment, health, or nutritional status is not well defined.[8,17,30] Others have urged that conclusions based upon studies of a single genotype should not be considered definitive until data on several genotypes or several species become available.[13] Although inbred mice and rats may be required for studies that involve tissue transplantation and for certain sorts of genetic analyses, their idiosyncracies in physiologic characteristics and late life pathology can lead to serious difficulties in reproducibility and interpretation of results derived from a single strain. The assumption that the absence of genetic variation must necessarily lead to diminished phenotypic variability (and thus diminish the amount of work needed to generate convincing evidence of an age-associated difference in some outcome) has also been refuted by data showing that phenotypic variability in F_1 hybrid animals, including rodents, is often lower than that of their inbred parents.[21] Studies that were limited to individuals of a single genotype would be considered seriously flawed for analyses of human aging but are almost universal in analysis of aging in rodents.

Use of a genetically heterogeneous population of mice thus presents an opportunity to address experimental questions with greater confidence that the findings may well apply to mice of diverse genotypes. Genetically heterogeneous mice are of particular value for research in which a central goal is to seek correlations among age-sensitive characters as tests of explanatory hypotheses or to seek evidence for genetic control of an age-dependent process. Recent examples include a demonstration in a 4WC mouse population of a correlation between early life immune status and lymphoma incidence[18] and the observation that high levels of early life humoral immunity were associated with increased longevity in a segregating backcross between heterogeneous strains selected for differences in immune competence.[6] Genetically heterogeneous mouse populations are not, however, routinely available from commercial suppliers or from the NIA contract colonies. Several commercial concerns sell mice labeled as outbred, but according to the companies' own published technical publications, these mice have been derived from extremely small numbers of founder animals. Charles River Laboratories' CF-1® Mice, for example, are described as "intensively inbred by Carworth for 20 generations. This line was then reduced to a single pair and outbred from that point forward. . . ." The same supplier's Swiss-Webster mice (Crl:CFW®(SW)BR) are said to have been derived from "the selective inbreeding by Dr. Leslie Webster . . . The line selected was a single pair from which all CFW mice are descended." It seems unlikely that such nominally outbred populations provide a great deal of genetic heterogeneity. Genetic variability established by cross-breeding among four or more inbred lines can be maintained by a breeding system that employs a large number of mating pairs at each generation and also carefully minimizes mating between closely related animals, but this approach is too laborious for most laboratories, requires many years of work before establishment of genetic equilibrium, and still results in gradual but inexorable loss of genetic variability. 4WC mice, in contrast, can be produced with minimal technical skill and in arbitrary numbers from commercially available F_1 progenitors and provide a population of mice whose genetic structure is essentially invariant from place to place and time to time.

Introduction of novel animal models for gerontologic research requires preliminary work, such as that reported here, to describe the life span and pathology of the specific animal population. Our data suggest that 4WC mice will, as expected, show substantial variation in longevity and disease spectrum. Various forms of lymphoma were a common cause of and incidental finding at death in these mice. Lymphoma was also frequently seen in other studies that acquired terminal necropsy data on genetically heterogeneous mice[6,12] and may indeed be a frequent cause of late life illness in mice of a wide range of genotypes, just as the sequelae of vascular narrowing and insulin resistance are a common cause of death in outbred humans. Lymphoma is also the most common cause of death in some but not all populations of laboratory aged mice originally captured in the wild;[11] further work on the genetic and

environmental factors that regulate life span in lymphoma-prone and lymphoma-resistant heterogeneous populations is clearly called for.

Are genetically heterogeneous mice also heterogeneous phenotypically? Phelan and Austad[21] have reviewed evidence that inbred animals exhibit relatively high levels of intrastrain phenotypic variance, and the theoretical argument that the genetic compromises entailed in inbreeding to homozygosity lead to diminished homeostatic control and thus hyperresponsiveness to environmental variation. It is not clear whether 4WC mice would be expected to exhibit more or less phenotypic variation than their inbred grandparents. One approach to this question would be to compare coefficients of variation (CV), the ratio of the standard deviation of a set of measurements to the mean of the same measurements, for a variety of relevant variables. For the 43 mice in this study, the CV for life span was 26%, and it was greater for males (23%) than for females (20%). In a previous study of longevity in groups of approximately 20 female mice in each of 20 recombinant inbred (RI) strains derived from the $B6D2F_1$ hybrid, the average CV, weighted to reflect the numbers of mice in each RI strain, was 24% (range: 17–60%),[12] which is comparable to but slightly greater than the CV for our own female animals. Thus, our life span data do not support the hypothesis that phenotypic variance in a heterogeneous population will always exceed that in inbred mice. Other morphometric and performance measures, however, do show greater variance in 4WC mice than in inbred lines (D. Harrison et al., in preparation), and the relative influence of homeostatic control and genetic influence on phenotype may well vary depending on the phenotype under study.

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