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Life Table Analysis and Pathologic Observations in Male Mice of a Long-Lived Hybrid Strain $(A_f \times C57BL/6)F_1$

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We have utilized a long-lived ($A_f \times C57BL/6)F_1$ hybrid strain of mice for a variety of aging studies. In this report we have characterized the life expectancy and pattern of spontaneous deaths in 202 mice, malignant and nonmalignant lesions in 64 male mice dying spontaneously, and organ weights and lesions in 39 male mice killed at selected ages. The maximum age observed was 41.5 months. The principal causes of death were malignant lymphoma and alveologenic neoplasms, which were present in 56.3% and 45.3%, respectively, of the mice dying spontaneously. A variety of other neoplastic and non-neoplastic lesions that are not infrequently seen in older mice were observed in these mice. Neoplasms seen in these mice that are rare in other mice included disseminated mast cell tumors in two mice and gastric adenocarcinoma in one mouse. In comparing the diseases observed in this hybrid strain with those reported for the parent strains, there was an incidence of malignant lymphoma similar to the C57BL/6 parent, an incidence of alveologenic neoplasms intermediate between the parent strains, and a markedly reduced incidence of amyloidosis. This study provides a detailed background of baseline hematologic and morphologic data in a long-lived hybrid of two commonly used strains of mice.

PATHOLOGICAL alterations may be considered as age-associated if they (a) are found at necropsy in animals that are sacrificed at varying ages or permitted to live out their natural life spans; (b) have not died of a single major disease; and (c) are raised in a colony that is free of life-shortening intercurrent infectious diseases. It is critical to compile these data for each strain of animals used in aging research so that investigators working with these animals can be adequately informed about the particular spectrum of pathologic changes that occur naturally (Zurcher et al., 1982).

The (A_f/StTrWo × C57BL/6JTrWo) mouse, herein abbreviated as $AB6F_1$, has been used extensively by one of us (NSW) for studies on bone marrow transplantation and hemopoiesis. We selected this hybrid strain for studies on the effects of aging on hemopoiesis because of our extensive accumulated experience with it. Survival data for (A × C57BL/6)F_1 and (C57BL/6 × A)F_1 hybrids have been reported by previous workers, as noted below.

In this report we examined $AB6F_1$ hybrid mice to characterize life span, pathologic alterations, organ weights, and hematologic values. This information was thought to be of value because of the relative lack of detailed pathological data in previous aging studies of the parent strains and their absence in the hybrid strain (Myers, 1978; Goodrick, 1975; Russell, 1966).

Materials and Methods

Animal history. — All mice in this study were (A_f/StTrWo × C57BL/6JTrWo)F_1 hybrids. The subscript *f* indicates that the parental A strain was foster-reared to be free of Bittner mammary tumor virus. The occurrence of mammary tumor has been less than 1/100 in breeding and nonbreeding females of ages 3 to 15 months. The parental strains had been inbred on these premises for 16 years (approximately 20 generations), having been obtained by one of us (Wo) from the breeding colony of J. J. Trentin (Tr), Baylor Medical College, where they had been inbred for a similar period of time previously. The A_fTrWo strain originated from the Strong subline (St) of the Jackson (Jax) laboratory (Bar Harbor, ME), while the C57BL/6TrWo were also of Jax (J) origin.

The animals were kept under closed colony, barrier-maintained specific pathogen-free (SPF) conditions with all contact materials sterilized by autoclaving and filtered unidirectional airflow. The drinking water was acidified to pH 2.5 by addition of HCl to give a final concentration of 0.005 N. Food (Wayne sterilizable rodent blox, Wayne Pet Food Division, Chicago) and bedding (1/8-in-diameter corncob) were autoclaved according to manufacturer's instructions. Animal handlers wore sterile masks, gowns, and gloves. During the 4-year period in which this study was conducted, no outbreaks of clinical disease were observed. Some animals in the parental breeding colony, which numbered around 1000 animals, were found on routine periodic serological testing to be seropositive for mouse hepatitis, Sendai, Reo 3, pneumonia virus of mice, and minute virus of mice. Among the contemporary population of $AB6F_1$ from which these aging study groups were drawn, 4/18 mice tested were positive for mouse hepatitis (fluorescent antibody test), 4 for PVM and 2 for MVM, all were negative for Sendai virus. The panel of organisms tested for consisted of mycoplasma, PVM, GDVII, Sendai, polyoma, MVM, KRV, H-1, MHV,

ectromelia, LCM, and Reo 3. While bacterial cultures of respiratory tract and abdominal organs produced only microorganisms normally considered to be nonpathogens, the facultative pathogens *Pseudomonas aeruginosa* and *Pasturella pneumotropica* were occasionally present. Mycoplasma was not isolated, nor were serum antibodies present. These mice were also negative for external and internal parasites. The tested animals were not included in this study. Fifty weanling male mice were set aside every 4 months for the aging studies, which included numerous experiments other than the survey reported here.

Pathology. — Animals for necropsy came from two sources within the group set aside for aging, those that died spontaneously and those that were euthanized to allow for pathologic evaluation of apparently healthy mice at selected ages. Of the 202 mice that died spontaneously over time, 64 were selected as showing no gross evidence of postmortem autolysis. Complete necropsies were performed on 39 euthanized mice. Mice chosen for euthanasia were taken in small groups of mixed ages over a 3-year period.

Tissues examined from mice that were euthanized included cerebrum, cerebellum, thyroid, parathyroid, larynx, trachea, heart, lung, thymus, salivary gland, esophagus, stomach, duodenum, ileum, cecum, colon, pancreas, liver, gall bladder, spleen, mesentric lymph node, kidney, adrenal, testicle, epididymus, bladder, skin, bone marrow, nasal turbinate, and any gross lesions. Only major abdominal and thoracic organs were collected on mice that died spontaneously. After anesthesia with Halothane, mice were euthanized by cervical dislocation followed by exsanguination.

Tissues were fixed in 10% neutral, phosphate-buffered formalin, embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin and eosin. Occasional sections were stained with Giemsa to demonstrate metachromatic granules of mast cells and with Gomori's methanamine silver to stain glomerular basement membranes (Thompson, 1966).

Organ weights were obtained only on the mice that were euthanized. Heparinized blood from some of these was collected from the retroorbital plexus and packed cell volume, and total white cell counts were obtained by the capillary tube microhematocrit method and Coulter counting, respectively.

Statistical method. — The chi square method was used to compare suitable groups for incidence of lesions. Organ and body weights were compared by the two-tailed Student's *t*-test.

RESULTS

A survival curve depicting the ages of 202 male mice at the time of spontaneous death is shown in Figure 1. The mean age of mice that died spontaneously was 32.6 months; the range was 10.0–41.5 months.

A total of 64 mice of the 202 that died spontaneously were chosen for necropsy on the basis of no gross evidence of postmortem changes. Also, 39 mice that were euthanized were necropsied. Mice in groups of 6–10 were euthanized at 6–7, 12–13, 18–24, 30–35, and 36–41.5 months of age. The principal lesions noted were neoplasms. A summary of observations on these mice is shown in Table 1.

The major cause of death in mice that died spontaneously was malignant lymphoma, which affected 56.3% of these mice. The mean age at death of mice dying of malignant lymphoma was 32.0 months, essentially the same as that for the whole group. In mice that were euthanized, the incidence of malignant lymphoma was much less (15.4%), but the mean age of diagnosis was only slightly earlier (31.2 months). Although malignant lymphomas were not subclassified, the great majority were composed of both lymphocytes and histiocytes, compatible with the reticulum cell sarcoma type B of Dunn (1954) or the malignant lymphoma, pleomorphic type of Squire et al. (1978). They were multicentric, affecting principally the spleen, liver, kidney and regional lymph nodes.

The second most common neoplasm observed was the alveologenic neoplasm, also known as bronchio-alveolar adenoma, bronchiolo-alveolar carcinoma, pulmonary adenoma, and alveologenic adenocarcinoma (Squire et al., 1978; Stewart et al., 1970). We did not attempt to classify these into benign or malignant categories because many investigators believe that all these neoplasms have malignant potential (Squire et al., 1978; Stewart et al., 1970). These neoplasms occurred as single or multiple nodules varying in diameter from 0.2 to 3.0 cm. No metastases to other organs were observed.

Alveologenic neoplasms were observed in 29/64 (45.3%) mice that died, and the mean age of affected mice was 32.9 months. These neoplasms were found in 9/39 (23.1%) of the

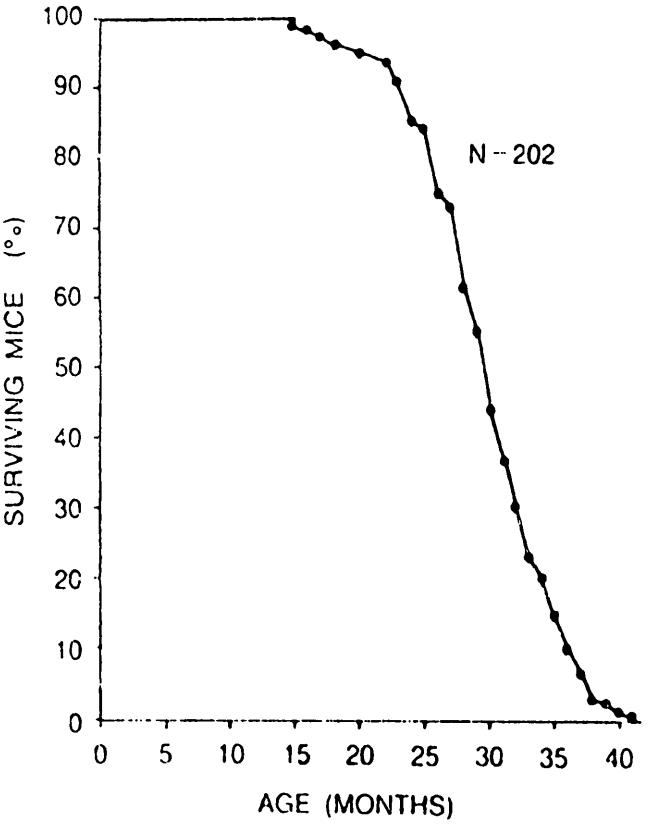

Figure 1. Age at time of death of 202 male AB6F$_1$ mice. Period from 0 to 16 months is truncated.

Table 1. Neoplasms Observed in Male AB6F$_1$ Mice

		Age Groups (months)						
		06–07	12–13	18–24	25–29	30–35	36–41	Combined age groups
Number of Mice Examined	Spontaneous deaths	0	0	2	12	34	16	64
	Euthanized	7	6	8	ND	10	8	39
Fraction With:		Occurrence by Age Grouping						
Malignant lymphoma	Spontaneous deaths	—	—	0	5/12	23/34*	8/16	
	Euthanized	0	0	1/8	ND	2/10*	3/8	
Alveologenic neoplasm	Spontaneous deaths	—	—	0	7/12	14/34	8/16	
	Euthanized	0	0	1/8	ND	4/10	4/8	
Hepatocellular adenoma	Spontaneous deaths	—	—	0	0	0	1/16	
	Euthanized	0	0	0	ND	1/10	0	
Hepatocellular carcinoma	Spontaneous deaths	—	—	0	0	3/34	1/16	
	Euthanized	0	0	0	ND	0	0	
Hemangioma	Spontaneous deaths	—	—	0	0	1/34	1/16	
	Euthanized	0	0	0	ND	0	0	
Hemangiosarcoma	Spontaneous deaths	—	—	0	1/12	0	1/16	
	Euthanized	0	0	0	ND	0	0	
Mast cell tumor	Spontaneous deaths	—	—	0	0	0	0	
	Euthanized	0	0	0	ND	1/10	1/8	
Astrocytoma	Spontaneous deaths	—	—	0	0	1/34	0	
	Euthanized	0	0	0	ND	0	0	
Adrenal adenoma	Spontaneous deaths	—	—	0	0	1/34	0	
	Euthanized	0	0	0	ND	0	0	
Myelosarcoma	Spontaneous deaths	—	—	0	0	1/34	0	
	Euthanized	0	0	0	ND	0	0	
Fibrosarcoma	Spontaneous deaths	—	—	0	0	0	0	
	Euthanized	0	1/6	0	ND	0	0	
Gastric carcinoma	Spontaneous deaths	—	—	0	0	0	0	
	Euthanized	0	0	0	ND	0	1/8	
Mice with at least one neoplasm	Spontaneous deaths	—	—	0	10/12	32/34	15/16	
	Euthanized	0	1/6	2/8	ND	7/10	8/8	
Mice with more than one neoplasm	Spontaneous deaths	—	—	0	3/12	11/34	4/16	
	Euthanized	0	0	0	ND	2/10	1/8	

*$p = < .05$ in comparing these two values. Other suitable comparisons by chi square method are N.S.

mice that were euthanized, and the mean age of diagnosis was 31.5 months.

Hepatocellular tumors were the third most frequently observed neoplasms. One of these was considered a hepacellular adenoma (Type A nodule of Walker et al., 1973) and was observed in a 38-month-old mouse that died spontaneously. The remaining four, also found in spontaneously dying mice, were considered hepatocellular carcinomas (Type B nodule of Walker et al., 1973), and three of these had metastisized to the lungs. The mean age of diagnosis in both classifications was 33.4 months.

A variety of other neoplasms were observed in these mice that are not infrequently seen in pathologic studies of older mice (Squire et al., 1978; Ward et al., 1979; Zurcher et al., 1982). These included two hemangiomas, two hemangiosarcomas, one astrocytoma, one adrenal cortical adenoma, one myelosarcoma, and one fibrosarcoma.

Three neoplasms deserve special mention because of their scarcity. In 2/39 mice that were euthanized, there were multiple mast cell tumors. These were characterized by deposits of mature mast cells in liver, spleen, lung, kidney and brain (Figure 2). The mast cells were well differentiated, did not have mitotic figures, and had metachromatic granules that stained with Giemsa. They displaced normal parenchymal tissue, but there was no suggestion of compression of surrounding tissues. The two affected mice were 30–36 months old. Another interesting and rare neoplasm was a gastric adenocarcinoma (Figures 3 and 4) observed in the greater curvature of the stomach of the oldest mouse in this study, a mouse which was euthanized when 41.5 months old. The neoplastic cells were anaplastic and formed pleomorphic acini. These cells had a high nuclear-to-cytoplasmic ratio, increased mitotic index, eosinophilic cytoplasm, and indistinct cell boundaries. It appeared to have originated in the fundus and had invaded the tunica muscularis and serosa of the greater curvature.

We have summarized the major non-neoplastic findings separately for euthanized and spontaneous death mice in Table 2. The most common finding was lymphocytic infiltration in visceral organs such as lung, kidney, salivary gland, and liver. This was diagnosed in a total of 21 mice. It was more often found in the euthanized group than in spontane-

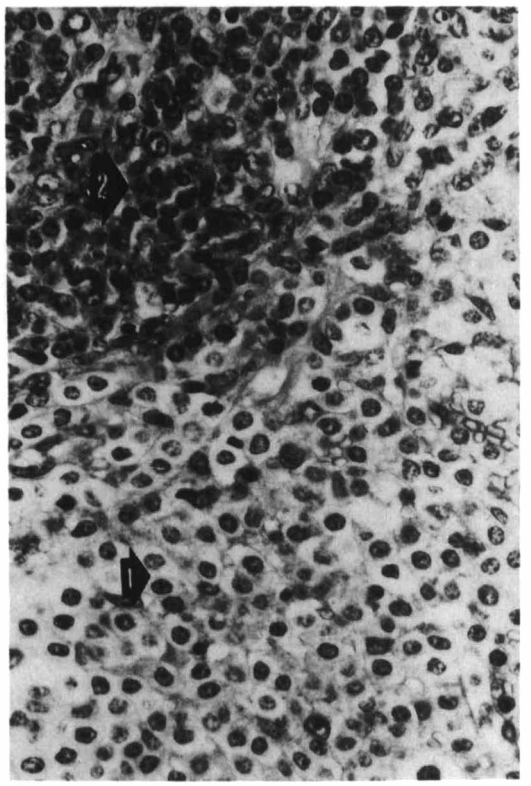

Figure 2. Mast cell tumor in spleen of a 30-month-old mouse. Mast cells (1) have replaced most of the red pulp, leaving the Malpighian corpuscles (2) intact. H + E × 384.

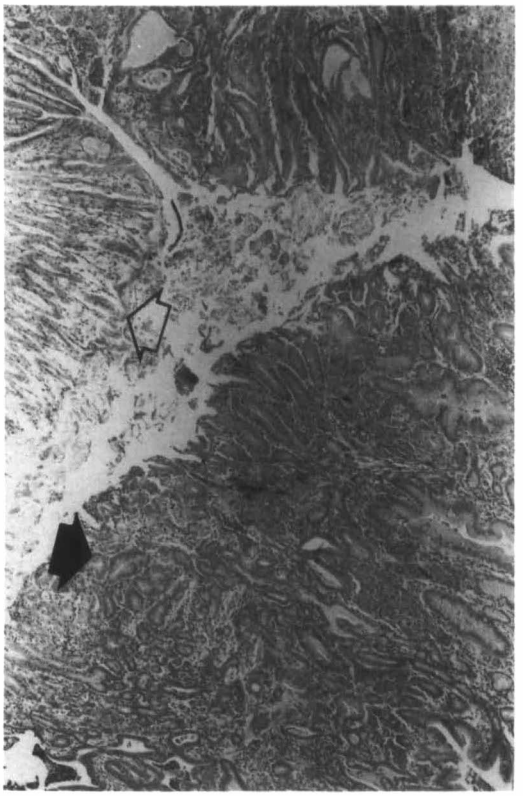

Figure 3. Gastric adenocarcinoma (dark arrow) affecting the fundus of the stomach of a 41.5-month-old mouse. A portion of the opposite wall of the stomach is normal (light arrow). H + E × 39.

ous deaths, even as early as 6 months of age. It represented a mirror image of the occurrence of lymphoma, since it could not be determined in animals dying with lymphoma. These infiltrates were non-neoplastic and occurred as small aggregates around blood vessels.

Glomerulonephritis of mild to moderate severity was observed in 20 mice. It was characterized by increase in mesangial cells and mesangial matrix, usually associated with accentuated lobularity of the glomerular tuft. It did not appear to be severe enough to cause renal dysfunction. Amyloidosis was observed in five mice, all of which had advanced neoplasia. Amyloid deposition was primarily in the glomeruli and interstitium of the kidneys, but it was observed around blood vessels of the heart in one animal.

Enlarged seminal vesicles were observed in six mice, all in the oldest group that was euthanized (not shown in Table 2). These organs became so large that they caused distension of the abdomen, occupying up to 50% of the space, and were filled with homogenous eosinophilic secretory product indistinguishable from that of normal seminal vesicles. A similar finding has been reported in detail in C57BL/6 mice by Finch and Girgis (1974).

Additional lesions observed sporadically in these mice were hydronephrosis, arteritis, mineralization of brain, and cardiomyopathy.

Organ weights and hematologic values. — Summarized in Table 3 are organ weights and hematologic values of the

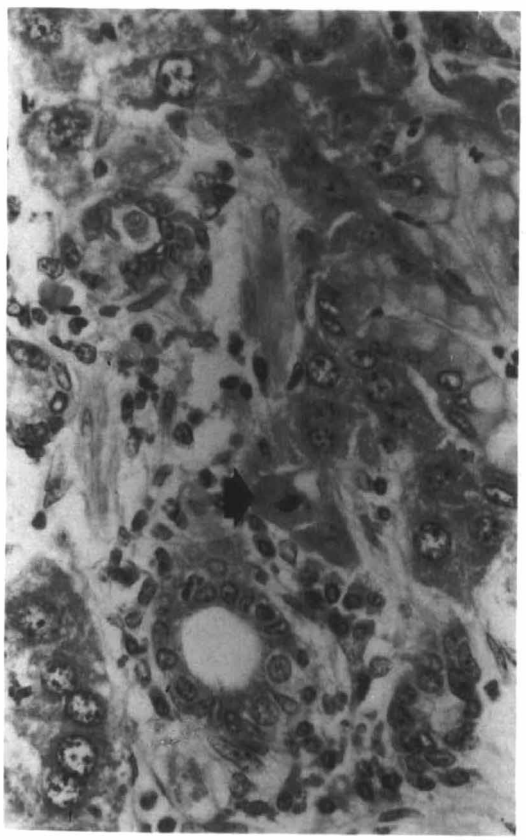

Figure 4. Higher power of the above neoplasm. Irregularly shaped acini are formed by pleomorphic epithelial cells with mitotic figures (arrow). H + E × 384.

Table 2. Non-neoplastic Lesions Observed in Male AB6F$_1$ Mice

		Age Groups (months)						
		06–07	12–13	18–24	25–29	30–35	36–41	Combined age groups
Number of Mice Examined	Spontaneous deaths	0	0	2	12	34	16	64
	Euthanized	7	6	8	ND	10	8	39
Fraction With:		Occurrence by Age Grouping						
Visceral lymphocytic infiltration	Spontaneous deaths	—	—	1/2	1/12	0/34*	2/16	
	Euthanized	3/7	3/6	2/8	ND	5/10*	4/8	
Glomerulonephritis	Spontaneous deaths	—	—	0	4/12	4/34	3/16	
	Euthanized	0	1/6	2/8	ND	2/10	4/8	
Hydronephosis	Spontaneous deaths	—	—	0	0	1/34	0	
	Euthanized	1/7	0	0	ND	0	0	
Amyloidosis	Spontaneous deaths	—	—	0	0	2/34	1/16	
	Euthanized	0	0	1/8	ND	1/10	0	
Mineralization of the brain	Spontaneous deaths	ND	ND	ND	ND	ND	ND	
	Euthanized	0	1/6	3/8	ND	1/10	0	
Arteritis/myocarditis	Spontaneous deaths	—	—	0	0	2/16	0	
	Euthanized	0	0	1/8	ND	0	0	
Hepatitis/necrosis	Spontaneous deaths	—	—	1/2	2/12	4/34	3/16	
	Euthanized	0	0	0	ND	0	0	

Note: Visceral lymphocytic infiltration was present in one or more organs of the majority of animals with frank lymphosarcoma, but was not listed as a separate entity there because of possible redundancy with the malignant entity. Eight of the 21 instances of this lesion in euthanized mice were limited to the lung. The other most common sites were liver and kidneys (these organs followed the spleen as the most common sites for lymphomas in the older mice). The paucity of classified lymphocytic infiltration cases among the spontaneously dying mice is directly related to the high incidence of frank lymphoma there, such mice being removed from the infiltration classification.

*The p value for 30–35 months comparing spontaneous deaths to euthanized = < .005, other values N.S.

Table 3. Body and Organ Weights (in grams) of Euthanized AB6F$_1$ Mice by Age

Age groups (months)		Body weight	Heart	Lung	Liver	L Kidney	R Kidney	Gonads	Spleen	Brain	Blood	
											Hct.	WBC
06–07	Mean	34.4	.148	.186	1.390	.216	.206	.195	.086	.417	ND	ND
	SD	3.2	.022	.041	.175	.038	.057	.005	.012	.029		
	n	7	8	7	8	8	8	8	8	7		
12–13	Mean	44.3	.168	.171	1.754	.281	.268	.180	.092	.424	ND	ND
	SD	5.1	.019	.037	.126	.033	.011	.008	.034	.030		
	n	5	5	5	5	5	5	5	5	5		
18–24	Mean	47.6	.198	.214	2.012	.340	.317	.182	.100	.424	ND	ND
	SD	7.1	.036	.092	.493	.054	.048	.020	.023	.048		
	n	5	5	5	5	5	5	5	5	4		
25–29	Mean	49.0	.236	.311	1.829	.311	.311	.200	.139	.462	47.8	7.8
	SD	6.7	.070	.111	.362	.043	.062	.006	.074	.023	3.5	1.3
	n	11	12	12	12	12	12	12	12	12	12	12
30–35	Mean	37.9	.225	.378	2.120	.330	.342	.199	.475	.451	43	14.9
	SD	7.0	.061	.173	.957	.060	.059	.046	.610	.028	10.8	13.9
	n	18	15	18	15	15	15	18	18	17	12	12
36–41	Mean	35.1	.194	.556	1.934	.275	.294	.183	.504	.459	48.3	12.1
	SD	3.8	.025	.293	1.029	.022	.026	.029	.639	.027	7.2	11.1
	n	9	8	9	9	9	9	9	9	8	9	9

Note: Organ weight values to three places correspond to actual measurements made on a milligram balance.

*p < .01 or better for:

Heart: 6–24 mo vs 25–28 mo and vs 30–35 mo

Lung: 6–24 mo vs all groups > 24 mo

Liver and Spleen: < 29 mo vs all groups > 30 mo

Kidney: < 29 mo vs 30–35 mo, also 30–35 vs 36–41 mo

Brain: 6–24 mo vs all groups > 24 mo.

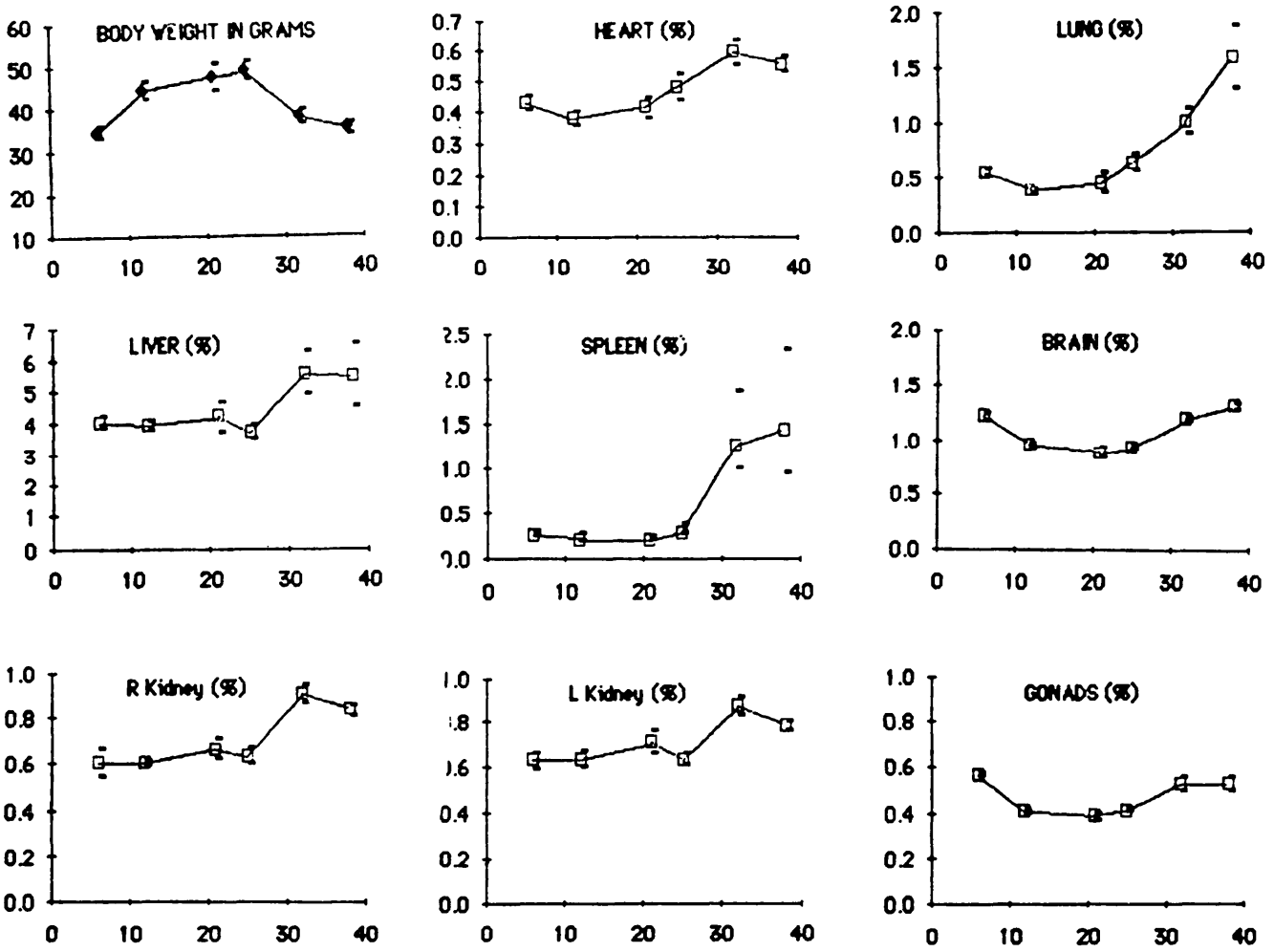

Figure 5. Organ weights as percent of body weight for AB6F$_1$ mice of various ages (months). Error bars are *SEM* for body weight graph and *SEM* of percent of mean body weight of the respective age group for the other graphs in the figure. Statistical Significance (relative organ weight): $p < .02$ or better for all organs for all points > 30 months when compared to pooled data for < 30 months with one exception: Gonads $p < .05$ comparing groups < 30 months with 36–41 months.

euthanized mice of varying age groups similar to Tables 1 and 2, except that a single cohort of animals available at 25 months of age has been added. They are presented by regression analysis as percent of body weight in Figure 5. There were four patterns of change in absolute organ weights with age: no change in organ weights, an increase reaching a plateau, a progressive increase followed by a sharp decrease in the oldest mice, and a progressive increase. The testes weights did not vary significantly as a function of age. Brain and liver increased in weight until midlife, then held steady. The organs or measurements which progressively increased in weight, reached their maximum in 25-month-old or 30- to 35-month-old mice, then declined in the oldest mice were total body weight, kidney, and heart. One organ, the spleen, increased progressively in weight with age.

When considered as percent of body weight, all organs showed relative increases in the later one-third of life span. Most marked were heart, lung, liver, kidney, and spleen. The large absolute and relative increases in lung and spleen may well have been related to premalignant or early malignant increases in tissue mass and were emphasized by the decrease in mean body weight after 25 months. In fact, 6 of 12, 8 of 18, and 6 of 9 euthanized mice in Table 3 of age groups 25–29, 30–35, and 36–41 months, respectively, contained early or progressing lung tumors. The increasing incidence of lymphoma, primary to the spleen, has been documented in Table 1.

No significant differences with age were noted in the hematocrit values, but the total mean white count was increased in the older mice because of quite high counts in several individual mice.

Discussion

Meyers (1978) has reviewed the mean life span (MLS) data, published by five authors for a total of 10 inbred strains. In three of the four publications which gave applicable data, the C57BL/6 strain ranked very high in survival, whereas strain A ranked low. His data, compiled from several sources, found the A/HeJ strain males to be shorter lived than the A/J (492 versus 604 days). His MLS for male C57BL/6 mice was 815 days, while Kunstyr and Leuenberger (1975) reported 878 days. MLS for the (A/J ×

C57BL/6)F$_1$ males was 892 days, which may reflect hybrid vigor. The latter figure is less than the MLS of 975 days of our AB6F$_1$ males. We note that Goodrick (1975) reported a MLS of 942 days for his study of male (C57BL/6 × A/J)F$_1$ mice, while Russell (1966) reported 673 days for the same hybrid strain, which differs from ours only in the male/female relationship of the parental strains and, of course, the continued inbreeding in different laboratories. The different times at which these two reports were published probably reflect the advances made in the quality of care and microbiological protection during recent years.

A large amount of data has been accumulated on the spectrum of lesions seen in a number of strains in mice, including the noninbred CD-1 HaM/ICR (Homburger et al., 1975; Percy and Jonas, 1971), inbred B6C3F$_1$ hybrids (Tarone et al., 1981; Ward et al., 1979), BALB/c (Cosgrove et al., 1978; Madison et al., 1968), and a variety of other mouse strains (Sher, 1974; Storer, 1966).

Because of the lack of pathological data on the (A × C57BL/6)F$_1$ hybrid in the literature, we focus here on that available for the two parent strains, the strain A and the C57BL/6, for comparative purposes. Strain A mice have been variously reported to have occurrence rates for lymphoreticular tumors of 10–43%, 0–9%, and less than 1%; for pulmonary tumors of 4–31%, 0–80%, and 0–2.5%; and for liver tumors of 0–12%, 0–12.5%, and 0–6.3% (Festing and Blackmore, 1971; Cohen et al., 1984; Kawada and Ojima, 1978; listed respectively for each tumor type). C57BL/6 mice have been variously reported to have occurrence rates for lymphoreticular tumors of 97%, 0–25%, and 0–17%; for pulmonary tumors of 8%, 0–50%, and 0%; and for liver tumors of 3%, 0%, and 0–0.6% (Hurvitz, 1978; Cohen et al., 1984; Kawada and Ojima, 1978; listed respectively for each tumor type). In most of the reports the highest occurrence rates of the tumors were in mice older than 18 months (thus the wide spread in several instances). Our occurrence rate of lymphoreticular neoplasms (0–68%), depending upon the age group, was near the high end of the findings for the C57BL/6 strain as reported by the above authors. Our occurrence rate of pulmonary tumors (0–58%) was in the middle range of findings for strain A. Our occurrence of hepatic tumors (0–9%) was somewhat higher than for the C57BL/6, but midrange for strain A. A high incidence of lymphoreticular tumors is characteristic of the C57BL/6 strain and that of pulmonary tumors is characteristic of strain A mice (Cohen et al., 1984; Murphy, 1966), while lymphoreticular disease is also not uncommon in strain A (Festing and Blackmore, 1971).

We studied a group of mice which were hybrids of C57BL/6 and strain A. Probably this genetic background has led to a spectrum of tumors and tumor incidence representative of both the parent strains (see above). Although previous studies have provided survival data on this hybrid (Myers, 1978; Goodrick, 1975; Russell, 1966), we believe ours is the first to provide extensive organ weight and pathological data.

We believe the higher overall incidence of malignant lymphomas and alveologenic neoplasms in our mice dying spontaneously is due to the rapidly fatal course which ensues once the frank malignancy appears. Thus, the incidence of these tumors in mice that died was not predictive of the incidence in mice that were euthanized, as was also found for several tumors in rats by Burek (1978). In particular, the occurrence both of malignant lymphoma and lymphocytic infiltration of organs was high in the spontaneous death and euthanized groups, respectively. The infiltration appeared possibly to be a forerunner of the lymphoma and was not listed separately in lymphomatous mice (see footnote, Table 2).

Two kinds of neoplasms of extreme rarity were observed in these mice. Two mice had multicentric mast cell tumors. These neoplasms are rare in mice (Furmanski and Rich, 1982), and it is unusual to find two in 39 euthanized mice. We can find no reports of spontaneous carcinomas of the stomach in mice, although they have been induced by various carcinogens (Newbern and McConnell, 1982). Perhaps these neoplasms are rare only because most mice do not live as long as those in our study. These three tumors were found in mice 30, 36, and 41.5 months old, respectively. The findings underscore the value of a careful pathologic study of long-lived mice.

None of the non-neoplastic lesions appeared to be age-related with the exception of the enlarged seminal vesicles. While Zurcher et al. (1982) found amyloidosis in 83% of male C57BL/6 mice, it was seen in only 7/104 of our AB6F$_1$ mice. We did not observe the "acidophilic macrophage pneumonia" which they saw in 30% of their males.

Body and organ weights of our F$_1$ hybrids might be compared with those of virgin male C57BL mice (a strain which diverged from common stock with the C57BL/6 several decades ago) as reported by Rowlatt et al. (1976), as we were unable to find any such previously recorded data for C57BL/6, strain A or AB6F$_1$ mice. Our mice had larger total body, kidney, spleen, and liver weights, but smaller brain and testicular weights. Although those authors did not examine mice older than 30 months of age, there was a similar pattern to our mice of rising, then falling, values with age for total body and kidney weights, but not for heart weights. There was also a similar progressive increase in splenic weights. Unlike our data, they reported a progressive decrease in testicular and seminal vesicle weights with age. We did not exclude animals with alveolar neoplasia or lymphoma from our organ weight data. We believe the real or relative increase in the lung and the spleen and kidney weights with age was related to an increase in incidence of tumors (adenocarcinoma and lymphoma, respectively) and the lymphocytic infiltration which appears to precede the latter. We believe the decline in body and organ weights in our oldest mice was due to decrease in body and organ fat. These mice were much thinner than the younger mice, thus emphasizing the organ/body weight ratio of these organs, as seen in Figure 5.

We suggest that the increase in total WBC count in older mice may have been due to the higher frequency of lymphoproliferative disease, which is often accompanied by leukemia, as reported in mice with C57BL/6 genetic background (Murphy, 1966; Frith and Wiley, 1981). Unfortunately, differential counts were not carried out in our mice.

In summary, five age-related parameters — mean life span, the pattern of spontaneous deaths, the lesions present

at the time of spontaneous death, the lesions present at timed sacrifices, and a progression of organ weights — have been reported for male AB6F$_1$ mice. These mice had a relatively long mean life span of 975 days despite the rather high incidence of late-appearing lymphoreticular tumors and lung tumors, as well as other age-associated lesions. These two tumors, in particular, are often associated with the parental C57BL/6 and strain A, respectively, and probably represent inheritance of genetic susceptibility. We noted an increase in body weight until the last few months of life, when a decrease became apparent. Most organs increased in weight with time. The kidneys, lungs, and spleen increased markedly. The later increases were almost certainly due to the presence of a slowly developing tumor or premalignant infiltration in many mice. Very large seminal vesicles were present in the older mice, sometimes interfering with research procedures. Our findings point out the differences which are found in the occurrence of mortality producing lesions between groups of euthanized mice and those dying spontaneously. The added value of presenting data for chronologically serial age groups rather than reporting findings only with mean age at time of death is also evident.

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