INTRODUCTION

On 3 March 1998, an apparently young but seriously emaciated seal, which had been observed outside the port on the previous day was stranded on the wharf of Ishida Fishing Port, Kurobe City, Toyama Bay, Sea of Japan. The Toyama Prefectural Government charged the staff of Uozu City Aquarium, Uozu City Family Park and Toyama Prefectural Tobu Livestock Health and Hygiene Service Center with the responsibility of caring for the animal.

The seal was quickly transported to a tepid bath at the aquarium and its recovery was assisted by the administration of a combination of intramuscular injections of antibiotics, reinforced hepatic mixture and oral administration of electrolyte drugs (200 ¥ 4). However, after intense shivering the animal died at 20.50 h on the third day of treatment. At that time the rectal temperature measured 35.5°C. By that time the animal had been identified as a harbor seal Phoca largha.

No report dealing with histopathology of organs and tissues of the pinnipeds stranded on the coast of the Sea of Japan has been published. Therefore, in order to determine the reason for stranding (or landing) of this seal and the possible cause of death, histopathological examinations were undertaken with emphasis on lesions and possible disease.

MATERIALS AND METHODS

The seal measured 1.35 m in length and weighed 44 kg (270 g brainweight). The body was lean with a very thin subcutaneous fat layer (1–2 cm thick being one-third to one-half of the normal condition). Moreover, no solid food or feces were detected in the digestive tract nor urine in the urinary bladder. Parasite worms such as Anisakis were not apparent.

During examination of the visceral organs tiny sand grain-like nodules were noticed in the kidney. The most obvious abnormality was the existence of a ‘hen’s egg’-sized, very hard tumorous swelling with a purulent inflammatory appearance in the outer wall of the stomach (Fig. 1). All of the visceral organs were removed and immersed in 10% formalin.

Several tiny blocks taken from each organ were refixed with Bouin’s fixative dehydrated through a graded alcohol series embedded in paraffin and cut at 8–10 µm thickness. The deparaffinized sections were stained chiefly with hematoxylin and eosin double stain, Masson-Goldner (MG) trichrome stain and aldehyde fuchsin (AF)-MG stain for examination by light microscope.
Histopathology of stranded harbor seal

places, ganglion cells and Schwann sheaths were observed.

No pathological features were encountered in the mucosal epithelia of the duodenum, jejunum or large intestine.

In contrast, the ileum showed remarkable aberrancy: the lamina propria was intensely invaded by lymphocytes and the anterior part of the mucosal epithelial cells heavily inflamed, collapsed and degenerated (eroded), some being fused with each other in a chymatous state. Following HE staining, all eroded cells were stained deep red (crimson). Just beneath these structures marked vacuolization was apparent (Fig. 4).

Kidney

The intact part of the kidney consisted of glomeruli and both convoluted and straight tubules.

A novel condition was found in the development of pale yellow nodules in the kidney. Each nodule was roughly divided into lobules comprising many segments of a homogeneous alveolar structure, composed of acidophilic cuboidal cells with a round or oval nucleus. A distinct nucleolus and much chromatin were apparent in each nucleus.

In the lumen diffuse AF-positive material was present. Also present were follicles comprising relatively large cells with a distorted contour. Each cell had a depressed (flat) nucleus and fine granular cytoplasm with glycolipid-like features.

In the center of each lobe, a large duct surrounded by the layers of squamous cells, fibrocytes and smooth muscles, was detected. In addition, a relatively large number of muscular arteries and veins were found variously scattered. Near and in the periphery of the ducts, ganglia were encountered, the axonal fibers of which were stained by dyes. These epitheliolar structures were diagnosed as benign cortical adenomas (Fig. 5).

Carcinoma in the stomach

The most noteworthy aberrancy in the visceral organs was the hard swelling developed in the stomach (Fig. 1). Sections of the swelling showed the existence of masses of signet ring cells arranged in acinar nests. The cells were polyhedral in shape and contained much cytoplasm and an eccentrically shifted ovoid nucleus, was present in large numbers while other cells with a signet ring shape, crescentic nucleus and fused granules were seldomly encountered. In the latter cells, the cytoplasm was confined exclusively to the marginal zone, a large proportion of the center being a cyst comprised of clotting (globular) mucus. Thus, the cells were identified as being of typical 'signet ring' (general type) (Figs 6,7).

Moreover, marked hyperplasia of the interstitial tissue

RESULTS

Lymph nodes

A serious pathological condition was apparent in the cortex of the lymph nodes. Collagenous capsules had invaded deeply into the cortex and medullary zone dividing into lymphoid follicles. The branches of the capsules (trabeculae) contained myofibroblasts, arterioles and venules. In the superficial cortical parenchyma, numerous lymph nodules were occupied exclusively by proliferative macrophages instead of lymphocytes indicating highly phagocytic activities as shown in the figures (Figs 2,3).

Near the medullary region (i.e. paracortex) the contents of the lymphocytes in the follicles were sparse. Vesicular endothelial cells, fibroblasts and a reticular framework were easily observed.

Stomach, duodenum, jejunum, ileum and large intestine

The most noticeable aberrancy encountered was a large swelling of stomach (Fig. 1), the pathological details of which are discussed later.

The normal portion of the stomach consisted of gastric mucosa and lamina propria containing lymphocytes. Two cell types were identified: large pyramidal cells with a centrally positioned nucleus (identified as acid-producing (parietal) cells), and more elongated AF-positive cells with a basally positioned nucleus (identified as enzyme-producing cells, being the chief cells). Eosinophilic cytoplasm in the former was pale and the cytoplasm of the latter, coarsely granular.

A few cells, with depressed nuclei that stained deeply with hematoxylin and rather vesicular cytoplasm, were located close to the basement membrane. In several

Fig. 1  Macroscopic appearance of *Phoca largha* stomach with swelling (hen's egg size, †).
Fig. 2  Section of an aberrant lymph node with a strong proliferation of active phagocytic macrophages. Note trabecula (t). HE stain, original magnification ×400.

Fig. 3  As in Fig. 2. Note aggregation of lymphocytes (l). HE stain, original magnification ×400.

Fig. 4  Section of villi of ileum showing intense inflammation and deterioration. HE stain, original magnification ×400.

Fig. 5  Section of kidney adenoma consisting exclusively of alveolar segments. HE stain, original magnification ×400.
Figs 6–9  Carcinoma in the stomach. Fig. 6. Typical signet ring cell carcinoma (general type). Note the aggregation of cells in acinar nests. HE stain, original magnification ×400. Fig. 7. As in Fig. 6 but Masson-Goldner (MG) stain. Note demonstration of aldehyde fuchsin (AF)-positive substance (dark) in cytoplasm and fibers ×400. Fig. 8. Scirrhous cancer consisting of marked hyperplasia of interstitial submucosal tissue. HE stain, original magnification ×100. Fig. 9. Signet ring cells (microcystic type) found in an AF-positive trabecular structure consisted to have been produced by carcinomatous cells. This structure is present in scirrhous cancer in the interstitial tissue. MG stain. ×400.
was apparent. Such proliferative growth of the submucosal tissue being diagnosed as scirrhous cancer. In this region, gigantic signet ring cells disposed in cords were detected. While two or three oval cells were sometimes aggregated to form a cyst, other single cells were also apparent, being of a microcystic type. However, it was difficult to positively identify goblet cell-type carcinomatous cells. Mucus stained positively by AF was demonstrated in and around the margin of the above-mentioned cells, and in the interstitial tissue, the cells were diagnosed as typical of an adenocarcinomatous mucocellularare (or mucocellular carcinoma).

Between the scirrhous cancer layers several masses of necrotic tissue were also apparent (Figs 8,9).

DISCUSSION

Although studies documenting the histological and histopathological characteristics of marine mammals are few, a comprehensive review of the macroscopic anatomy and histopathology of several organs and tissues of selected cetaceans and pinnipeds has been published. In addition, Smith considered the stomach morphology of condition of empty and full and noted a nematode infestation. Very recently, 50 beach-stranded lymphoid organs of the bottlenose dolphin were described macroscopically and microscopically.

A comparison of the present findings with those of the above reports found no essential differences in the normal histological conditions of the various organs considered. Therefore, there is no need for further discussion of the normal (intact) organ architecture of the harbor seal examined here.

However, novel pathological conditions were found in the lymph nodes, ileum, kidney and stomach. Schroeder and Wegeforth, after macroscopic examination, reported the occurrence of gastric ulcers in three species of seals and one of sea lion, originally caught on islands off Baja, California, and thereafter maintained in captivity. Similarly, a report of ulcers caused by larval Anisakis in the forestomach of a harbor porpoise and observed by naked eye was given.

Rewell and Willis described histologically a uterine fibroma, tongue papilloma, ovarian cystadenoma and ovarian granulosa cell tumor found in whales. In addition, Stolk reported many cases of tumors, various inflammation and a hepatic cirrhosis also in whales. A further pathological symptom, phocine distemper infection, which resulted in immunodepression lymph node aberrancy and eventual mass death of harbor seals has been reported. It is likely that the inflammatory and erosive villi of the ileum may have contributed to the debility and emaciation of the present harbor seal.

Although benign cortical adenoma of the kidney is relatively well known in humans nothing has been reported of kidney adenoma in marine mammals. Furthermore, Simpson and Gardner stated that surprisingly few instances of renal diseases in pinnipeds and cetaceans were known, and Uys and Best mentioned that in spite of a study of 2000 whales fished at Saldanha Bay, South Africa, a kidney pathological condition was found in only two Bryde's whales.

A search of the literature has revealed no cases reported on signet ring cell carcinoma associated with scirrhous cancer in the stomach of marine mammals. In humans this carcinoma is classified as a common early gastric cancer; its occurrence being recognized sporadically in young children. Therefore, it is very likely that the present report is the first documentation of a signet ring cell carcinoma in a marine mammal; the disease possibly being the leading cause of death of the present specimen.

Cytologically, being at an early stage, most of the aberrant cells did not show the typical signet ring shape in the present case. However, when cancerous cells infiltrate the submucosa or deeper fibroblasts reacting to such cells characteristically they produce a large amount of collagen. Accordingly, marked hyperplasia of the interstitial fibrous tissue occurs in this case as a hard swelling in the stomach wall. Accordingly, it was noted that a superficial cancer (i.e., the signet ring cell carcinoma) changes into a poorly differentiated adenocarcinoma and subsequently to a non-solid cancer. The swelling developed in the present harbor seal could thus be classified as a form of progressive cancer.

REFERENCES