Laboratory Animals (1990) **24**, 332–340

Background pathology in BDF_1 mice allowed to live out their life-span

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Summary

Fifty male and 50 female BDF_1 mice were observed allowing them to live out their life-span. Mortality up to 104 weeks of age was higher in males (42%) than in females (34%), and the 50% survival age was 112 weeks for males and 118 weeks for females. Body weight reached the peak at 82 weeks of age in males and 92 weeks of age in females, showing the mean body weight of 54·3 g for males and 48·0 g for females. The incidence of calculi and proteinaceous casts in the kidneys, that were not associated with exposure to chloroform, cell-alteration in the adrenal cortex, and islet cell hyperplasia in the pancreas was significantly higher in males than in females. On the other hand, hyaline droplet degeneration of the renal tubular epithelium, spindle cell proliferation in the adrenal cortex and milk-retention in the mammary glands occurred at a significantly higher incidence in females than in males. Cerebral mineralization in both sexes, atrophy and calcification of the testes and enlargement of the seminal vesicles of males, as well as cyst-formations in the ovary and endometrium of females developed at a very high incidence. Frequent neoplasms in males were hepatocellular adenomas and carcinomas, blood vessel tumours, pulmonary adenomas and carcinomas, and malignant lymphomas. In females, malignant lymphomas were the most common neoplasm, followed by blood vessel tumours, chromophobe pituitary adenomas and hepatocellular adenomas. Hepatocellular carcinomas developed only in males, whereas the histiocytic and lymphocytic types of malignant lymphomas and chromophobe cell adenomas arose solely or at a significantly higher incidence in females than in males.

Keywords: Life-span data; BDF_1 mice

Received 5 May 1988; accepted 29 January 1990

It is important to establish base-line data on age-related changes occurring in individual strains of mice, because there are strain-differences in the incidence of spontaneous lesions (Rowlatt *et al.*, 1969; Percy & Jonas, 1971; Smith *et al.*, 1973; Homburger *et al.*, 1975; Ward *et al.*, 1979; Frith *et al.*, 1983; Dragani *et al.*, 1984; Yamate *et al.*, 1986). Interlaboratory variations have also been shown in the incidence of some naturally occurring tumours in mice and rats, probably depending on diets, environmental conditions, length of experiments, and the process of pathological examinations (Tarone *et al.*, 1981; Sher *et al.*, 1982).

BDF_1 mice are produced by the crossing of C57BL/6 females and DBA/2N males, and have frequently been used in screening the antitumour activities of chemicals (Rao *et al.*, 1985; Abdallah *et al.*, 1987). To the best of our knowledge, however, there has been only one life-time study of the incidence of spontaneous tumours in this strain, and that was in vasectomized BDF_1 mice, compared with sham-operated animals (Anderson *et al.*, 1983).

In the present study, therefore, we investigated mortalities, body weight changes and spontaneous lesions in male and female BDF_1 mice maintained under barrier conditions throughout their life.

Materials and methods

Animals and environment

Fifty male and 50 female CRJ : BDF_1 [(C57BL/6 × DBA/2N)F_1] specific pathogen-free mice were

Table 1. Mortalities in male and female BDF_1 mice

Sex	No. of mice examined	Cumulative mortalities (%) at indicated weeks 52	78	104	130	Age (week) of 50% survival	Age (week) of the longest lived mouse
Male	50	4	14	42	74	112	158
Female	50	2	8	34	82	118	150

obtained from the Charles River Japan Co., Ltd, Kanagawa Prefecture, Japan, at the age of 4 weeks, weighing $11{\cdot}9 \pm 0{\cdot}9$ g for males and $11{\cdot}6 \pm 1{\cdot}0$ g for females. The animals were acclimatized for one week before commencement of the experiment. Throughout the experiment, 2 mice of the same sex were housed to a polycarbonate cage ($29 \times 14 \times 15$ cm) with sterilized wood shavings for bedding. The animal room was kept under positive air pressure and ventilated 10–12 times per hour with filtered fresh air. Temperature and relative humidity were controlled at 23 ± 2°C and 50 ± 20%, respectively, and lighting controlled to give 12 h light and 12 h dark per day. All mice had free access to standard commercial pelleted diet (CRF-1 prepared by Oriental Yeast Co., Ltd, Tokyo, Japan, containing 23·2% crude protein, 5·7% crude fat, 6·4% ash, and 2·3% crude fibre) and tap water.

Experimental design

The animals were inspected daily and weighed once a month between 5 and 130 weeks of age. Complete necropsies were performed on all mice. Moribund animals were killed by exsanguination under anaesthesia with a mixture of alcohol, ether and chloroform in a dissecting room separated from the animal room. The following tissues were examined histologically: the liver, spleen, kidneys, heart, lungs, thymus, stomach, small and large intestines, pancreas, brain, femoral bone marrow, trachea, aorta, skeletal muscle, skin, spinal cord, femoral bone, eyeballs, testes, ovaries, uterus, tongue, urinary bladder, and the adrenal, pituitary, thyroid, parathyroid, salivary, mammary, Harderian, lacrimal and prostate glands as well as any other tissues with gross lesions. All the tissues, except the eyeballs which were fixed in Bouin's fluid, were fixed in 10% formalin. They were embedded in paraffin wax, sectioned at 4–6 μm and stained with haematoxylin and eosin (H & E). Selected sections were also stained with periodic acid-Schiff, von Kossa's method or toluidine blue.

Statistical method

Pathological findings were statistically evaluated between males and females using one-sided Fisher's exact probability test. Differences were considered significant at $P < 0{\cdot}05$.

Results

Mortality

Death began to occur at 37 weeks of age in males and 51 weeks in females, and thereafter the mortalities of both sexes gradually increased (Table 1).

Body weight changes

As shown in Fig. 1, mean body weights determined during a period from 5–130 weeks of age were at all times greater in males than in females. At the age of 5 weeks, mean body weights were 18·8 g for males and 16·1 g for females. Thereafter, body weight gradually

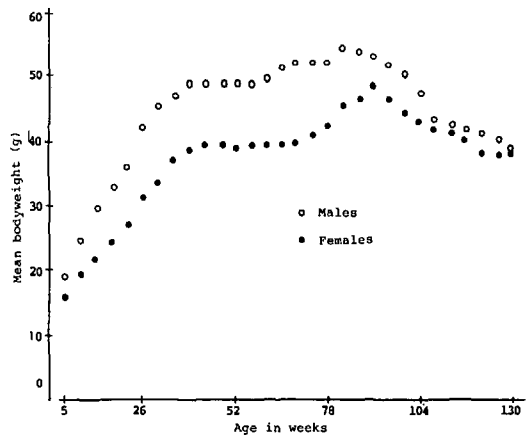

Fig. 1. Mean body weights of male and female BDF_1 mice.

Table 2. Incidence of nonneoplastic lesions in BDF_1 mice

Organ: Lesion	*No. of mice with lesions in 50 males*	*50 females*
Liver:		
Cell-alteration foci	6	6
Angiectasis	1	3
Amyloidosis	0	1
Kidney:		
Hyaline droplet degeneration	4	13*
Renal calculi	31***	1
Proteinaceous casts	16***	3
Pelvic dilatation	5	7
Glomerulosclerosis	2	6
Interstitial fibrosis	3	5
Cyst-formation	5	1
Spleen:		
Lymphoid hyperplasia	0	1
Heart:		
Myocardial fibrosis	3	7
Myocardial calcification	0	2
Lung:		
Foam cell aggregation	9	6
Adrenal gland:		
Cortical cell-alteration	13*	4
Spindle cell proliferation	28	48***
Pituitary gland:		
Chromophobe cell hyperplasia	1	4
Cyst-formation	3	2
Pancreas:		
Islet cell hyperplasia	14***	0
Brain:		
Mineralization	31	32
Abdominal adipose tissue:		
Focal necrosis	6	2
Skeletal muscle:		
Calcification	0	3
Mammary gland:		
Milk-retention	0	9**
Systemic panarteritis	2	5
Testis:		
Calcification	43	NA
Atrophy with aspermatogenesis	25	NA
Seminal vesicle:		
Enlargement	41	NA
Ovary:		
Cyst-formation	NA	24
Uterus:		
Endometrial cyst-formation	NA	34

NA, not applicable. Asterisk indicates sex-difference, $*P<0{\cdot}05$, $**P<0{\cdot}01$, $***P<0{\cdot}001$.

increased, attaining the maximum mean body weight of 54·3 g in males at 82 weeks and 48·0 g in females at 92 weeks of age. Subsequently, mean body weights of both sexes decreased gradually, reflecting a reduction in body weight of individual animals.

Nonneoplastic lesions

As shown in Table 2, renal calculi and proteinaceous casts in the kidneys, focal cell-alteration in the adrenal cortex, and islet cell hyperplasia in the pancreas developed at a significantly higher incidence in males. On the other hand, hyaline droplet degeneration of the renal tubular epithelium, spindle cell proliferation in the adrenal cortex and milk-retention in the mammary glands were seen more commonly in females. Principal lesions tended to increase in incidence with age, except pancreatic islet cell hyperplasia most of which developed in male mice 130 weeks old and under (Tables 3 and 4).

Hyaline droplet degeneration in the renal tubular epithelium was characterized by the appearance of eosinophilic droplets or granules in the cytoplasm (Fig. 2), and found in 4 males and 13 females. Of these animals, 3 males and 11 females had diffuse infiltration of the kidneys by histiocytic lymphoma. Renal calculi stained strongly with haematoxylin, showing a distinct laminar structure (Fig. 3), and calculi were detected one to 5 per cross section of the kidney. No cellular reactions were seen around them. Proteinaceous casts in the renal tubules stained uniformly with eosin and could not be related with any other lesions.

Foci of cortical cell-alteration in the adrenal gland were composed mostly of basophilic cells. Although there was a slight compression of the surrounding adrenal tissue, neither infiltrative proliferation nor alterations in the architectural pattern were seen. Proliferation of spindle-shaped cells with fusiform nuclei developed beneath the adrenal capsule and sometimes extended to the medulla in a wedge-shape.

The hyperplastic pancreatic islets became several times larger than normal, possibly by clustering or coalescing of neighbouring islets. In what was described as milk-retention, milk was retained in the dilatated ducts lining by cuboidal epithelial cells. Eight of 9 females with milk-retention had pituitary adenoma.

Foci of cellular alteration in the liver consisted of basophilic hepatocytes and occasional foci

Table 3. Age-related incidence of principal nonneoplastic lesions in male mice died or killed when moribund

	No. (%) of mice with lesions in indicated age periods (week)				
	~52	*53~78*	*79~104*	*105~130*	*131~158*
Site: Lesion	n=*2*	n=*5*	n=*14*	n=*16*	n=*13*
Kidney:					
Renal calculi	0 (0)	0 (0)	7 (50·0)	12 (75·0)	12 (92·3)
Proteinaceous casts	1 (50·0)	0 (0)	2 (14·3)	7 (43·8)	6 (46·2)
Adrenal gland:					
Cortical cell-alteration	0 (0)	1 (20·0)	3 (21·4)	4 (25·0)	5 (38·5)
Spindle cell proliferation	1 (50·0)	1 (20·0)	8 (57·1)	9 (56·3)	9 (69·2)
Pancreas:					
Islet cell hyperplasia	2 (100)	4 (80·0)	5 (35·7)	2 (12·5)	1 (7·7)
Brain:					
Mineralization	0 (0)	4 (80·0)	8 (57·1)	11 (68·8)	8 (61·5)
Testis:					
Calcification	0 (0)	3 (60·0)	12 (85·7)	15 (93·8)	13 (100)
Atrophy with aspermatogenesis	0 (0)	0 (0)	2 (14·3)	11 (68·8)	12 (92·3)
Seminal vesicle:					
Enlargement	1 (50·0)	2 (40·0)	10 (71·4)	15 (93·8)	13 (100)

Table 4. Age-related incidence of principal nonneoplastic lesions in female mice died or killed when moribund

	No. (%) of mice with lesions in indicated age periods (week)				
	~52	*53~78*	*79~104*	*105~130*	*131~150*
Site: Lesion	n=*1*	n=*3*	n=*13*	n=*24*	n=*9*
Kidney:					
Hyaline droplet degeneration	0 (0)	1 (33.3)	5 (38·5)	6 (25·0)	1 (11·1)
Adrenal gland:					
Spindle cell proliferation	1 (100)	2 (66·7)	12 (92·3)	24 (100)	9 (100)
Brain:					
Mineralization	0 (0)	0 (0)	8 (61·5)	17 (70·8)	7 (77·8)
Ovary:					
Cyst-formation	0 (0)	0 (0)	5 (38·5)	12 (50·0)	7 (77·8)
Uterus:					
Endometrial cyst-formation	0 (0)	1 (33·3)	7 (53·8)	19 (79·2)	7 (77·8)
Mammary gland:					
Milk-retention	0 (0)	0 (0)	0 (0)	7 (29·2)	2 (22·2)

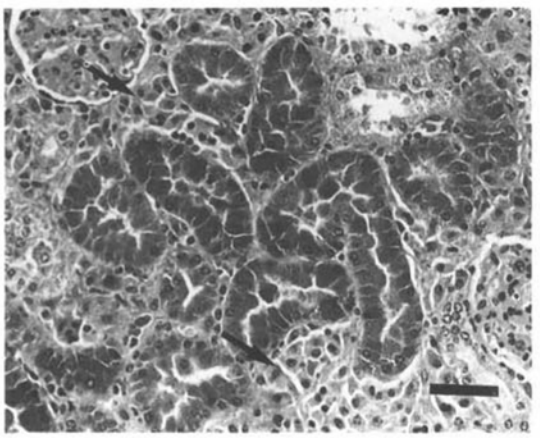

Fig. 2. Female mouse, 132-week-old. Hyaline droplet degeneration of the renal tubular epithelium and neoplastic cell infiltration of malignant lymphoma, histiocytic type (arrows). (H & E; bar = 50 μm.)

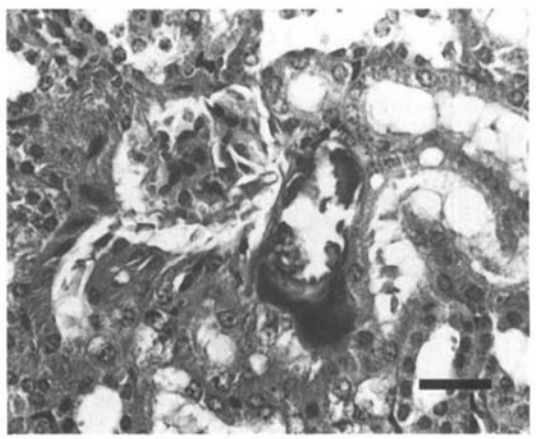

Fig. 3. Male mouse, 144-week-old. Tubular calcification with a laminar structure in the kidney. (H & E; bar = 30μm.)

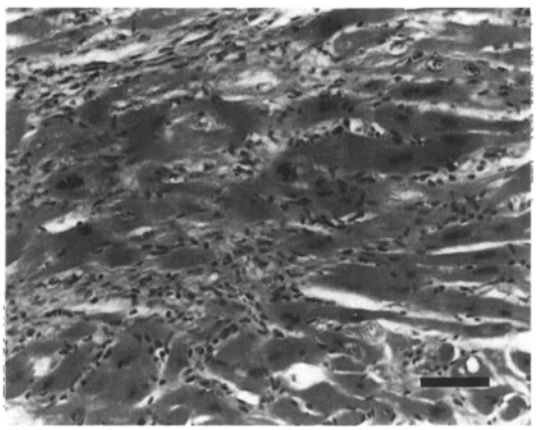

Fig. 4. Male mouse, 144-week-old. Myocardial fibrosis accompanying with atrophy and degeneration of myocardial fibres. (H & E; bar = 30 μm.)

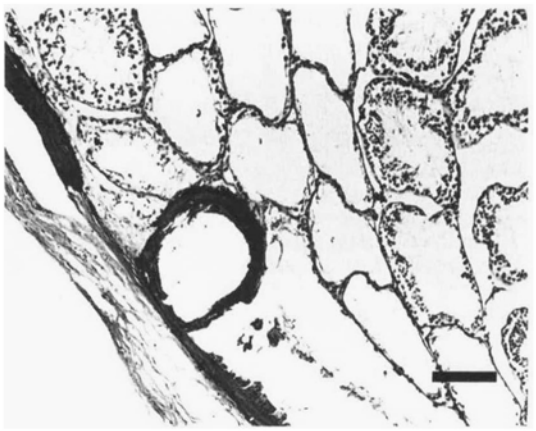

Fig. 5. Male mouse, 144-week-old. Testicular atrophy consisting of seminiferous tubules with aspermatogenesis, and calcification in the arterial wall and tunica albuginea. (H & E; bar = 100 μm.)

were composed of eosinophilic or vacuolated hepatocytes. They differed from hepatocellular adenomas which showed an expansive growth compressing the surrounding tissues. Myocardial fibrosis was found in the left ventricular wall or interventricular septum. It was accompanied with atrophy and the Anitschkow pattern of nuclear chromatin of myocardial fibres (Fig. 4). Cerebral mineralization occurred at a high incidence in ageing mice. It was mainly localized around blood vessels in the thalamus, stained strongly with haematoxylin and was positive for the periodic acid-Schiff reaction. Systemic panarteritis was characterized by fibrinoid degeneration and necrosis of the arterial walls as well as infiltration by inflammatory cells. The lesions occurred in the heart, urinary bladder, tongue, ovary, uterus, adrenal gland, thyroid gland and small intestine.

Nonneoplastic lesions in the reproductive system developed at a high frequency in both sexes, the incidence being age-related (Tables 3 and 4). Calcification in the testes occurred in the tunica albuginea and arterial walls and gave a positive reaction to the von Kossa's method. Atrophic testes contained seminiferous tubules with reduced spermatogenesis (Fig. 5). The enlarged seminal vesicles contained whitish firm secretion and filled the abdominal cavity but did not compress any of the visceral organs. Ovarian cysts developed unilaterally or bilaterally. Endometrial cysts ranged in size from microscopic to gross lesions up to about 1·0 cm in diameter, and usually contained transparent or reddish fluid.

Aggregations of lymphocytes and histiocytes were observed in the interstitium of the kidneys and liver, in the submucosa of the urinary bladder, as well as in periarterial tissues of the salivary, lacrimal and Harderian glands in 3–30 males and 9–23 females. These lesions could not be related to any other particular changes.

Neoplastic lesions

The type and incidence of neoplastic lesions are shown in Table 5. Principal tumours developed exclusively in old male and female mice, 79 weeks of age or over. Twenty-seven males and 23 females bore 2–4 different types of tumours in their liver, lungs, haematopoietic tissue or blood vascular system. The most frequent combination was hepatic tumour and blood vessel tumour in males, and malignant lymphoma and blood vessel tumour in females. Such combinations developed in 9 males and 6 females.

Hepatocellular adenoma was composed of uniform sized hepatocytes with basophilic, vacuolated or clear cytoplasm and did not show infiltrative growth. Carcinomas consisted of hepatocytes assuming trabecular, papillary and

Table 5. Incidence of neoplastic lesions in BDF_1 mice

Organ: Tumour type	*No. of mice with tumours in 50 males*	*50 females*
Liver:		
Hepatocellular adenoma	7	6
Hepatocellular carcinoma	21***	0
Hepatoblastoma	3	0
Lung:		
Alveolar-bronchiolar adenoma	5	2
Alveolar-bronchiolar carcinoma	6	3
Haematopoietic tissue:		
Malignant lymphoma of histiocytic type	7	15*
Malignant lymphoma of lymphocytic type	0	15***
Malignant lymphoma of mixed cell type	2	5
Mast cell tumour	2	0
Kidney:		
Adenoma	0	1
Adrenal gland:		
Cortical adenoma	1	0
Spindle cell tumour	2	0
Phaochromocytoma	1	0
Pituitary gland:		
Chromophobe cell adenoma	0	8**
Harderian gland:		
Adenoma	1	1
Skin:		
Fibrosarcoma	0	2
Malignant fibrous histiocytoma	0	1
Round cell sarcoma	1	0
Body cavity:		
Malignant mesothelioma	1	0
Undifferentiated carcinoma	1	0
Prostate gland:		
Adenocarcinoma	1	NA
Mammary gland:		
Adenocarcinoma	0	4
Ovary:		
Adenoma	NA	1
Fibrosarcoma	NA	1
Uterus:		
Polyp	NA	1
Blood vascular system:		
Solitary blood vessel tumour	9	5
Multiple blood vessel tumour	10	9
Total number of tumours	81	80

NA, not applicable. Asterisk indicates sex-difference, $*P<0{\cdot}05$, $**P<0{\cdot}01$, $***P<0{\cdot}001$.

acinar patterns and invaded the adjacent parenchyme and blood vessels. Seventeen of the 21 carcinomas metastasized to the lung (Fig. 6).

In alveolar-bronchiolar adenomas or carcinomas, the papillary, solid and mixed types

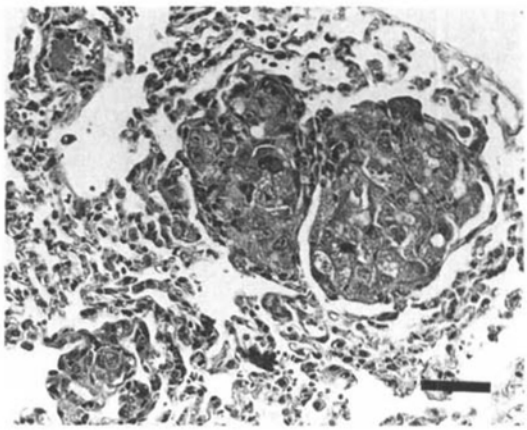

Fig. 6. Male mouse, 121-week-old. Metastatic foci of hepatocellular carcinoma in the lung. (H & E; bar = 40 μm.)

were observed, as described by Heath *et al.* (1982). Most adenomas were smaller than carcinomas, which exhibited a distinct cellular atypia and more expansive growth. Lymphoma was classified according to Frith & Wiley (1981). The histiocytic type was the most common, and showed massive or diffuse infiltration in the kidneys, liver, and uterus, leading to an increase in their size. Neoplastic cells of this type had abundant, eosinophilic cytoplasm and small nuclei. Neoplastic cells of the lymphocytic type almost always infiltrated into the organs, such as the liver, spleen, kidneys, lymph nodes, lungs and skeletal muscle (Fig. 7). Mast cell tumours were found in two males of 123 and 158 weeks old. Extensive infiltration of tumour cells, which contained many, cytoplasmic basophilic granules staining metachromatically with toluidine blue, was present in the spleen, liver and lymph nodes.

The pituitary adenomas were composed of chromophobic cells and slightly compressed the surrounding tissues.

Blood vessel tumours were classified in accordance with a previous study of such tumours in $B6C3F_1$ (Yamate *et al.*, 1988). The solitary type developed at a single, discrete site, whereas the multiple type arose at more than two sites. The solitary type was seen most commonly in the liver, but also occurred in the femoral bone marrow, subcutis, skeletal muscle and ovary.

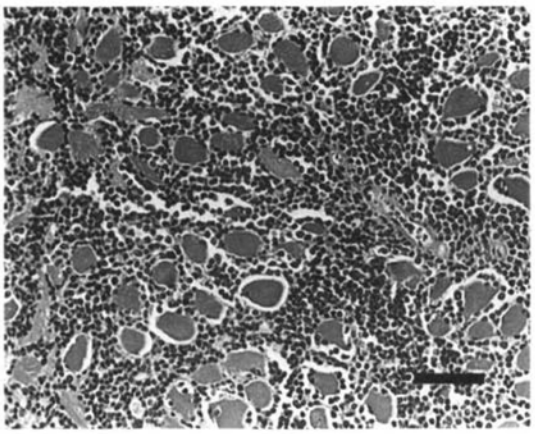

Fig. 7. Female mouse, 113-week-old. Diffuse infiltration of the skeletal muscle by neoplastic cells of malignant lymphoma, lymphocytic type. (H & E; bar = 70 μm.)

The multiple type occurred in 10 males and 9 females at up to 6 sites including the liver, spleen, heart, femoral bone marrow, subcutis, kidneys, skeletal muscle, parietal peritoneum, ovary and uterus. Both types of blood vessel tumours were similar histologically and in sites of development to each other. Most parts of blood vessel tumours showed features of cavernous haemangioma consisting of well-developed vascular channels lined by flattened and elongated endothelial cells with hyperchromatic nuclei. The haemangioendotheliomatous growth, in which endothelial cells proliferated without forming any vascular channels and exhibited a slight cellular atypia, was occasionally observed in a portion of blood vessel tumours arising from the spleen and liver.

The other tumours listed in Table 5 were not common in BDF_1 mice. Two hepatoblastomas and one adenocarcinoma of the prostate gland metastasized to the lungs.

Discussion

The longevity and body weight changes of our BDF_1 mice were not noticeably different from those of other strains of mice maintained under similar environmental conditions (Smith *et al.*, 1973; Yamate *et al.*, 1986).

Histological features of nonneoplastic and neoplastic lesions observed in BDF_1 mice agreed well with those reported previously in aged mice (Percy & Jonas, 1971; Ward *et al.*, 1979; Frith & Wiley, 1981; Heath *et al.*, 1982; Yamate *et al.*, 1986). The incidence of most nonneoplastic lesions increased with age, but pancreatic islet-cell hyperplasia tended to occur more frequently in younger males as observed in C3H mice (Sass *et al.*, 1978) and $B6C3F_1$ mice (Yamate *et al.*, 1986). Renal calculi developed at a significantly higher incidence in male BDF_1 mice than in females, as reported in $B6C3F_1$ mice (Yamate *et al.*, 1986). Chloroform nephrosis in susceptible male mice has been associated with accidental exposure resulting from euthanasia of mice with chloroform in the animal room (Carlton & Engelhardt, 1986). Such accidental exposure was not possible in the present study, because the anaesthesia using a mixture of alcohol, ether and chloroform was done in a separate dissecting room. The pathogenesis of renal lesions in our BDF_1 mice remains to be solved. Hyaline droplet degeneration in the renal tubular epithelium, which developed exclusively in females with histiocytic lymphoma, may be due to malreabsorption by the renal tubular epithelium, resulting from extensive infiltration of neoplastic cells of the histiocytic type. Milk-retention was found only in females with pituitary adenoma. Since prolactin-secreting pituitary tumours have been reported to develop in rats (Lee *et al.*, 1982), prolactin hormone may be produced in BDF_1 mice with pituitary adenomas. It would be worth examining these tumours immunohistochemically.

Anderson *et al.* (1983) reported that tumours in the liver and lung were more common in long-lived male BDF_1 mice, but they did not document the development of malignant lymphomas and blood vessel tumours which occurred at a high incidence in our BDF_1 mice. This may be due to differences in the origin of BDF_1 mice and environmental factors. Both sexes of the parent mouse strains, C57BL and DBA, have been reported to develop lymphoma at a high incidence (Smith *et al.*, 1973; Veninga *et al.*, 1984), and females of DBA strain frequently developed mammary tumours (Smith *et al.*, 1973). In this study, mammary

adenocarcinomas were found only in 4 female BDF_1 mice.

The hepatic tumours were classified according to Frith & Ward (1980). The incidence of hepatocellular carcinomas was three times as great as that of hepatocellular adenomas in male BDF_1 mice allowed to live out their life-span, suggesting a shift from benign to malignant (Reuber, 1975; Frith & Ward, 1980).

Malignant lymphoma was the most common neoplasm in our female BDF_1 mice. Female mice of other strains also show a high incidence of malignant lymphoma (Frith & Wiley, 1981; Frith *et al.*, 1983; Yamate *et al.*, 1986). The histiocytic type was least common among these other strains of mice, whereas the histiocytic type showed the highest incidence in BDF_1 mice.

Mast cell tumours have rarely been found in mice (Frith & Wiley, 1981), and this is the first report of spontaneous mast cell tumours in BDF_1 mice. The cytological features and organ distribution of mast cell tumours described here were similar to those reported by previous workers (Frith & Wiley, 1981; Lewis & Offer, 1984).

Acknowledgments

We gratefully acknowledge the technical assistance of Mr Y Ogata and the animal care of Messrs T Inoue, K Yamashita and H Watanabe.

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