Spontaneous Lesions in Cynomolgus Monkeys Used in Toxicity Studies

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Spontaneous lesions in wild-caught, laboratory-maintained cynomolgus monkeys used in drug-toxicity studies were examined histopathologically in an effort to better distinguish toxic changes from spontaneous lesions and assess the toxicity of drugs more exactly. In the liver and kidney, where toxic changes are observed frequently, many spontaneous lesions were observed. Infiltration of mononuclear cells, vacuolization of the hepatocytes, dilatation of the renal tubules, and vacuolization of the renal epithelia were observed at a relatively high frequency. It is considered important to examine these changes carefully, because they closely resemble the changes recognized as toxic. Deposition of brownish pigment was observed in various organs such as the liver, kidney, spleen, intestinal tract, lung and brain, however the type of pigment differed among the organs, and histochemical examination revealed anthracosis or accumulation of hemosiderin, or melanin. Since the monkeys were caught in the wild, many parasitic lesions were observed especially in the large intestine and liver. Helminthous worms were frequently observed in the granulomas in the large intestine, however no parasites were observed in the granulomas in the liver. Such lesions in the liver may be misinterpreted as toxic changes, when only scars or inflammatory lesions are observed. — KEY WORDS : cynomolgus monkeys, spontaneous lesions

In pre-clinical toxicity studies of new drug candidates for human use, the use of primates which closely resemble humans is important [35]. However, many monkeys used in toxicity studies have bacterial or parasitic lesions, because almost all are wild-caught. Therefore, the accumulation of background data in hematology, blood chemistry, and histopathology and the recognition of spontaneous lesions are very important. Cynomolgus monkeys (*Macaca fascicularis*), which have been caught in Indonesia, the Philippines, or Malaysia, are used in toxicity studies in our laboratories. Though there are several reports [2, 10, 24, 26, 28, 32] on the parasitic lesions in this animal, no histopathological background data have been reported.

In the present study, the histopathology of spontaneous lesions found in 442 cynomolgus

monkeys (221 per sex) used in toxicity studies which were performed in our laboratories from 1979 through 1990 is described.

Materials and Methods

A. Animals : Four hundred and forty-two cynomolgus monkeys (221 per sex, assumed to be more than 3 years old, caught in Indonesia, the Philippines, or Malaysia) used in 17 toxicity studies performed in our laboratories from 1979 through 1990 were the subjects of this study. These animals were quarantined at CLEA Japan, Inc. after import and were confirmed to be normal by clinical observation, measurement of body weight, microbiological examinations (tuberculin reaction, serological examinations for *Salmonella*, *Shigella*, and *Entamoeba*), parasitological examinations

Table 1. Treatment-related lesions in cynomolgus monkeys examined in this study

Study No.	Finding
1	Dilatation of renal tubules
2	None
3	Vacuolization or hypertrophy of hepatocytes / Multinucleated hepatocytes / Vacuolization or hypertrophy of renal tubular epithelia
4	None
5	Necrosis of hepatocytes / Necrosis or regeneration of renal tubular epithelia / Infiltration of eosinophils in various organs
6	Infiltration of eosinophils in various organs
7	Necrosis of hepatocytes / Hypertrophy of germinal center of spleen
8	Necrosis or vacuolization of hepatocytes / Hyaline deposition of renal tubular epithelia
9	Necrosis, vacuolization or pigmentation of hepatocytes
10	Pigmentation of hepatocytes
11	Necrosis or vacuolization of hepatocytes
12	Pigmentation of hepatocytes
13	Necrosis, vacuolization, regeneration or hypertrophy of renal tubular epithelia
14	Hypercellularity of bone marrow / Infiltration of eosinophils in various organs
15	Congestion and follicular atrophy of spleen
16	Atrophy or vacuolization of hepatocytes / Degeneration, regeneration or hyaline deposition of renal tubular epithelia / Atrophy of myocardium / Hypercellularity of bone marrow
17	Hypertrophy of hepatocytes

(parasite-ova), hematological examinations (hematocrit value, leukocyte count), and blood chemistry (urea nitrogen, alanine aminotransferase, aspartate aminotransferase) in our laboratories.

The animals were housed in individual metal cages in an air-conditioned room with a temperature of $24 \pm 2^\circ\text{C}$, a relative humidity of $55 \pm 10\%$, air-exchanges 10–20 times/hr, and a 12 hr fluorescent illumination light/dark cycle. Each animal was fed daily 150g of a pellet diet (AB, Oriental Yeast Co., Ltd. or Certified Primate Chow (5048), Ralston Purina Co.) and was allowed free access to tap water.

B. Histopathology : At the end of the dosing period in each toxicity study, each animal was anesthetized with ketamine hydrochloride (Ketalar®, Sankyo Co., Ltd.) and was sacrificed by bleeding from the common carotid artery. The following organs were observed visually and fixed in 10% neutral buffered formalin : liver, gallbladder, kidneys, heart, bronchus, lungs, spleen, trachea, tongue, esophagus, stomach, duodenum, small intestine, large intestine, submaxillary glands, sublingual glands, pancreas, thymus, mesenteric lymph node, bone marrow, testes, prostate, seminal vesicles, ovaries, uterus, vagina, urinary bladder,

brain, spinal cord, lacrimal glands, pituitary, thyroids, parathyroids, adrenals, skin, mammary gland, sternum, and femurs. The eye balls were fixed in 4% glutaraldehyde and post-fixed in 5% neutral buffered formalin. All organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H-E). As a rule, a left organ of paired organs was examined. Pigments were differentiated by Berlin blue stain [7], Masson stain for argen-tophile granules [19], the ferrous ion uptake method for melanin [16], Nile blue stain for melanin [15], bleaching by potassium per-manganate-oxalic acid [17], the periodic acid-Schiff reation (PAS) [20], and observation of autofluorescence with ultraviolet radiation [34]. To identify the cells with pigment in the brain, immunohistochemistry against glial fibrillary acidic protein (GFAP) was performed using an immunoperoxidase staining kit (HistoScan, Biomedica Co., USA).

Findings other than the lesions shown in Table 1, which were considered to be treatment-related, were assumed to be spontaneous.

Differences between the sexes in the incidence of each lesion were statistically analyzed using the chi-square test.

Table 2. Incidence of spontaneous histopathological lesions in cynomolgus monkeys

	Male (%)	Female (%)
Liver	n=221	n=221
Mononuclear cell infiltration	176 (79.6)	192 (86.9)
Vacuolization of hepatocyte	45 (20.4)	47 (21.3)
Inclusion body in hepatocyte	13 (5.9)	10 (4.5)
Necrosis of hepatocyte	14 (6.3)	6 (2.7)
Pigmentation of sinusoidal cell	42 (19.0)	32 (14.5)
Gallbladder	n=221	n=220
Mononuclear cell infiltration	32 (14.5)	26 (11.8)
Tongue	n=209	n=209
Mononuclear cell infiltration	41 (19.6)	37 (17.7)
Esophagus	n=157	n=156
Mononuclear cell infiltration	28 (17.8)	25 (16.0)
Stomach	n=221	n=221
Mononuclear cell infiltration	81 (36.7)**	44 (19.9)
Dilatation of gastric gland	19 (8.6)	16 (7.2)
Duodenum	n=157	n=157
Mononuclear cell infiltration	19 (12.1)	13 (8.3)
Pigmentation	40 (25.5)	40 (25.5)
Small intestine	n=221	n=221
Mononuclear cell infiltration	23 (10.4)	23 (10.4)
Large intestine	n=221	n=220
Mononuclear cell infiltration	20 (9.0)	34 (15.5)
Pigmentation	17 (7.7)	28 (12.7)
Submandibular gland	n=221	n=221
Mononuclear cell infiltration	113 (51.1)	113 (51.1)
Sublingual gland	n=157	n=157
Mononuclear cell infiltration	59 (37.6)	51 (32.5)
Pancreas	n=221	n=221
Mononuclear cell infiltration	21 (9.5)	19 (8.6)
Kidney	n=221	n=221
Pigmentation of tubular epithelium	56 (25.3)	42 (19.0)
Basophilic tubular epithelium	35 (15.8)	33 (14.9)
Calcium deposition in papilla	16 (7.2)	20 (9.0)
Dilatation of tubule	12 (5.4)	15 (6.8)
Vacuolization of tubular epithelium	18 (8.1)*	7 (3.2)
Hyaline cast	11 (5.0)	9 (4.1)
Glomerulosclerosis	10 (4.5)	14 (6.3)
Mononuclear cell infiltration	143 (64.7)	125 (56.6)
Urinary bladder	n=221	n=220
Mononuclear cell infiltration	34 (15.4)	36 (16.4)
Eosinophilic inclusion in epithelium	29 (13.1)	35 (15.9)

Table 2. Incidence of spontaneous histopathological lesions in cynomolgus monkeys (continued)

	Male (%)	Female (%)
Heart	n=221	n=221
Mononuclear cell infiltration	97 (43.9)	91 (41.2)
Pigmentation	3 (1.4)	2 (0.9)
Coronary arteritis	1 (0.5)	0
Lung	n=221	n=221
Pigmentation	141 (63.8)	150 (67.9)
Mononuclear cell infiltration	65 (29.4)	61 (27.6)
Aggregation of macrophage	21 (9.5)	17 (7.7)
Granuloma	14 (6.3)	13 (5.9)
Emphysema	16 (7.2)*	6 (2.7)
Spleen	n=221	n=221
Hyaline deposition	43 (19.5)	40 (18.1)
Hypertrophy of follicle	11 (5.0)	9 (4.1)
Pigmentation	25 (11.3)	23 (10.4)
Congestion	11 (5.0)	13 (5.9)
Bone marrow	n=221	n=221
Lymphoid follicle	19 (8.6)	27 (12.2)
Thymus	n=216	n=219
Involution	63 (29.2)	58 (26.5)
Cyst	52 (24.1)	40 (18.3)
Mesenteric lymph node	n=221	n=221
Pigmentation	21 (9.5)**	45 (20.4)
Hyaline deposition	15 (6.8)	15 (6.8)
Testis	n=221	
Atrophy of seminiferous tubule	11 (5.0)	
Immature seminiferous tubule	15 (6.8)	
Multinucleated giant cell	5 (2.3)	
Prostate	n=221	
Mononuclear cell infiltration	76 (34.4)	
Ovary		n=221
Calcium deposition		18 (8.1)
Uterus		n=221
Mononuclear cell infiltration		21 (9.5)
Vagina		n=157
Mononuclear cell infiltration		54 (34.4)
Brain	n=221	n=221
Pigmentation	28 (12.7)	24 (10.9)
Calcium deposition	8 (3.6)	10 (4.5)
Eye	n=209	n=209
Mononuclear cell infiltration	6 (2.9)	4 (1.9)
Skin	n=157	n=157
Mononuclear cell infiltration	3 (1.9)	0
Granuloma	1 (0.6)	1 (0.6)

Table 2. Incidence of spontaneous histopathological lesions in cynomolgus monkeys (continued)

	Male (%)	Female (%)
Pituitary	n=209	n=209
Cyst	11 (5.3)	15 (7.2)
Adrenal	n=221	n=221
Mononuclear cell infiltration	14 (6.3)	16 (7.2)
Pigmentation	24 (10.9)	17 (7.7)
Thyroid	n=221	n=221
Mononuclear cell infiltration	18 (8.1)	18 (8.1)
Pigmentation	25 (11.3)	21 (9.5)
Cystic dilatation of follicle	9 (4.1)	8 (3.6)

* : Significant difference in incidence between male and female ($p < 0.05$).

** : Significant difference in incidence between male and female ($p < 0.01$).

Results

Incidence of spontaneous lesions, except for hyperplastic, neoplastic or parasitic lesions, observed in each organ and their incidences are shown in Table 2.

A. Non-neoplastic, non-parasitic lesions

1. Alimentary system : In the liver, infiltration of mononuclear cells, such as lymphocytes or plasma cells, was most commonly observed in the hepatic lobules, and the incidence was high : 79.6% of the males and 86.9% of the females. Among the changes in the hepatocytes, vacuolization (Fig. 1) which is considered to be fatty change was most common (male, 20.4 % ; female, 21.3%). This finding was almost always seen focally, however its localization in a hepatic lobule was not definite. Eosinophilic inclusion bodies in hepatocytes (male, 5.9 % ; female, 4.5%) and focal necrosis of hepatocytes (male, 6.3 % ; female, 2.7%) (Fig. 2) were seen but at lower incidences. Deposition of brown pigment, which was positive for Berlin blue stain, was observed in sinusoidal cells at a relatively high frequency (male, 19.0 % ; female, 14.5 %).

In the alimentary tract from the tongue through the large intestine, infiltration of mononuclear cells was observed in the lamina propria or subserosa at the incidence of 8.3% to 36.7%. Dilatation of the gastric gland (male, 8.6 % ; female, 7.2%), deposition of yellowish brown pigment in the lamina propria of the duodenum (25.5% in each sex), and deposition of brown pigment in the large

intestine (male, 7.7 % ; female, 12.7 %) were observed. The pigment in the duodenum was negative for Berlin blue stain, however that in the large intestine was positive. In the submaxillary gland, sublingual gland, and pancreas, infiltration of mononuclear cells was also observed.

2. Urinary system : In the kidney, incidence of the tubular lesions was higher than one of the glomerular lesion. Deposition of brown pigment which was positive for Berlin blue stain in the tubular epithelia (male, 25.3 % ; female, 19.0%) (Fig. 3) and basophilic tubular epithelia (male, 15.8 % ; female, 14.9%) were observed frequently. Calcium deposition in papilla (male, 7.2 % ; female, 9.0%) (Fig. 4), dilatation of the renal tubules (male, 5.4 % ; female, 6.8%), vacuolization of the tubular epithelia (male, 8.1 % ; female, 3.2%), and hyaline casts (male, 5.0 % ; female, 4.1%) were observed in a small number of animals. Infiltration of mononuclear cells in the interstitial tissue was observed frequently (male, 64.7 % ; female, 56.6%).

3. Cardiovascular system : In the heart, infiltration of mononuclear cells was observed frequently (male, 43.9 % ; female, 41.2%) ; pigmentation (male, 1.4 % ; female, 0.9%) and coronary arteritis (male, 0.5%) were observed in a few animals.

4. Respiratory system : In the lung, deposition of dark-brown pigment in the interstitial tissue was most common (male, 63.8 % ; female, 67.9%). Infiltration of mononuclear cells in the interstitial tissue (male, 29.4 % ; female 27.6%), accumulation of macrophages

Table 3. Incidence of hyperplastic or neoplastic lesions in cynomolgus monkeys

Organ	Finding	Male (%)	Female (%)
Gallbladder	Hyperplasia of epithelium	0	1 (0.5)
Tongue	Papillary hyperplasia of epithelium	1 (0.5)	0
Stomach	Polyp	1 (0.5)	1 (0.5)
Ovary	Teratoma		1 (0.5)
Uterus	Papillary hyperplasia of endometrium		1 (0.5)
Adrenal	Nodular hyperplasia of zona fascicularis cells	10 (4.5)	3 (1.4)

in the alveoli (male, 9.5% ; female, 7.7%), and granuloma (male, 6.3% ; female, 5.9%) were observed.

5. Hematopoietic system : In the spleen, hyaline deposition (male, 19.5% ; female, 18.1%) and hypertrophy of lymphoid follicles (male, 5.0% ; female, 4.1%) were seen in the white pulp, and deposition of brown pigment, which was positive for Berlin blue stain (male, 11.3% ; female, 10.4%) were observed in the red pulp.

In the bone marrow, formation of lymphoid follicles was observed frequently (male, 8.6% ; female, 12.2%) (Fig. 5). In the thymus, involution (atrophy of both the cortex and medulla) (male, 29.2% ; female, 26.5%) and cysts (male, 24.1% ; female, 18.3%) (Fig. 6) were often seen. In the mesenteric lymph node, pigmentation in the medullary cord (male, 9.5% ; female, 20.4%) and hyaline deposition in lymphoid follicles (6.8% in each sex) were observed.

6. Genital system : In the testis, immature seminiferous tubules (6.8%) and multinucleated giant cells in the seminiferous tubules (2.3%) (Fig. 7) were observed. In the ovary, calcium deposition (8.1%) (Fig. 8) was observed.

7. Nervous system : In the brain, deposition of brown pigment was observed at a fairly high frequency (male, 12.7% ; female, 10.9%) (Fig. 9). The pigment was positive for Masson stain, ferrous ion uptake method, and Nile blue stain, and bleached by potassium permanganate-oxalic acid, while it was negative for either PAS reaction or Berlin blue stain, and emitted no autofluorescence with ultraviolet radiation. The cells retaining pigment with radial cytoplasmic projections were positive for GFAP. Most of the lesions were observed in the cortex of the frontal or parietal lobes. In the diencephalon, calcium deposition was seen around the small blood vessels (male, 3.6

% ; female, 4.5%) (Fig. 10).

8. Endocrine system : In the pituitary, cysts were observed in the anterior or intermediate lobe (male, 5.3% ; female 7.2%). Pigmentation of the zona reticularis cells male, 10.9% ; female, 7.7%) in the adrenal and of the follicular epithelia (male, 11.3% ; female, 9.5%) in the thyroid were observed.

B. Hyperplastic and neoplastic lesions

Incidence of hyperplastic or neoplastic lesions is shown in Table 3.

The most frequent hyperplastic lesion was nodular hyperplasia of the zona fascicularis cells in the adrenal (male, 4.5% ; female, 1.4%) (Fig. 11). Hyperplasia of epithelia in the gallbladder (female, 0.5%), papillary hyperplasia of mucosa in the tongue (male, 0.5%), polyps in the stomach (0.5% in each sex), and papillary hyperplasia of the endometrium (0.5%) were rarely observed. The only neoplastic lesion was a teratoma observed in the left ovary of one female. The teratoma was like a cyst (2×2×1.5cm) with black spots and contained hair and a mucous substance. In the wall of the cyst, mature tissues of bone, bone marrow, cartilage, tooth, skin, and hair follicle were observed (Fig. 12).

C. Parasitic lesions

Incidence of lesions related to parasites is shown in Table 4.

In the liver, granulomas were seen in 12.7% of the males and 17.2% of the females. The granulomas were cysts with a thick wall consisting of fibroblasts, epithelioid cells, lymphocytes and eosinophils, contained an amorphous substance (Fig. 13), and were frequently calcified. Helminths with a sucker were seen in the intrahepatic bile ducts (male, 1.4% ; female, 0.9%), however no changes were observed in the hepatic parenchyma.

In the large intestine, large cystic granu-

Table 4. Incidence of spontaneous lesions related to parasites in cynomolgus monkeys

Organ	Finding	Male (%)	Female (%)
Liver	Parasitic granuloma	28 (12.7)	38 (17.2)
	Parasite in bile duct	3 (1.4)	2 (0.9)
Tongue	Parasite in muscle fiber	25 (12.0)	36 (17.2)
	Parasitic granuloma	2 (1.0)	0
Esophagus	Parasite in muscle fiber	1 (0.6)	1 (0.6)
	Parasitic granuloma	1 (0.6)	0
Stomach	Parasitic granuloma	10 (4.5)	12 (5.4)
	Hyperplasia of epithelium with parasite in lumen	1 (0.5)	1 (0.5)
Small intestine	Parasitic granuloma	0	1 (0.5)
	Parasite in omentum	1 (0.5)	1 (0.5)
Large intestine	Parasitic granuloma	71 (32.1)	66 (30.0)
	Parasite in lumen	2 (0.9)	1 (0.5)
Lung	Parasite/Parasitic granuloma	12 (5.4)	13 (5.9)
Spleen	Parasitic granuloma	3 (1.4)	1 (0.5)
Thymus	Parasite	1 (0.5)	3 (1.4)
Mesenteric lymph node	Parasitic granuloma	2 (0.9)	1 (0.5)
Prostate	Parasite in muscle fiber	1 (0.5)	
Skin	Parasite in muscle fiber	4 (2.5)	1 (0.6)

Table 5. Number of animals with parasitic lesion (s)

Number of Parasitic Lesion(s) / Animal	1	2	3	4	5
Male	78	22 (1)	9 (2)	1	0
Female	75	22 (1)	6	3	1

() : Number of animals with the same lesion in 2 or 3 organs.

lomas were seen in the lamina propria, submucosa, or muscularis (male, 32.1% ; female, 30.0%) (Fig. 14). The inner side of the granulomas were covered by multinucleated giant cells and contained threadworms and an amorphous substance.

In the stomach, papillary hyperplasia of the mucosa was observed in 0.5% in each sex, and helminths were found in the gastric pits in the lesions (Fig. 15). Another parasitic lesion was a granuloma, consisting of fibrous tissue in the subserosa (male, 4.5% ; female, 5.4%), however the presence of helminth was not confirmed. The same granulomas were observed

in the subserosa of the small intestine (male, 0.5%) and in the omentum (0.5% in each sex).

In the lung, a granuloma accompanied by necrosis of the parenchyma around mites and wide-spread infiltration of inflammatory cells was seen in 5.4% of the males and 5.9% of the females (Fig. 16).

In the spleen, a granuloma accompanied by proliferation of reticuloendothelial cells and infiltration of eosinophils was observed in 1.4% of the males and 0.5% of the females. Microfilariae, occasionally engulfed by multinucleated giant cells were found in the granulomas.

Cysts of sporozoon were observed in the

muscle fibers of the tongue (male, 12.0% ; female, 17.2%), esophagus (female, 0.6%), prostate (0.5%), and subcutaneous tissue (male, 2.5% ; female, 0.6%), however no biological reaction against the parasites was observed.

Number of animals with parasitic lesions is shown in Table 5. Individually, a few animals had 4 or 5 parasitic lesions although most of animals had single lesion. A small number of animals had same parasitic lesion, cysts of sporozoan, in 2 or 3 organs individually.

Discussion

The liver and kidney are the organs in which toxic effects of chemicals are induced frequently, because in many cases a drug is metabolized in the liver and excreted in the urine via the kidney. Therefore, it is noteworthy that many spontaneous lesions occur in these organs : infiltration of mononuclear cells was most common in both organs, and vacuolization of the hepatocytes, dilatation of the renal tubules, vacuolization of the renal tubular epithelia were observed to a lesser extent. Care must be taken to differentiate the spontaneous infiltration of mononuclear cells in the liver from the obvious hepatitis which can be caused by the administration of chemicals such as diphenylhydantoin [23] and para-aminosalicylic acid [14]. The storage of proteins [4] or fat droplets [27] in alcoholism and the storage of fat droplets with tetracycline treatment [5, 8] or exposure to carbon tetrachloride [22] are observed histopathologically as vacuolization of hepatocytes. Therefore, it is important to observe the size and/or distribution of the fat droplets in the lobulus and the presence or absence of degeneration or necrosis of hepatocytes or inflammatory changes in order to distinguish a toxic change from a spontaneous one. In a few animals, focal necrosis of hepatocytes was observed along with infiltration of mononuclear cells and multinucleated giant cells. The lesions were similar to those seen in parasitism by *Hepatozystis* spp. ; however, a definitive diagnosis could not be made because the lesions were not identified as a typical lesion caused by *Hepatozystis* spp. Dilatation of the renal tubules by cephaloridine [31] is easily distinguished from spontaneous dilatation because degenerative changes in the renal epithelia are accompanied in the case of

drug-induced dilatation but not spontaneous dilatation. Vacuolization of the renal tubular epithelia caused by exposure to carbon tetrachloride [13] can be distinguished from spontaneous vacuolization by the presence of degenerative changes.

In various organs, deposition of yellowish to dark brown pigment was observed. The brown pigment in the hepatic sinusoidal cells, renal tubular epithelia, splenic red pulp, and around parasitic granulomas in the large intestine was positive for Berlin blue stain, indicating deposition of hemosiderin. The yellowish brown pigment in the duodenal lamina propria and mesenteric lymph node was negative with Berlin blue stain, indicating probably deposition of bile pigment. The dark brown pigment seen in the interstitial connective tissue around the lung bronchioles resembles the pigment seen in anthracosis in humans ; the pigment in the lungs of monkeys is reported to be a discharge of lung mites, *Pneumonyssus* sp. [12]. The brown pigment in the brain was considered to be melanin because of a positive reaction with Masson stain for argentophile granules [19], ferrous ion uptake [16], positive Nile blue staining [15], bleaching by potassium permanganate-oxalic acid [17], a negative PAS reaction [20], negative Berlin blue staining [7], and no autofluorescence upon ultraviolet radiation [34]. Since the cells retaining the melanin-like pigment had radial cytoplasmic projections and gave a positive GFAP immunohistochemical stain, they are considered to be astrocytes. Astrocytes have no phagocytic ability [21], and the lesions were located far from the *substantia nigra* and the *locus coeruleus*. Therefore, it is considered that the melanin pigment is produced by astrocytes. Neuronal melanin [3, 6, 9, 18, 21, 29] or neuronal ceroid-lipofuscin [1, 11, 33] have been reported in mammals, including monkeys, as brown pigment in the central nervous system, however melanin pigment has not been reported in the astrocytes. On the other hand, difficulty in identifying the substance producing the brown pigmentation in H-E stained sections of various organs emphasized the importance of histochemical techniques.

Statistically significant sex differences were seen in the incidence of infiltration of mononuclear cells in the stomach ($p < 0.01$), pigmentation in the mesenteric lymph node (p

<0.01), and vacuolization of the renal tubular epithelia ($p < 0.05$); however, the meaning of the sex differences in these changes is not clear as there are no reports of the involvement of sex hormones in these changes.

Reports of neoplastic lesions in monkeys are rare, and a teratoma of the ovary was observed in one female in this study. The teratoma formed a cyst containing hair and a mucous substance. In the wall of the cyst, ectodermal tissues such as tooth, skin, and hair follicle and mesodermal tissues such as bone, cartilage, and bone marrow were observed histologically, indicating a bidermoma. Scott *et al.* [30] and Ninomiya [25] reported this tumor in the ovary of a rhesus monkey and in the adrenal of a Wistar rat, respectively. The former was a dermoid cyst consisting of only ectodermal tissues such as skin, sweat gland, and hair follicle; the latter was a tridermoma consisting of ectodermal tissues such as nervous tissue and skin, mesodermal tissue such as striated muscle, bone, and cartilage, and endodermal tissues such as pancreatic acinus.

The most common parasitic lesion in this study was a cystic granuloma in the large intestine with an incidence of more than 30% in each sex. Since a threadworm was often found in the cyst, the lesion is considered to be formed by a larva (in the third phase) of *Oesophagostomum* sp. [2]. In the liver, cyst-like granulomas consisting of fibroblasts, epithelioid cells, lymphocytes, and eosinophils were frequently observed. Though no parasite was observed in these granulomas in most cases, they are considered to be related to schizogony of *Hepaticocystis* spp. because their morphology is similar to that in one of the cases reported by Ryan *et al.* [28]. Threadworms, considered to be *Noctia nocti*, were found in the gastric pits with papillary hyperplasia of the regional mucosa in one animal of each sex. Hashimoto *et al.* [10], observed papillomas caused by threadworms in the stomach at a relatively high frequency. We observed fibrous granulomas without evidence of parasites in the subserosa of the stomach, small intestine, and mesenterium. Abbott *et al.* [2] reported a relationship between similar lesions and peritoneal migration of *Paragonimus westermani*; therefore, the granulomas might be related to *Paragonimus westermani* or other

parasites. In the spleen of a few animals, granulomas with microfilariae and proliferation of reticuloendothelial cells were observed. Nonoyama *et al.* [26] and Narama *et al.* [24], reported the presence of mature filariae under the peritoneum or pleura in cynomolgus monkeys with similar splenic lesions, and Tada *et al.* [32] identified morphologically a mature filaria and microfilariae in the blood as *Edesonfilaria malayensis*. In this study, no mature filariae were found at autopsy, however the granuloma in the spleen is considered to related to *Edesonfilaria malayensis*, because the histological features are similar to those in one of the cases in the reports of Nonoyama *et al.* and Narama *et al.* Though several other parasitic lesions were observed, they would not affect the interpretation of chemical toxicity, because no abnormalities were seen in any animal in general condition, hematology, or blood chemistry. Nevertheless, there is the possibility of misinterpretation when no parasites are detected or only inflammatory lesions around a parasite are observed. Therefore, it is necessary to accumulate knowledge about parasitic lesions or to perform three-dimensional observation of serial sections.

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毒性試験に用いられたカニクイザルにおける 自然発生病変

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薬物による変化と自然発生病変との鑑別を正確に行い、より精度の高い毒性評価を行うことを目的として、毒性試験に用いられた雌雄各 221 匹のカニクイザルの自然発生病変について病理組織学的に検討した。肝臓及び腎臓は毒性所見が多く発現する臓器であるが、自然発生病変もまた多く認められた。単核細胞浸潤、肝細胞の空胞化、尿細管の拡張、尿細管上皮の空胞化が比較的高頻度にみられたが、これらの所見は毒性変化として認められるものに類似していた。各種臓器において褐色調の色

素沈着が認められたが、その本態はヘモジデリン、炭粉あるいはメラニンと多様であった。また、野生で捕獲した動物であるため寄生虫による病変が多数みられ、特に大腸と肝臓における肉芽腫が高頻度に認められた。大腸の肉芽腫では内腔に線虫の虫体を認める場合が多いのに対し、肝臓の肉芽腫では寄生虫は認められないため、瘢痕病変や周辺の炎症性病変のみ観察した場合毒性病変と誤認する可能性が示唆された。

Explanation of Figures

Fig. 1. Liver, Female, Vacuolization of hepatocytes H-E stain $\times 100$

Fig. 2. Liver, Male, Focal necrosis of hepatocytes with infiltration of multinuclear giant cells (arrows) and mononuclear cells H-E stain $\times 200$

Fig. 3. Kidney, Female, Pigmentation of epithelial cells in proximal straight tubule (arrow) H-E stain $\times 200$

Fig. 4. Kidney, Male, Deposition of calcium (arrow) in interstitial tissue of renal papilla H-E stain $\times 200$

Fig. 5. Bone Marrow, Male, Formation of lymphoid follicle H-E stain $\times 100$

Fig. 6. Thymus, Male, Involution of thymus, and formation of cyst (arrow) H-E stain $\times 40$

Fig. 7. Testis, Male Multinuclear giant cells (arrows) in seminiferous tubule H-E stain $\times 200$

Fig. 8. Ovary, Female, Deposition of calcium in ovarian follicles (arrows) H-E stain $\times 100$

Fig. 9. Cerebrum, Male Pigmentation of glial

cells (arrows) H-E stain $\times 200$

Fig. 10. Cerebrum, Male, Deposition of calcium (arrow) around blood vessel H-E stain $\times 200$

Fig. 11. Adrenal Gland, Male, Nodular hyperplasia (N) of cortical cells in zona fasciculata H-E stain $\times 100$

Fig. 12. Ovary, Female, Teratoma. In the wall of the cyst, mature tissues of tooth (T), bone (B), skin (S), and hair follicle (HF) are observed. H-E stain, $\times 15$

Fig. 13. Liver, Male, Parasitic granuloma beneath capsule H-E stain $\times 20$

Fig. 14. Large Intestine, Male, Parasitic granuloma in submucosa H-E stain $\times 20$

Fig. 15. Stomach, Male, Papillary hyperplasia of mucosa H-E stain $\times 10$ Inset: Magnified photograph of the area surrounded by lines, $\times 40$ Parasites in gastric pits

Fig. 16. Lung, Male, Parasite (arrow) in bronchus, and granuloma with infiltration of inflammatory cells around the parasite H-E stain $\times 20$