SPONTANEOUS TUMORS IN F344/DuCrj RATS FROM 12 CONTROL GROUPS OF CHRONIC AND ONCOGENICITY STUDIES

Keizo MAITA, Masahiro HIRANO, Takanori HARADA,
Kunitoshi MITSUMORI, Akiyoshi YOSHIDA, Kimimasa TAKAHASHI,
Nobuaki NAKASHIMA, Toshiaki KITAZAWA, Akiko ENOMOTO,
Kosei INUI, and Yasuhiko SHIRASU

Toxicology Division, Institute of Environmental Toxicology,
Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan

Accepted February 14, 1987

Abstract.....The types and incidences of spontaneous tumors in F344/DuCrj rats were examined in 960 males and 959 females served as the control groups of separate twelve 2-year chronic and oncogenicity studies carried out during a 1978~1983 period. The major tumors occurred at more than 5% incidence were leukemia (mononuclear cell), testicular interstitial cell tumor, preputial gland adenoma, pituitary anterior adenoma, thyroid C-cell adenoma, adrenal pheochromocytoma and subcutis fibroma for males, and leukemia, uterine endometrial polyp, pituitary anterior adenoma, thyroid C-cell adenoma and mammary gland adenoma/fibroadenoma in females. Analyses on age-related occurrence of tumors revealed that the incidences of most of the major tumors in males attained already to the plateau between 85 and 97 weeks of age while those in females showed a steep rise during the last interval of observation, 98 to 110 weeks of age. An intralaboratory heterogeneity in incidence was observed in the thyroid C-cell adenoma and the adrenal pheochromocytoma for males, and the anterior pituitary adenoma for females.

Key words : Spontaneous tumors, F344/DuCrj rats, age-related occurrence, intralaboratory heterogeneity.

INTRODUCTION

Increasing demands for oncogenicity studies with various species of chemicals have been promoting accumulation of informations on spontaneous tumors in mice and rats of various strains. While these data give an overview of types and incidences

of spontaneous tumors occurring in a certain strain of mice or rats, they also reveal significant heterogeneity in incidences of particular tumors between different laboratories. Numerous intrinsic and extrinsic factors including genetic background, geographical situation, food, sanitary condition, and other various factors of animal husbandry are considered to be responsible for the discrepancy. In addition to these physiological conditions of animals, the incidences of tumors may be much influenced by different diagnostic criteria among pathologists. F344/DuCrj have been widely used in the oncogenicity studies since Carcinogenesis Testing Program of the National Cancer Institute in USA nominated them as the standard strain of rat for oncogenicity study with chemicals in early '70. Among largescale accumulation of documents on types and incidences of spontaneous tumors in F344/DuCrj rats, several authors have indicated the interlaboratory discrepancies of spontaneous tumor genesis and pointed out inadequacy of simple comparison of oncogenicity data from a laboratory with historical control data collected in other laboratories (Tarone et al., 1981). Besides the discrepancy among laboratories, recent investigations have elucidated that a significant heterogeneity may exist even in a laboratory where the pathologists have the same protocol for diagnosis (Haseman et al., 1984). It is necessary, therefore, to be concerned with intralaboratory heterogeneity as well as interlaboratory heterogeneity when the historical tumor occurrence in animals are analyzed.

Although there is an urgent demand to provide historical control data on spontaneous tumors for F344/DuCrj rats in Japan, only limited descriptive reports have been available so far (Maekawa et al., 1983).

In this report we mention the types and incidences of spontaneous tumors in 960 males and 959 females F344/DuCrj rats which were used as controls in twelve 2-year chronic toxicity and oncogenicity studies, and also deal with age-related occurrence of the major tumors and their heterogeneity between the different groups.

MATERIALS AND METHODS

F344/DuCrj rats of both sexes were purchased at 4 weeks of age from Charles River Japan Inc. (Atsugi, Kanagawa) and transferred to the barrier sustained animal rooms with a controlled temperature at $22 \pm 2^\circ\text{C}$, relative humidity at $55 \pm 10\%$ and an illumination cycle of 14 hrs light and 10 hrs dark. On access to the animal room, wet shower bathing was required. These animals were used as control groups in twelve 2-year chronic toxicity and oncogenicity studies with agrochemicals carried out from 1978 to 1983. After one week acclimatization period, 80 males and 80 females (79 females for one study) were randomly selected and allotted to each control group. Five animals of either sex were housed in one cage, $310 \times 440 \times 230$ mm in size and equipped with wire-meshed floor, and were allowed free access to local tap water in a glass bottle and a powdered chow Diet-M which was made by pulverization of pellet Diet-MF (Oriental Yeast Industry Co. Itabashi-ku, Tokyo). The standard constitution of nutrients and suspectible contaminants in Diet-MF was shown in Table 1.

Spontaneous tumors in F344/DuCrj rats

Table 1. Standard constitution of nutrients and suspective contaminants in Diet MF

Nutrients (in 100 g of diet)					
Water	7.0g	Crude protein	24.0g	Crude lipid	5.1g
Ash	6.2g	Fiber	3.2g		
Soluble non-nitrogen substances			54.5g	Calorie	359.9kcal
Vitamin A	1100 IU	Vitamin D ₃	80 IU	Vitamin E	6.3mg
Vitamin K ₃	0.04mg	Vitamin B ₁	1.9mg	Vitamin B ₂	1.3mg
Vitamin B ₆	0.8mg	Niacin	4.0mg	Pantothenic acid	1.9mg
Biotin	0.02mg	Folic acid	0.04mg	Inositol	60mg
Choline	100mg	Vitamin B ₁₂	0.004mg	Vitamin C	5.4mg
Contaminants (ppm)					
Lead	0.51	Cadmium	0.10	Arsenic	0.40
Total chrome	0.38	Total mercury	0.01	Nitrite	0.60
Aflatoxin	ND*	PCBs	ND	BHC	ND
Aldrin	ND	Heptachlor	ND	Dieldrin	ND
Parathione	ND	Marathione	0.48		

* ; not detected

After 26, 52 and 78 weeks of treatment, in 11 out of 12 studies 10 males and 10 females were randomly selected and sacrificed to obtain current informations on toxicity or oncogenicity of the chemicals. In one study the interim sacrifice after 26 weeks of treatment was not conducted while 52-and 78-week sacrifice were performed. All animals were subjected to weekly careful palpation to detect tumor mass in addition to daily cage-side watching. The animals found dead or killed in extremis were autopsied immediately to minimize autolysis. The organs and tissues examined were the brain, spinal cord (cervical, thoracic and lumbar region), sciatic nerve, pituitary, thymus, thyroids with parathyroids, adrenals, spleen, sternum and femur with bone marrow, tibio-femoral joint, lymph nodes (cervical and mesenteric), heart, aorta (thoracic), salivary gland, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, nasal cavity, paranasal sinus, buccal cavity, inner ear, pharynx, larynx, trachea, lung, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles and coagulating glands, ovaries, uterus, eyes and contiguous glands, skeletal muscle (M. triceps surae), skin (lumbodorsal region), mammary gland (abdominal region ; female only) and gross lesions including tumor masses. All collected samples were fixed in neutral buffered 10% formalin. Paraffin embedded sections were stained with hematoxylin and eosin for the routine slide

reading. When needed for diagnosis of the tumors, special stainings were applied. The basic criteria for classification of the tumors were followed to the text book published by WHO (Turusov et al., 1973). Heterogeneity of tumor incidence among different groups was analyzed by Fisher's exact probability test and the 5% level of probability was used as the criterion of significance.

RESULTS

1. *Histological types and incidences of spontaneous tumors* : Table 2 shows the histological types of spontaneous tumors and their overall incidences in males and females served as control dietary groups in twelve 2-year chronic studies. In cases that tumor masses with same histological feature originated in multiple locations, the tumors were given a single diagnosis. Fibroadenoma of the mammary gland and papilloma of the skin were examples of the tumors diagnosed by this criterion. When there were multiple and discrete nodules in one organ which apparently originated from same tissue component but were different in degree of malignancy, plural diagnoses were recorded, such as adenoma and adenocarcinoma in the lung.

Cardiovascular system : Schwannoma of the heart occurred in two males and two females. Although the endocardial disease of the heart characterized by tumor like proliferation of the subendothelial tissue was found in several animals of both sexes in each control group, the lesion was not classified into the neoplasm.

Hematopoietic system : Mononuclear cell leukemia occurring in 68 males and 91 females shared 5.7% and 9.5% of total number of animals for males and females examined in the present study, respectively. It was one of the major cause for moribundity or death of the animals after 88 weeks of age. The clinical signs in the animals killed in extremis or found dead consisted of ruffled hair, decreased motility, red lacrima on eyelid, and paleness of eye followed by a rapid development of anemic condition and dyspnea. The animals usually manifested debility one or two weeks after showing the first sign of disease. Jaundice was rather rare in these animals. Splenomegaly was most common and striking finding at autopsy. The abdominal cavity was sometimes occupied by the extraordinarily enlarged spleen which was friable, purplish and partially mottled, and occasionally adhesive to the omentum, mesenterium or abdominal wall. Discoloration of visceral organs and tissues, mottled surface of the lung, occasional hepatomegaly, slight ascites and/or hydrothorax, and gelatinous, discolored bone marrow were also noted in the affected animals. Enlargement of the lymph node was slight or moderate. The leukemia cells were similar to monocytes or large (histiocytic) lymphocytes which had ovoid, dark, partially dimpled nuclei and sordid, slightly eosinophilic cytoplasm. In the spleen which is suspicious of the primary organ for tumor growth, there were scattered foci of the leukemia cell proliferation at the boundary between the red pulp and white pulp in the early phase of growth. When the leukemia cells began to show vigorous proliferation in the sinus, congestion became prominent resulting in enlargement of

Spontaneous tumors in F344/DuCrj rats

Table 2. Histological types and incidences of spontaneous tumors observed in 960 male and 959 female F344/DuCrj rats.

Histological types of tumors	No. of tumor bearers (%)	
	Male	Female
Cardiovascular system		
Heart :		
Schwannoma	2 (0.2)	2 (0.2)
Hematopoietic system		
Thymus :		
Thymoma	3 (0.3)	1 (0.1)
Spleen :		
Hemangioma	1 (0.1)	—
Malignant histiocytoma	1 (0.1)	—
Lymph node :		
Malignant lymphoma	1 (0.1)	1 (0.1)
Bone marrow :		
Mastocytoma	1 (0.1)	1 (0.1)
Multiple organs :		
Malignant lymphoma	1 (0.1)	—
Malignant histiocytoma	1 (0.1)	1 (0.1)
Leukemia (mononuclear cell)	68 (5.7)	91 (9.5)
Respiratory system		
Lung :		
Adenoma	40 (4.2)	21 (2.2)
Adenocarcinoma	5 (0.5)	1 (0.1)
Adenocarcinoma of bronchial gland	1 (0.1)	—
Digestive system		
Tongue :		
Papilloma	1 (0.1)	2 (0.2)
Squamous cell carcinoma	1 (0.1)	—
Palate :		
Squamous cell carcinoma	2 (0.2)	—
Salivary gland :		
Adenoma	1 (0.1)	—
Fore stomach :		
Squamous cell carcinoma	—	1 (0.1)
Small intestine :		
Adenocarcinoma	1 (0.1)	1 (0.1)
Leiomyoma	2 (0.2)	—
Leiomyosarcoma	—	1 (0.1)
Large intestine :		
Lipoma	—	2 (0.2)
Leiomyoma	1 (0.1)	—
Leiomyosarcoma	2 (0.2)	1 (0.1)

Table 2. (continued 1)

Histological types of tumors	No. of tumor bearers (%)	
	Male	Female
Digestive system		
Liver :		
Hyperplastic (neoplastic) nodule	25 (2.6)	16 (1.7)
Hepatocellular carcinoma	3 (0.3)	—
Cholangiocellular adenoma	1 (0.1)	2 (0.2)
Pancreas :		
Acinar cell adenoma	3 (0.3)	—
Islet cell adenoma	14 (1.5)	2 (0.2)
Urinary system		
Kidney :		
Nephroblastoma	1 (0.1)	—
Transitional cell carcinoma	2 (0.2)	—
Lipoma	1 (0.1)	—
Urinary bladder :		
Papilloma	1 (0.1)	—
Transitional cell carcinoma	1 (0.1)	2 (0.2)
Leiomyoma	1 (0.1)	—
Urethra :		
Squamous cell carcinoma	1 (0.1)	—
Genital system		
Testis :		
Interstitial cell tumor	716 (74.6)	—
Seminal vesicle :		
Adenoma	1 (0.1)	—
Prostate :		
Adenoma	2 (0.2)	—
Adenocarcinoma	1 (0.1)	—
Preputial gland :		
Adenoma	51 (5.3)	—
Adenocarcinoma/Carcinoma	2 (0.2)	—
Ovary :		
Granulosa/Theca cell tumor	—	4 (0.4)
Adenocarcinoma	—	1 (0.1)
Leiomyosarcoma	—	1 (0.1)
Uterus :		
Papilloma	—	1 (0.1)
Squamous cell carcinoma	—	1 (0.1)
Adenoma	—	3 (0.3)
Adenocarcinoma	—	2 (0.2)
Endometrial polyp	—	121 (12.6)
Endometrial sarcoma	—	9 (0.9)

Spontaneous tumors in F344/DuCrj rats

Table 2. (continued 2)

Histological types of tumors	No. of tumor bearers (%)	
	Male	Female
Genital system		
Uterus :		
Leiomyoma	—	6 (0.6)
Leiomyosarcoma	—	11 (1.1)
Hemangioma	—	1 (0.1)
Vagina :		
Leiomyoma	—	1 (0.1)
Leiomyosarcoma	—	1 (0.1)
Clitoral gland :		
Adenoma	—	10 (1.0)
Adenocarcinoma/Carcinoma	—	3 (0.3)
Endocrine system		
Pituitary :		
Anterior adenoma	119 (12.4)	270 (28.2)
Anterior adenocarcinoma	4 (0.4)	7 (0.7)
Adenoma in pars intermedia	1 (0.1)	1 (0.1)
Hamartoma	1 (0.1)	—
Thyroid :		
Follicular adenoma	8 (0.8)	3 (0.3)
Follicular adenocarcinoma	4 (0.4)	2 (0.2)
C-cell adenoma	120 (12.5)	79 (8.2)
C-cell adenocarcinoma	4 (0.4)	2 (0.2)
Undifferentiated carcinoma	1 (0.1)	—
Parathyroid :		
Adenoma	—	1 (0.1)
Adenocarcinoma	1 (0.1)	—
Adrenal :		
Cortical adenoma	7 (0.7)	21 (2.2)
Cortical adenocarcinoma ?	1 (0.1)	2 (0.2)
Pheochromocytoma	114 (11.9)	31 (3.2)
Malignant pheochromocytoma	3 (0.3)	—
Ganglioneuroma	4 (0.4)	1 (0.1)
Neuroblastoma	1 (0.1)	—
Nervous system		
Brain :		
Granular cell tumor	1 (0.1)	—
Glioma	3 (0.3)	4 (0.4)
Meningioma	1 (0.1)	—
Sense organs		
Ear :		
Papilloma	1 (0.1)	2 (0.2)
Adenoma of Zymbal's gland	1 (0.1)	1 (0.1)
Adenocarcinoma of Zymbal's gland	5 (0.5)	1 (0.1)

Table 2 (continued 3)

Histological types of tumors	No. of tumor bearers (%)	
	Male	Female
Musculo-skeletal system		
Bone :		
Osteoma	—	1 (0.1)
Osteosarcoma	2 (0.2)	—
Skin/Subcutis		
Skin/Subcutis :		
Papilloma	19 (2.0)	6 (0.6)
Keratoacanthoma	8 (0.8)	2 (0.2)
Trichoepithelioma	2 (0.2)	1 (0.1)
Squamous cell carcinoma	13 (1.4)	8 (0.8)
Basal cell carcinoma	7 (0.7)	2 (0.2)
Anal gland adenoma	—	1 (0.1)
Sebaceous carcinoma	2 (0.2)	1 (0.1)
Fibroma	69 (7.2)	11 (1.1)
Fibrosarcoma	7 (0.7)	1 (0.1)
Lipoma	8 (0.8)	2 (0.2)
Hemangioma	1 (0.1)	—
Hemangiopericytoma	2 (0.2)	1 (0.1)
Leiomyoma	1 (0.1)	—
Leiomyosarcoma	2 (0.2)	—
Rhabdomyosarcoma	1 (0.1)	3 (0.3)
Schwannoma	—	1 (0.1)
Malignant schwannoma	—	1 (0.1)
Fibrous histiocyteoma	1 (0.1)	—
Malignant histiocyteoma	2 (0.2)	1 (0.1)
Malignant fibrous histiocyteoma	—	2 (0.2)
Mammary gland :		
adenoma	4 (0.4)	59 (6.2)
Fibroadenoma	8 (0.8)	68 (7.1)
Adenocarcinoma	1 (0.1)	10 (1.0)
Harderian gland :		
Adenoma	1 (0.1)	—
Body cavities		
Thoracic cavity :		
Malignant mesothelioma	1 (0.1)	1 (0.1)
Malignant schwannoma	1 (0.1)	—
Abdominal cavity :		
Malignant mesothelioma	28 (2.9)	—
Fibroma	1 (0.1)	1 (0.1)
Paraganglioma	1 (0.1)	—
Leiomyosarcoma	1 (0.1)	—
Rhabdomyosarcoma	—	1 (0.1)
Malignant fibrous histiocyteoma	1 (0.1)	—
Undetermined origin		
Osteosarcoma	1 (0.1)	—

the spleen. The T-cell area of the white pulp was progressively replaced by the tumor cells. At this stage of tumor growth, the diagnosis of mononuclear leukemia was given to the animal, although there was still no evidence of involvement in the liver, lung and other organs and were probably no leukemia cells on the smear of the peripheral blood (Stromberg et al., 1983).

Other types of the tumors of the hematopoietic system were extremely rare, and there was no granulocytic leukemia in this series.

Respiratory system : Most of adenomas of the lung were considered to originate from the terminal bronchiolar region. One adenocarcinoma of the lung from a male found dead at 106 weeks of age metastasized to the muscle of the diaphragm.

Digestive system : There has been an increasing argument on the criteria of proliferative lesions of the liver in mouse, rat and hamster (Stewart et al., 1980). In the present study, hyperplastic or neoplastic nodule was employed instead of classical nomenclature of adenoma or nodular hyperplasia to the proliferative lesions of hepatocytes. They were round to spheroidal in shape, more than two lobules in size, and proliferated massively compressing the surrounding tissue. Although they maintained substantially normal architecture of the hepatic lobule, there were occasional dysplastic areas where the hepatic cords were slightly or moderately distorted resulting in congestion or hemorrhage. The tumor cells sometimes considerably enlarged with swollen nuclei and vacuolated or coarsely granular cytoplasm containing spheroidal hyaline bodies. It was not rare that a localized group of the tumor cells disclosed a sheet like proliferation with smaller or larger cell size and a slight degree of atypia of nuclei. The proliferative lesions sizing up to two lobules or less were diagnosed as foci or area of cell alteration. The signs of malignancy of the tumor included frequent and large necrotic areas with hemorrhage, marked dissociation or disruption of cell cords, trabecular pattern of cell arrangement, frequent mitosis, and a marked atypia of the tumor cells. One hepatocellular carcinoma from a male killed in extremis at 105 weeks of age metastasized to the lung.

The diagnostic criteria of the proliferative lesions in the pancreatic islet have also raised a lot of debate. In most of the proliferative lesions largely composing of B cells, the cells appeared slightly larger than normal ones and sometimes a nucleus/cytoplasm ratio was decreased. They disclosed the same distribution of cytoplasmic granules as seen in intact cells. The basis for criteria of adenoma included size of the tumor mass more than twice as large as average islet, compression of surrounding tissue, or distorted vascular system with occasional hemorrhage. There were sometimes a few foci of smaller or larger cells which showed various degree of atypia of nuclei with large and prominent nucleoli, localized condensation of chromatin, and considerable irregularity in shape.

A variety of tumors occurred in all course of the digestive tract although their incidences were very low.

Urinary system : The occurrence of tumors in the urinary system was vary rare. In females only two cases of transitional cell carcinoma were seen in the urinary bladder in this series.

Genital system : Interstitial cell (Leydig cell) tumors were the most common ones in male rats in this series and the incidence was nearly 100% during 98 and 110 weeks of age (Table 3). Although malignant features of the tumor such as frequent mitosis, cellular atypia, or invasion to the tunica were occasionally seen, no metastasis was detected.

Preputial gland adenoma formed a discrete nodule 0.5 to 1 cm in diameter at the base of the penis. The nodule usually contained flaked materials inside and gave sometimes a cauliflower-like appearance when its surface was ulcerated. The tumor cells contained numerous hyaline granules in the cytoplasm.

In females, endometrial polyp was the most common tumor in the genital system. Since the lesions are largely composed of simple growth of the endometrium with no progressive proliferation of stromal cells, they are sometimes classified into a non-neoplastic lesion. However, in large tumor masses there were sometimes proliferative foci of various endometrial components which showed a considerable degree of malignancy such as mitosis, cellular atypia, vascular deformation with hematoma or hemorrhage, or necrosis. Recent carcinogenicity studies with various classes of chemicals have revealed a dose-dependent increase in incidence of this lesion, which lead to be positive conclusion with carcinogenic potentiality of the chemicals (Mennear et al., 1985).

Endocrine system : Anterior adenoma of the pituitary, C-cell adenoma of the thyroid and pheochromocytoma of the adrenal were not only the three commonest tumors in the endocrine system but also shared the largest population next to interstitial tumor of the testis in male in this series.

Anterior adenomas of the pituitary are sometimes classified into several subtypes depending on their morphological feature, functional characteristic, or immunohistochemical markers. In the present study they were grouped into one category, anterior adenoma, because in the practical aspect of the histopathological investigation in the carcinogenicity study it would be more important to give a comprehensive information on the tumor occurrence from particular tissue component than to provide the cytological details on subclassification of the tumors. Malignant feature of the tumors was defined to the invasive proliferation of the tumor cells beyond the capsule, although there were many other morphological features characteristic of malignant tumors such as a marked distortion of capillaries with frequent hemorrhage, mitosis, cellular atypia, necrosis, or extreme expansion of the tumor as much as inducing marked compression of the brain and obstruction of cerebrospinal fluid flow. Anterior adenoma occurred predominantly in females.

C-cell adenoma of the thyroid usually occurred unilaterally but it was not rare to arise bilaterally. The criteria to define between hyperplasia and adenoma are much debatable. Our criterion for C-cell adenoma was based on size of the lesion, and

more than one follicular size was diagnosed as adenoma. When the tumor cells showed a proliferation beyond the capsule, or metastasis to the neighboring lymphnode or lung, adenocarcinoma was given as a diagnosis. C-cell adenoma was seen predominantly in males.

The incidence of pheochromocytoma in the adrenal was extremely higher in males than in females while the occurrence of cortical adenoma was rather predominant in females. The criterion of pheochromocytoma was defined as tumor cell proliferation occupying more than one third of the medulla. Although the pheochromocytoma showed sometimes various figures of malignant nature such as predominant proliferation of small cells with dark, chromatin rich nuclei and frequent mitosis or distinctive destruction of normal architecture with large area of hemorrhage and necrosis, the only one evidence for malignant pheochromocytoma was metastasis to the remote organs.

Nervous system, Sense organs, Musculo-skeletal system : Seven gliomas observed in the brain were of astrocytic origin except for one oligodendroglioma which occurred in a female killed in extremis at 103 weeks of age.

Zymbal's gland (ceruminous gland) tumors sometimes formed a large cystic nodule with flaked materials inside producing striking swelling or deformity of the temporal area of the animal. It was not uncommon that the animals showed various degrees of neurological disturbance suggestive of bearing brain tumor. Histological examinations disclosed a vigorous proliferation of the epithelial cells which still maintained the characteristic features of the modified sebaceous gland in spite of frequent metaplasia to squamous or keratinizing cells.

Skin/Subcutis : While the tumors originating from the mammary gland were seen predominantly in females, those of skin or subcutis origin were generally rather more common in males except for a few tumor types. Fibromas formed a hard and elastic, lumpy, and partially lobulated mass consisting largely of thick bundles of well developed collagen fibers. Fibrosarcomas displayed rather smooth surfaced, solid mass with flashy appearance on cut surface showing a sheet-like or diffuse proliferation of fibroblasts with rather diverse cell arrangement and considerably frequent mitosis, and high cellularity and poorly developed collagen fibers. The tumor cells rarely showed a high pleomorphism in the structure of nucleus or cytoplasm. In case that a subcutaneous tumor displayed a high diversity in types of constituting cells which included small mesenchymal blastic cells, fibroblastic cells with considerable amount of collagen fibers, monocytic cells with frequent erythrophagia, and giant cells with bizarre nuclei and/or plural nuclei, fibrous histiocytoma or malignant fibrous histiocytoma was given to diagnosis. Although in a large fibrous mass of fibroma there were sometimes fibroblasts with atypical nuclei, occasional foci of mesenchymal blastic cells, or small aggregates of xanthomatous cells with erythrophagia, they were considered to be degeneration of the tumor cells or inflammatory reaction to necrosis.

Body cavities : Malignant mesothelioma was the most prominent tumor in the body cavities with a predominant occurrence in males. On laparotomy there were small, dark greyish brown nodules, 1 mm to 3 mm in size, disseminating all over the surface of the omentum or mesenterium. In some cases which were considered to be the early phase of the tumor development, distribution of the nodules was limited in the scrotum or retroperitoneal cavity. Serous ascites dark brown in color was frequently associated with the tumor. Although most of the tumors were comprised of papillary or nodular proliferation of the epithelium, a few cases were predominated by proliferation of stromal components of the mesothelium.

2. Age-related occurrence and incidence variability of major tumors : Age-related occurrence of the tumors in males or females at more than 5% incidence and variability in incidence among 12 control groups are shown in Table 3 or 4. For analysis of age-related occurrence of the tumors, the examined period was divided into 4 intervals ; 0 to 58, 59 to 84, 85 to 97, and 98 to 110 weeks of age. Each interval included animals killed on schedule except for third interval, 85 to 95 weeks of age. The mononuclear cell leukemia showed the highest incidence in the third interval, which was 21% and 20% for males and females, respectively, and was regarded as one of the major cause of death for those animals. Besides mononuclear cell leukemia, the incidences of the major tumors in males were already high in the third interval of the study as much as those in the last interval, except for C-cell adenoma in the thyroid. Among them, the

Table 3. Age-related occurrence of tumors in male F344/DuCrj rats at more than 5% incidence and variability in incidence among 12 control groups

Histological types of tumors	Overall incidence of tumors and variability in incidence	Age-related occurrence of tumors			
		0-58 wks (%)	59-84 (%)	85-97 (%)	98-110 (%)
No. of animals examined during each interval of age	960	198	144	57	591
Leukemia (mononuclear cell)	7.1 (3.8-12.5)	3(1.5) ^a	6(5.3)	12(21.1)	47(8.0)
Testis: interstitial cell tumor	74.6(63.8-82.5)	4(2.0)	88(77.2)	41(71.9)	583(98.6)
Preputial gland: Adenoma	5.3 (1.3-12.5)	1(0.5)	3(2.6)	4(7.0)	43(7.3)
Pituitary: anterior adenoma	12.4 (7.5-18.8)	—(—)	6(5.3)	7(12.3)	106(17.9)
Thyroid: C-cell adenoma	12.5 (3.8-21.3)*	—(—)	6(5.3)	5(8.8)	109(18.4)
Adrenal: pheochromocytoma	11.9 (1.3-22.5)*	3(3.8)	6(5.3)	9(15.8)	96(16.3)
Subcutis: fibroma	7.2 (5.0-11.3)	—(—)	2(1.8)	6(10.5)	61(10.3)

a: Parenthesis shows percentage of the tumor to the number of animals examined during each interval of age.

*; Significant heterogeneity $p < 0.05$

Table 4. Age-related occurrence of tumors in female F344/DuCrj rats at more than 5% incidence and variability in incidence among 12 control groups

Histological types of tumors	Overall incidence of tumors and variability in incidence	Age-related occurrence of tumors			
		0-58 wks (%)	59-84 (%)	85-97 (%)	98-110 (%)
No. of animals examined during each interval of age	959	204	142	45	568
Leukemia (mononuclear cell)	9.5(3.8-15.0)	—(—)	4(2.8) ^a	9(20.0)	78(13.7)
Uterus: endometrial polyp	12.6(6.3-23.8)	3(1.5)	9(6.3)	3(6.7)	106(18.7)
Pituitary: anterior adenoma	28.2(15.0-41.3)*	—(—)	25(17.6)	9(20.0)	236(41.5)
Thyroid: C-cell adenoma	8.2(2.5-16.3)	1(1.3)	4(2.8)	2(4.4)	72(12.7)
Mammary gland: adenoma/ fibroadenoma	13.2(5.0-22.5)	—(—)	3(2.1)	2(4.4)	122(21.5)

a: Parenthesis shows percentage of the tumor to the number of animals examined during each interval of age.

*: Significant heterogeneity $p < 0.05$

interstitial cell tumor in the testis was already common in the second interval of the study. In females, on the contrary, most of the major tumors showed a steep elevation of incidence between the third and last interval of the study.

The significant heterogeneity of tumor incidence among the different control groups was seen in the C-cell adenoma of the thyroid and pheochromocytoma of the adrenal in males and the anterior adenoma of the pituitary in females.

DISCUSSION

There have been a considerable number of reports on spontaneous tumors in F344 rats (Jacobs et al., 1967 ; Sass et al., 1975 ; Coleman et al., 1977 ; Goodman et al., 1979). Besides those reports which dealt with rather small number of animals and stressed on the elucidation of histopathological characteristics of the tumors in F344 rats including comparative analysis of tumor incidences with those of other strains of rats (Masoro et al., 1980) or other species of animals (Sher et al., 1982), several authors have recently contributed a large scale of data on the incidences of spontaneous tumors which would be served as baseline data for evaluation of the carcinogenicity studies with chemicals (Tarone et al., 1981 ; Maekawa et al., 1983 ; Solleveld et al., 1984). The types of the tumors observed in the present study were almost comparable to these large scale baseline data. The incidences of the tumors in the present study were comparable to or lower than the previous reports. As the present data were collected from ordinary 2-year chronic and oncogenicity studies, they included the materials from young animals subjected to the interim-kill at 32 weeks of age when no tumor

occurrence was usually expected. If only survivors after 59 weeks of age were accounted as the effective number of animals for the analysis, the incidence of each tumor might be comparable to that of the historical incidence.

Various factors or conditions have been considered to be responsible for fluctuation of the tumor occurrence (Everett et al., 1984); diet, hormonal state, environment of the animal room, housing condition, genetic background, and age factor. In addition to these factors, recent reports have been concerned with difference of diagnostic criteria between laboratories or pathologists (Haseman et al., 1984). A lot of arguments have been raised on criteria for tumorous conditions of the endocrine system between hyperplasia and adenoma (Jacobs et al., 1967). The incidences of C-cell adenoma in the present study were higher for males and females than those reported by Goodman et al. or Solleveld et al. The difference could be attributable to different diagnostic criteria, although dietary and environmental differences between North America and Japan must be taken into consideration.

Analyses on age-related occurrence of tumors revealed that the incidences of most of the major tumors in males had attained already to the plateau between 85 and 97 weeks of age while those in females showed a steep rise during the last interval of observation, 98 to 110 weeks of age. These results are very interesting in term of the sex difference of tumorigenesis.

The heterogeneity of tumor incidence between different control groups has been concerned by several authors (Tarone et al., 1981; Maekawa et al., 1983; Haseman et al., 1984). Tarone et al. reviewed the data of the carcinogenesis bioassay program sponsored by NCI for the analysis of variation of the tumor occurrence in 72 control groups from six laboratories, and pointed out the significance of intralaboratory heterogeneity as well as interlaboratory variation. Maekawa et al. found the intralaboratory heterogeneity in several major tumors of both sexes. In the present study, the heterogeneity was also noted in the C-cell adenoma of the thyroid and pheochromocytoma of the adrenal for males, and the anterior adenoma of the pituitary for females. F344 rat is an inbred strain with same genetic backgrounds. Besides, those data providing the basis of the debates on the intralaboratory heterogeneity were obtained in a given laboratory during a period of recent several years where the animals were reared under the same controlled conditions, including diet, sanitary care, handling procedure and etc. The pathologists in each laboratory might have the same criteria for diagnosis. These would indicate that it seemed to be inevitable to be aware of the intralaboratory heterogeneity as well as the interlaboratory heterogeneity when discussing the tumor occurrence of the rats.

The intralaboratory and interlaboratory heterogeneity sometimes obscure the significance of the historical background data of tumors in the evaluation of the results of the oncogenicity studies, and may address the argument that historical background data could become efficient only when a statistical analysis between the treated group and the control group gave an equivocal result. It is absolutely true that the tumor incidence of the treated group shall be compared primarily with that of the concurrent

control. However, even in the modern laboratories providing with the strictly controlled system for animal husbandry, there are still a lot of unknown factors which would happen to bring a significant fluctuation of the tumor occurrence among the animals in control or treated groups. Therefore, when a statistically significant elevation in incidence of a certain tumor is obtained in a treated group, it would always become an important procedure to see whether or not the incidences of both the control and the treated group remain within double SD (standard deviation) range of the historical background incidence of the tumor. In case that there is no accumulation of the tumor incidence in-house, the result should be referred to the outside data from several different sources. These motivations are still encouraging pathologists to expand the accumulation of historical background data on the tumor occurrence in rats.

REFERENCES

- Coleman, G. L., Barthold, S. W., Osbaldiston, G. W., et al. (1977) : Pathological changes during aging in barrier-reared Fischer 344 male rats. *J. Gerontol.*, **32**(3), 258–278.
- Evert, R. (1984) : Factors affecting spontaneous tumor incidence rates in mice : A literature review. *CRC Critical Rev. Toxicol.*, **13**, 235–251.
- Goodman, D. G., Ward, J. M., Squire, R. A., et al. (1979) : Neoplastic and nonneoplastic lesions in aging F344 rats. *Toxicol. Appl. Pharmacol.*, **48**, 237–248.
- Haseman, J. K., Huff, J., and Boorman, G. A. (1984) : Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pharmacol.*, **12**(2), 126–135.
- Jacobs, B. B. and Huseby, R. A. (1967) : Neoplasms occurring in aged Fisher rats, with special reference to testicular uterine, and thyroid tumors. *J. Natl. Cancer Inst.*, **39**, 303–309.
- Maekawa, A., Kurokawa, Y., Takahashi, M., et al. (1983) : Spontaneous tumors in F-344/DuCrj rats. *Gann*, **74**, 365–372.
- Masoro, E. J. (1980) : Mortality and growth characteristics of rat strains commonly used in aging research. *Exp. Aging Res.*, **6**(3), 219–233.
- Menear, J. H., Boorman, G. A., Chhabra, R. S., et al. (1985) : Toxicology and carcinogenesis studies of HC Blue No. 1 in F344/N rats and B6C3F1 Mice. National Toxicology Program, Technical Report Series No. 271, National Toxicology Program, Research Triangle Park, North Carolina.
- Sass, B., Rabstein, L. S., Madison, R., et al. (1975) : Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. *J. Natl. Cancer Inst.*, **54**, 1449–1456.
- Sher, S. P., Jensen, R. D., and Bokelman, D. L. (1982) : Spontaneous tumors in control F344 and Charles River-CD rats and Charles River CD-1 and B6C3HF1 mice. *Toxicol. Letters*, **11**, 103–110.
- Solleveld, H. A., Haseman, J. K., and McConnell, E. E. (1984) : Natural history of

- body weight gain, survival, and neoplasia F344 rat. J. Natl. Cancer Inst., **72**, 929–940.
- Stewart, H. L., Williams, G., Keysser, C. H., et al. (1980): Histologic typing of liver tumors of the rat. J. Natl. Cancer Inst., **64**, 178–206.
- Stromberg, P. C. and Bogtsberger, L. M. (1983): Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. Vet. Pathol., **20**, 698–708.
- Tarone, R. E., Chu, K. C., and Ward, J. M. (1981): Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N x C3H/HeN) F1 (B6C3F1) mice. J. Natl. Cancer Inst., **66**, 1175–1181.
- Turusov, V. S., Chesterman, F. C., Della Porta, G., et al. (1973): Pathology of tumours in laboratory animals. Vol. I: Tumours of the rat. International Agency for Research on Cancer, Lyon.