

Life-span Studies on Spontaneous Tumor Development in the Medaka (*Oryzias latipes*)

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A total of 961 medaka, separated chronologically from the first to the fifth year of life, were examined for spontaneous tumor development. While no liver tumors were found in either male or female medaka under the age of 1 year and the incidence in 2-year-old fish was relatively low (males 1.9% and females 1.7%), they became more common with advancing age. The incidence was higher in females than in males from 3 to 5 years of age, reaching 7.1% in 5-year-old female stock. These liver tumors included a total of 12 adenomas and 9 hepatocellular carcinomas. The hepatocellular carcinomas were histologically well differentiated and were all observed in female medaka. Spontaneous tumors occurring in organs other than the liver were rare and sporadic. Four squamous cell carcinomas, 5 melanomas and 4 lymphosarcomas were observed with no sexual or pronounced age bias being evident. The squamous cell carcinomas developed in the surface epithelium with local invasion into the dermis. Melanomas occurred in the abdominal cavity and demonstrated systemic invasion into various parts of the body. Three out of the 4 lymphosarcomas arose from the inner part of the operculum suggesting that these tumors were of thymic origin. They also showed extensive invasion. The data indicate a particular susceptibility of older female medaka to liver but not other tumor development.

Key words: Life-span study — Spontaneous tumors — Medaka — Liver tumors

The medaka, *Oryzias latipes* (Temminck et Schlegel) is a small fresh water fish, inhabiting rice paddies, ponds, and brooks throughout Japan. Although they have been used widely in the laboratory for studies in various fields such as genetics, physiology and biology,^{1,2)} their application in cancer research is relatively recent, as with other small aquarium fishes.³⁻⁵⁾ After Ishikawa *et al.* (1975)⁶⁾ first succeeded in inducing liver tumors in medaka with diethylnitrosamine (DNA), Aoki and Matsudaira (1977)⁷⁾ reported that liver tumors developed in this fish species within a short period of time after exposure to methylazoxymethanol (MAM) acetate for only 1 day. Since the medaka was thus found to be highly susceptible to chemical carcinogens this fish species has been widely used by several research groups.⁸⁻¹⁶⁾

Not only liver tumors,^{8,9,11,14,15)} but also gill tumors,¹²⁾ intraocular tumors,^{13,14)} melanomas¹⁰⁾ and rhabdomyosarcomas¹⁶⁾ have been induced in medaka by various carcinogens. However, only a few previous reports^{14,17)} concerning spontaneous tumors in medaka are available. Hawkins *et al.*¹⁴⁾ in 1988 found that the incidence of spontaneous tumors in young medaka was almost zero, only 1 liver tumor and 1 lymphosarcoma being observed in several thousand fish.¹⁴⁾ An olfactory neuroepithelioma in a medaka was also recently reported by Torikata *et al.* in 1989.¹⁷⁾

The present investigation is, to our knowledge, the first life-span approach to spontaneous tumor development in medaka and is based on our surveillance of this freshwater fish at the Cancer Institute, Tokyo between 1976 and 1989. Particular attention was given to the influence of aging on the incidence of spontaneous liver tumors.

MATERIALS AND METHODS

All medaka surveyed in this study were domesticated orange-red variety (himedaka), raised and randomly bred under artificial conditions. The medaka stocks examined for liver tumors were separated chronologically in special experimental ponds at the Division of Biology, National Institute of Radiological Sciences, Chiba-shi, from the first to the fifth year of life. Fish were maintained on artificial diet (TetraMin, Tetra, Melle, West Germany) in these out-door aquaria (ponds) constantly supplied with well water, and randomly collected at intervals. They were fixed in 10% neutral formalin solution or Bouin's fluid.

After macroscopic examination of the major internal organs livers from medaka from 1 to 5 years of age were dissected, embedded in paraffin and sectioned at 3 to 4 μ m. The medaka liver is a one-lobe organ (less than 3 to 4 mm in maximum diameter) localized in the anterior

abdomen. Sections were stained with hematoxylin and eosin (H-E), Mallory-Azan and periodic acid-Schiff (PAS) stains. Medaka with tumors other than liver tumors were also occasionally obtained from three different institutions (Zoological Institute, University of Tokyo; Division of Biology, National Institute of Radiological Sciences; Department of Experimental Pathology, Cancer Institute) between 1976 and 1989 (Table III). All fish were raised in out-door or in-door aquaria on artificial diets and processed for histological examination for tumors as above.

At the time of autopsy, the sex of medaka was determined from the shape of the anal fin^{1, 2)} (Fig. 1). Data for fish with liver or other tumors are given in Tables I, II and III.

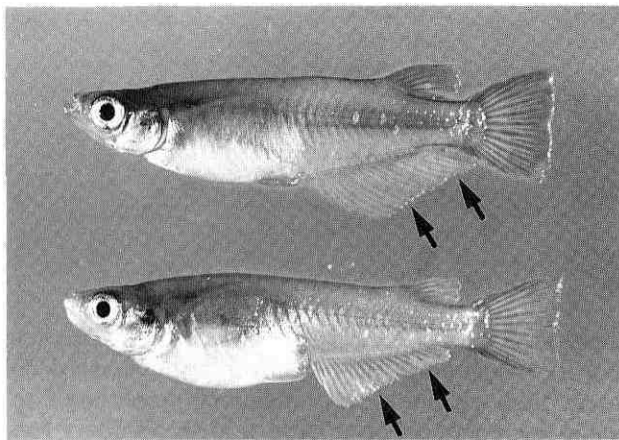


Fig. 1. Two normal medaka, male (top) and female (bottom). Sex can be determined from the shape and length of the anal fin (arrows). $\times 2$.

RESULTS

Life-span studies on liver tumors The incidences of spontaneous liver tumors as surveyed in medaka of various ages (1 to 5 years old) are shown in Table I. In 1-year-old (57 males and 67 females) medaka, no neoplastic lesions were found in the liver. In a total of 223 fish aged 2 years, the incidence of liver tumors was similar between males (1.9%) and females (1.7%) but thereafter the liver lesions became increasingly more common in females. Thus the incidence for liver tumors in 3- to 5-year-old male medaka was 0 to 3.8% whereas the respective figures for females were 1.3 to 7.1%.

A total of 12 adenomas and 9 hepatocellular carcinomas were diagnosed. The hepatocellular carcinomas all developed in females (Table II). Some liver tumors were palpable from the outside as abdominal swellings at autopsy. Grossly they presented as gray-whitish nodules (Fig. 2).

Adenomas measured within the range of 0.25 to 1.8 mm in maximum diameter microscopically. Histologically, the adenomas were noted as a round nodule that contrasted with the surrounding normal liver tissue showing sheetlike arrangement of parenchymal cells (Fig. 3). The adenomas were composed of large cells with little variation in size or shape, demonstrating greater amounts of cytoplasmic eosinophilic granules than the surrounding normal liver cells (Fig. 3). The hepatocellular carcinomas varied from 0.8 to 6.0 mm in maximum diameter and were arranged in rather compact, rounded masses with irregular boundaries, generally larger than the adenomas (Table II). The constituent cells presented numerous eosinophilic and PAS-positive granules in the cytoplasmic compartment and most were characterized by a round nucleus and large nucleolus. All hepato-

Table I. Incidence of Spontaneous Liver Tumors in Medaka (*Oryzias latipes*) with Age

Age (year)	No. of fish	Sex	No. of fish with liver tumors (%)	Diagnosis (No. of cases)
1	57	M	0 (0)	
	67	F	0 (0)	
2	108	M	2 (1.9)	Adenoma (2)
	115	F	2 (1.7)	Adenoma (1), hepatocellular carcinoma (1)
3	103	M	0 (0)	
	151	F	2 (1.3)	Hepatocellular carcinoma (2)
4	138	M	2 (1.4)	Adenoma (2)
	140	F	8 (5.7)	Adenoma (4), hepatocellular carcinoma (4)
5	26	M	1 (3.8)	Adenoma (1)
	56	F	4 (7.1)	Adenoma (2), hepatocellular carcinoma (2)

Table II. Spontaneous Liver Tumors in Medaka

Fish No.	Sex	Age (year)	Tumor size (mm)	Diagnosis
1	M	2	0.5×0.6	Adenoma
2	M	2	0.4×0.4	Adenoma
3	F	2	0.25×0.25	Adenoma
4	F	2	6.0×5.0	Hepatocellular carcinoma
5	F	3	2.5×1.9	Hepatocellular carcinoma
6	F	3	2.5×1.4	Hepatocellular carcinoma
7	M	4	0.5×0.3	Adenoma
8	M	4	1.4×0.8	Adenoma
9	F	4	0.7×0.5	Adenoma
10	F	4	0.5×0.3	Adenoma
11	F	4	0.8×0.8	Hepatocellular carcinoma
12	F	4	1.8×1.8	Adenoma
13	F	4	1.8×0.8	Hepatocellular carcinoma
14	F	4	1.2×0.8	Hepatocellular carcinoma
15	F	4	2.6×2.5	Hepatocellular carcinoma
16	F	4	1.1×0.6	Adenoma
17	M	5	1.1×0.8	Adenoma
18	F	5	1.0×0.8	Adenoma
19	F	5	1.0×1.0	Adenoma
20	F	5	2.0×1.6	Hepatocellular carcinoma
			0.4×0.3	Adenoma
21	F	5	1.5×0.9	Hepatocellular carcinoma

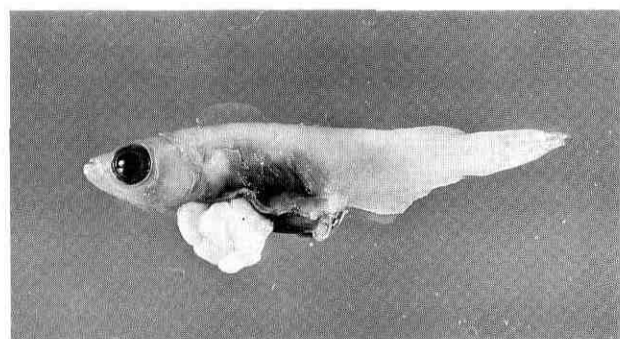


Fig. 2. Large spontaneous liver tumor, 6×5 mm in size with several smaller nodules in a 2-year-old medaka. ×2.

cellular carcinomas were diagnosed as well differentiated (Fig. 4). One liver tumor measuring 1.5 mm in maximum diameter, found in a 5-year-old female medaka, demonstrated a different appearance with an atypical hepatocellular carcinoma (Fig. 5). The tumor cells had basophilic cytoplasm. In the livers of the oldest medaka (5 years old), degenerated or necrotic foci were frequently observed (Fig. 6) suggesting age-associated changes.

Other spontaneous tumors Spontaneous tumors occurring in organs other than the liver were observed only rarely. Four squamous cell carcinomas measuring 5 to 6 mm in maximum diameter were located as solitary hemispheric nodules on the head skin (1 case)(Fig. 7), outer or inner operculum (2 cases) and on the abdominal skin (1 case). Histologically, the neoplastic epithelial cells were arranged variously in the form of sheets or cords in a poorly developed collagenous matrix (Fig. 8). In all squamous cell carcinomas examined, a few PAS-positive mucous cells which also exist in the normal fish epidermis, were seen.

Melanomas were found in the abdominal cavity of 5 medaka (2 to 3 years old), three of the lesions being evident externally (Fig. 9). Composed of aggregates of heavily pigmented polyhedral or spindle shaped melanocytes diffused into the peritoneal wall (Fig. 10), these melanomas showed systemic invasion into the gill, heart, spleen, connective tissue, muscle or the peri-spinal cord tissues (Fig. 11). The melanoma in fish #30 was a mass (6×5×5 mm) embedded in the muscle with local invasion to the ovary. Although in most areas cells were heavily laden with melanin pigments some tumor cells included newly formed sparse melanin pigments or were amelanotic (Fig. 12).

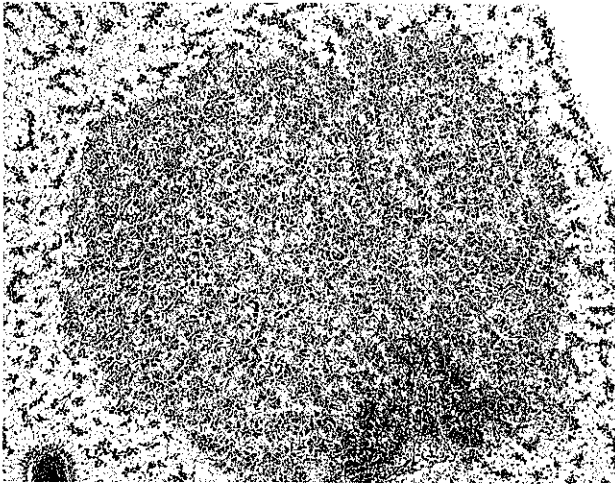


Fig. 3. Histologic appearance of a liver cell adenoma showing small round nodular character. Note the uniformity of the cells. H-E stain, $\times 70$.

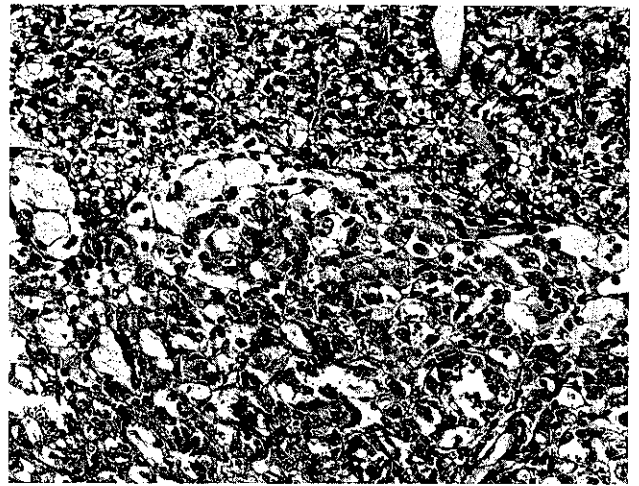


Fig. 5. Histologic appearance of a spontaneous liver tumor demonstrating an atypical hepatocellular carcinoma. H-E stain, $\times 200$.

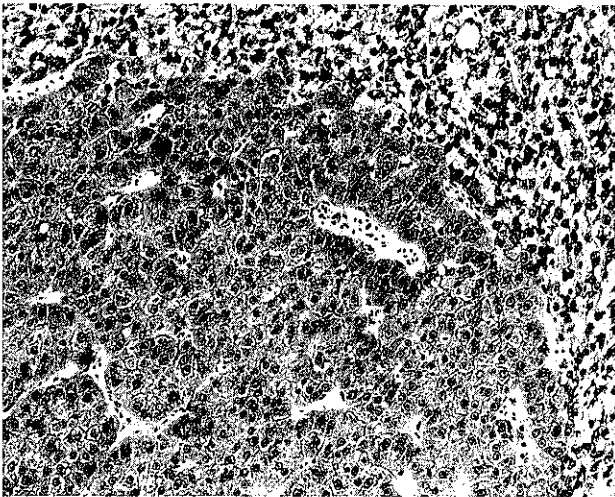


Fig. 4. Histologic appearance of a liver cell carcinoma showing a trabecular pattern and moderate basophilia. H-E stain, $\times 200$.

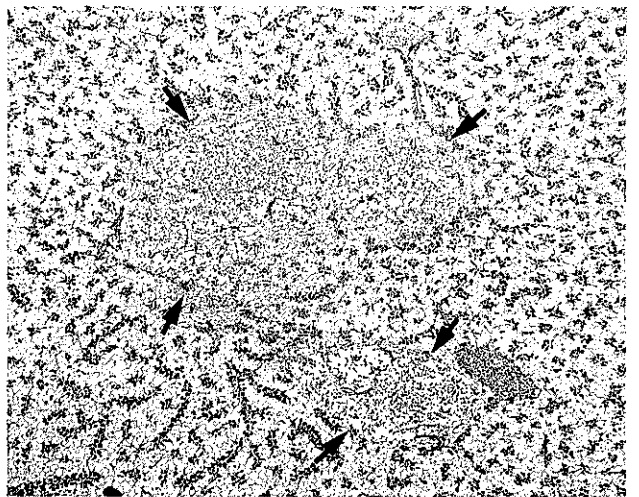


Fig. 6. Liver of an old medaka (5 years old) showing focal liver cell necrosis suggestive of age-associated change (arrows). H-E stain, $\times 70$.

Lymphosarcomas were found in 4 medaka as protruding nodules, measuring 4 to 7 mm in maximum diameter. Three out of 4 were observed in the operculum (Fig. 13), the other being found on the head. The ages of the tumor-bearing medaka were unknown except for one case (Table III). The lymphosarcomas consisted of round small lymphoid cells and in some parts of the tumors a starry sky pattern was observed (Fig. 14). Lymphosarcoma cells invaded the gill, thyroid, kidney,

ovaries, pharyngeal wall, peritoneum, muscle and peri-vertebral tissues in a diffuse manner (Fig. 15).

DISCUSSION

The present data suggest a clear sex- and age-dependency for liver but not other tumor development in the medaka. Reported life span data for this species¹⁸⁾ indicate that the 5-year-old stock used in the present study

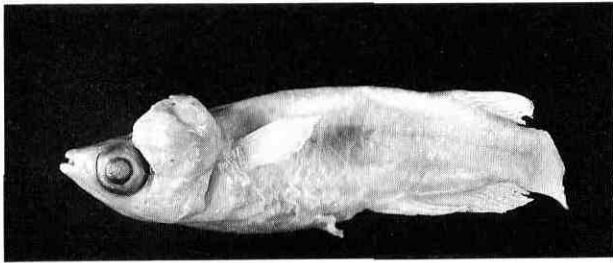


Fig. 7. Spontaneous squamous cell carcinoma in the head region. $\times 2$.

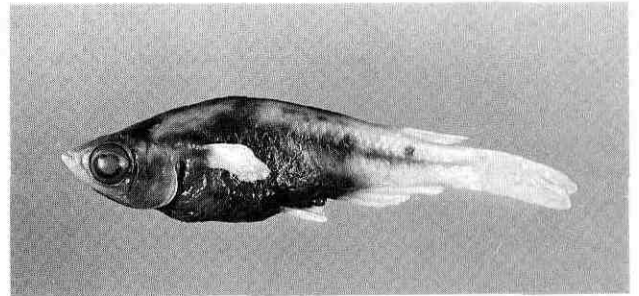


Fig. 9. Melanoma of the abdominal cavity showing systemic invasion which is visible from the outside. $\times 2$.



Fig. 8. Histologic appearance of a squamous cell carcinoma showing irregular arrangement of epithelial cells. H-E stain, $\times 70$.

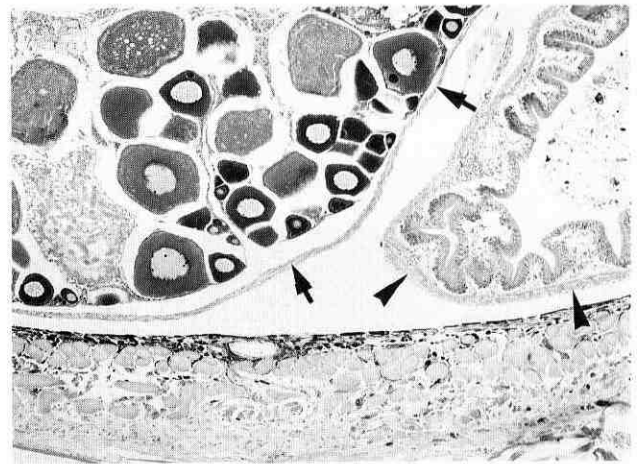


Fig. 10. Histologic appearance of a melanoma showing aggregation of pigmented tumor cells in the peritoneal wall. Note the ovary (arrows) and intestine (arrowheads). H-E stain, $\times 70$.

can be considered as at near-maximal age. Therefore, the present investigation is the first complete life-span approach to spontaneous tumor development in this fish. The incidence of liver tumors in young fish of both sexes was almost zero, as also noted by Hawkins *et al.*¹⁴⁾ However, with increasing age (from 3 to 5 years) and particularly in 4- and 5-year-old female medaka, relatively high incidences were observed. Therefore, an aging factor may play an important role in the development of spontaneous liver tumors in the medaka, as earlier reported for erythrophoromas in goldfish.¹⁹⁾ Histologically, the spontaneous liver tumors were less malignant than those induced in the medaka by chemical carcinogens,^{6,7)} all of the hepatocellular lesions observed being classified as adenomas or early stage carcinomas. The fact that carcinomas preferentially developed in the female medaka is in contrast to the earlier observations regard-

ing spontaneous liver tumors in man²⁰⁾ and mouse²¹⁾ in which a preponderance in males rather than in females had been noted. However, the report of higher incidences of spontaneous liver tumors in female than in male rainbow trout (*Oncorhynchus mykiss*) and river resident masu salmon (*Oncorhynchus masou masou*)²²⁾ suggests a similar sex-dependence for fish species. The development of liver tumors in female rainbow trout also increased between 2 and 4 years of age.²²⁾ A parallelism was suggested between liver tumor incidence and degree of ovarian maturation in the two kinds of salmonid fishes²²⁾ and when ovaries of river resident masu salmon were kept immature under artificial photoperiod conditions, the development of liver tumors was suppressed.²²⁾ The ovaries of medaka become mature at about 4 to 6 months of age.¹⁾ However, there are no data concerning the relation between incidence of liver tumors and develop-

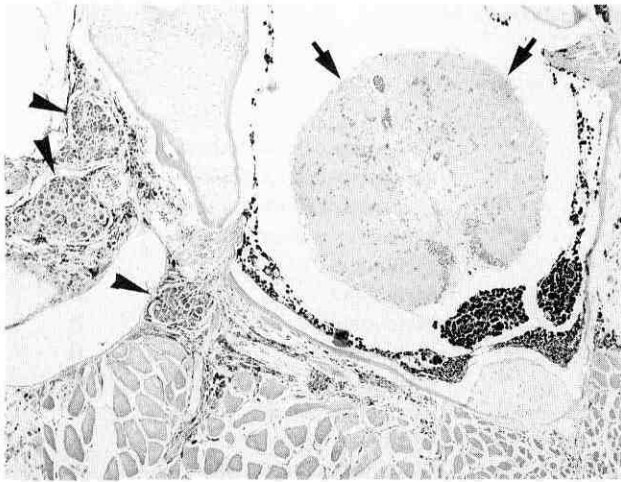


Fig. 11. Histologic appearance of a melanoma demonstrating systemic invasion into peri-spinal cord tissues. Note the spinal cord (arrows) and ganglions (arrowheads). H-E stain, $\times 70$.

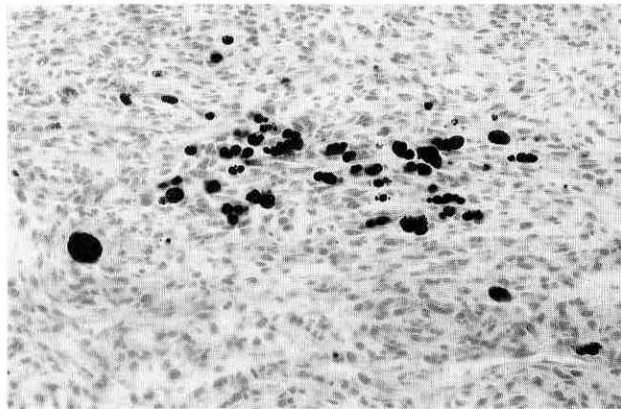


Fig. 12. Histologic appearance of an abdominal melanoma composed of spindle-shaped amelanotic cells and scattered heavily pigmented cells. H-E stain, $\times 300$.

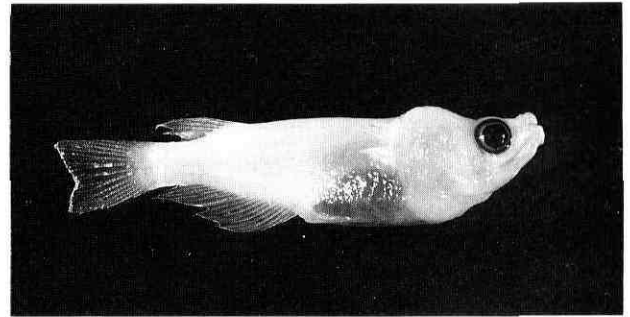


Fig. 13. Lymphosarcoma of the operculum suggesting thymic origin. $\times 2$.

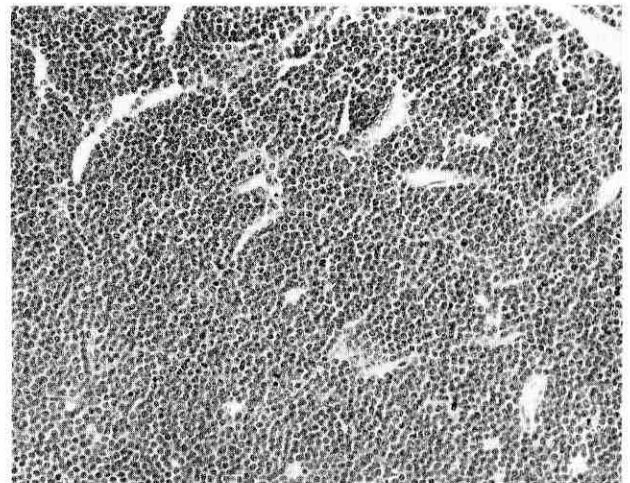


Fig. 14. Histologic appearance of a lymphosarcoma composed of small, round lymphoid cells. Note the starry sky pattern. H-E stain, $\times 150$.

ment of ovaries in this species. While both medaka^{6,7,23)} and rainbow trout²⁴⁾ have proven very sensitive to chemical carcinogens, whether different mechanisms might operate between spontaneous and induced hepatocarcinogenesis remains unclear. All liver tumor-bearing medaka used in this study were raised in constantly supplied well water and evidence of possible environmental contamination is presently not available.

Since only 4 squamous cell carcinomas, 5 melanomas and 4 lymphosarcomas were found in the medaka of this study and these tumors are rather common among

various species²⁵⁻²⁷⁾ their incidences would appear to be very low in the medaka. At least the 3 lymphosarcomas derived from the operculum might be considered as of thymic origin as mentioned by Hawkins *et al.*¹⁴⁾ Lymphosarcomas of thymic origin have also been observed in salmonid fishes.²⁸⁻³¹⁾ Melanomas and lymphosarcomas were truly malignant tumors which eventually killed the fish.

At present, the medaka is one of the most appropriate fish species for screening of possible environmental carcinogens. The findings detailed above suggest that medaka which are younger than 2 years old should be used in bioassay systems since spontaneous tumor development at this age is negligible. Favorable characteristics of this fish for laboratory experimentation^{23,32)} warrant its increasing application in cancer research.

Table III. Spontaneous Tumors Occurring in Organs Other than the Liver in Medaka

Fish No.	Total length (mm)	Sex	Age ^{a)} (year)	Tumor size ^{a)} (mm)	Tumor site	Invasion	Diagnosis	Source
22	35	M	—	6×5×5	Head skin	+ ^{b)}	Squamous cell carcinoma	A
23	33	F	2	5×3×3	Operculum	+ ^{b)}	Squamous cell carcinoma	B
24	40	F	3	5×4×4	Operculum	+ ^{b)}	Squamous cell carcinoma	B
25	35	F	4	5×5×5	Abdominal skin	+ ^{b)}	Squamous cell carcinoma	B
26	30	F	2	—	Abdominal cavity	+ ^{c)}	Melanoma	B
27	31	F	3	—	Abdominal cavity	+ ^{c)}	Melanoma	B
28	30	F	3	—	Abdominal cavity	+ ^{c)}	Melanoma	B
29	35	F	3	6×3×3	Abdominal cavity	+ ^{b)}	Melanoma	B
30	33	F	2	6×5×5	Abdominal cavity	+ ^{b)}	Melanoma	C
31	37	M	—	7×7×6	Operculum	+ ^{c)}	Lymphosarcoma	C
32	38	F	—	6×6×6	Operculum	+ ^{c)}	Lymphosarcoma	A
33	32	M	—	4×4×4	Operculum	+ ^{c)}	Lymphosarcoma	A
34	35	M	1	5×5×5	Head	+ ^{c)}	Lymphosarcoma	B

A=These fish were kept in the aquaria of the Zoological Institute, University of Tokyo, Tokyo. B=These fish were kept in the aquaria of the Division of Biology, National Institute of Radiological Sciences, Chiba-shi. C=The fish were kept in an aquarium of the Department of Experimental Pathology, Cancer Institute, Tokyo.

a) —=not determined.

b) Local invasion.

c) Systemic invasion.

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