Some Histological Observations on a Harbor Porpoise, *Phocoena phocoena*, Stranded on Nishiyama Beach, Niigata Prefecture, Sea of Japan

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Abstract: Histological observations to determine physical condition were performed on some organs of a young female harbor porpoise, *Phocoena phocoena*, stranded on the beach at Nishiyama-machi, Niigata Prefecture, Sea of Japan. Most of the hepatic cells appeared normal, although some areas of fatty liver, heavy hemorrhaging and necrotic tissue were detected. The exocrine pancreas consisted of acini comprising granular (= zymogen) acinar cells and a centroacinar cell. On the other hand, the endocrine pancreas (islets of Langerhans) were composed of 3 types of endocrine cells, A- (glucagon producing), B- (insulin producing) and a few D (somatostatin producing) cells. The cortical region of the spleen appeared normal, with healthy lymphocytes, while the medullary region contained numerous macrophages. Considerable pigment (lipofuscins) were apparent in the macrophages, but no plasma cells were encountered. No kidney abnormalities were observed in the distal and collecting tubule cells, and most of the glomeruli, although a few of the latter had collapsed. Noticeably, several epithelial portions of the urinary bladder were in an early papillomatous phase. All of the lung alveolar cells had collapsed due to stranding, although the peripheral region (cartilagenous rings supporting the bronchi) was unchanged. A longitudinal section of anastomosed cardiac muscle fibers revealed intercalated discs, while in a cross (transverse) section Cohnheim fields were detected.

Introduction

As reported previously, a young female harbor porpoise, *Phocoena phocoena*, was stranded ashore at Nishiymama-machi, west of Niigata City on 26 February, 2001 (Honma et al., 2002). Because of a heavy visible injury, the finders buried the individual in the sandy beach.

Soon after this action, several aquarists and a veterinarian exhumed the specimen and removed the visceral organs (preserved in 10% formol) for further examination. The organs were forwarded to the senior author, a histological examination of the urino-genital organs having already been reported (Honma et al., 2002).

The remaining organs were also examined histologically, the former still appearing fresh despite the animal having been buried for one day. Because histological criteria for several organs of harbor porpoise have only been documented for a sole male individual (Honma et al., 1992), the study of a female will be a valuable addition to our present knowledge data base.

Materials and Methods

A number of organs (preserved in 10% formalin), including the liver, pancreas, spleen, kidney, urinary bladder, lung and heart were used in this study. Blocks removed from each organ were fixed secondarily in Bouin's solution, dehydrated in an alcohol series, embedded in paraffin, cut at 5 μm thickness, stained chiefly with hematoxylin-eosin (HE) double stain and Masson-Goldner (MG) associated with aldehyde fuchsin (AF) tetrachrome stain, and observed under a light microscope (Orthoplan, Leitz).

Results of Observations

Liver: Although a lobular pattern was indistinct, the liver was composed of lobules of epithelial (parenchymal hepatic) cells arranged radially in a central vein, associated in parallel with sinusoids (Fig. 1). The polyhedral hepatic cells were coarsely granular, each with an ovoid and/or round nucleus. However, in some parts, the cell mass included a considerable amount of adipose components, the congestive portions being surrounded by deeply-stained hepatic cells (Fig. 2). Nodules containing such cells were also stained deeply.

Each hepatic cell characteristically had a clearcut round nucleus, including distinct nucleoli. However, necrotic
portions were encountered in the nodules. The bile duct, consisting of columnar cells, in addition to Kupffer and dicaryonate cells, was observed only with difficulty.

Exocrine pancreas: The organ included numerous acini in the lobules, vascular components and interlobular ducts. Each acinus comprised several (or more) pyramidal cells arranged around a centroacinar cell. Two types of acinal cells were found: a larger cell containing coarse granules (=zymogen granules) and a smaller pale cell that lacked granules (i.e., granule exhausted). No anomalies were detected in the exocrine pancreas (Fig. 3).

Endocrine pancreas: Numerous small masses of islets of Langerhans were scattered throughout the exocrine pancreas. MG-AF stain disclosed three types of endocrine cells in the islets, the former being included within or in contact with tubuloacinar tissues.

The abundant polyhedral cells showed a strong affinity for AF, being categorized as insulin-producing B cells, whereas the smaller acidophile (phloxinophil) cells with peripherally-shifted nuclei were considered to be glucagon-producing A cells. Cells stained with light green were located sporadically, being identified as somatostatin-producing D cells (Fig. 3).

Spleen: The spleen consisted of a collagenous framework, a well-developed trabeculae, and roughly divided cortical and medulary portions. The organ was covered with a comparatively thick, loose connective tissue containing arteries and veins. Separation of the white (mass of lymphocytes) and red (circulating blood cells) pulps was incomplete (Fig. 5).

The cortical region appeared normal, with healthy lymphocytes, whereas a considerable number of macrophages much yellow brownian pigment (probably lipofuscin) both within and around the latter were detected in the medullary region. The texture of the medulla was rough and lacunal. However, no areas of highly active phagocytosis were encountered (Fig. 6).

Kidney: Although only one reniculus was examined, no notably aberrant and/or pathological conditions were found in the renal tissues, except for minor changes in a few renal corpuscles. Just beneath and at the entrance of the capillary glomerulus (the vascular pole), juxtapglomerular cells with granules and the macula densa consisting of low columnar cells were clearly defined (Fig. 7).

Glomerular necrosis was very seldom encountered. The proximal and collecting tubules were distinguishable adjacent to or near the renal corpuscle. The apices of the former cells were each equipped with a brush border, while the cytoplasm of the latter cells was light. The juxtapglomerular and Goormaghtigh cells, corresponding to smooth muscular cells, contained rich cytoplasm (Fig. 8).

Urinary bladder: The bladder was constructed from (1) a serous membrane of loose connective tissue, (2) an outer layer of longitudinal smooth muscle, (3) a middle layer of circular smooth muscle, (4) an inner layer of longitudinal smooth muscle, (5) a lamina propria of collagenous connective tissue and (6) transitional epithelium. It is notable that slight swellings in the epithelium, diagnosed as benign papillomatous growths, were encountered. The comparatively large, polyhedral cells, each epoating a round or ovoid nucleus, were very weakly positive with AF. The cell membrane was equipped with intercellular bridges (Fig. 9), and the epithelial margin showed keratinization. However, no mitotic figures and/or pyknotic features were detected (Fig. 10).

Lung: Unfortunately, most of the alveolar cells, with long ovoid nuclei, were congested and/or collapsed. However, interlobular loose connective tissues existed in the alveolar ducts, bronchioles and arteries, and alveolar septa were apparent.

Rings of hyaline cartilage, constituting part of the myoelastic sphincter accompanying muscle fiber bundles and epithelial cells were apparent at the bottom of alveolar duct (Fig. 11). The surface of the cartilage ring fragments was covered with a mucosal epithelium of cuboidal cells and lined with smooth muscle fiber bundles, as mentioned above. The cuboidal cells each had a deeply-stained nucleus. Between the sphincters and veins masses of circular mucous glands consisting of tall cells, were seen, the mucus showing an affinity to AF (Fig. 12).

Cardiac muscle: This involuntary striated muscle consisted of the muscle cells joined end to end at junctional zones, the so-called intercalated discs (Fig. 13). A single cardiac muscle cell comprised an elongated nucleus and sarcoplasm. However, branching muscle fibers were anastomosed. In cross section, so-called Cohnheim fields were seen (Fig. 14), the nuclei and pale portions without myofilibrils being located in the central portions of fibers, each fiber being associated with myofilibrils. Between the muscle fiber strands, flat, spindle-shaped cells were recognized.

Discussion

All organ specimens used in this study were removed
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from a young female harbor porpoise, together with the genital apparatus examined reported previously (Honma et al., 2002).

Kikkawa (1943～1944), who reported histological characteristics of several visceral organs of cetaceans, briefly mentioned that the liver of sei and blue whales contained a comparatively small amount of connective tissue, had a disrupted radial arrangement of hepatic cords toward the peripheral region, and lacked a defined gall bladder. Similar characteristics were reported for a senile male harbor porpoise (Honma et al., 1992) and were evident in the present examination.

Cockrill (1960) noted fat storage of fats in the livers of pregnant cetaceans, as did Honma et al. (1992). However, in the present young specimen, the amount of fat deposited was small.

Kikkawa (1944), Suito (1969), Simpson and Gardner (1972) and Honma et al. (1992) described the exocrine and endocrine pancreas of cetaceans, and emphasized that they contained a smaller amount of stromatous connective tissues than found in terrestrial tetrapod animals. Excepting Kikkawa (1944), they also pointed out the relatively large number (but smaller size) of islet tissues comprising A, B and D cells. The present examination confirmed these findings.

Lymphoid organs, including the spleen of marine mammals, were described and reviewed by Kikkawa (1943), Simpson and Gardner (1972), Schmacher and Welsch (1987), Honma et al. (1992), Nakamine et al. (1992) and Cowan and Smith (1999). Cowan (1966), Schmacher et al. (1990) and Honma et al. (2001) also noted respective pathological conditions of marine mammal spleens. Among others, Nakamine et al. (1992) documented a type of primitive mammalian spleen in an odontocetous whale. Cowan and Smith (1999), who dissected 50 beach-stranded bottlenose dolphins noted many smaller accessory spleens, some even embedded in the pancreas. Such a condition was not apparent in the present specimen.

It is well documented that the kidney of marine mammals is composed of many reniculi : such (lobate) structures (Lappenbildung) having been often described (e.g., Beauregard et Boulart, 1882 : Ping, 1926 : Kamiya, 1958 : Slijper, 1962 : Honma, et al., 1992). However, no histologically-different architecture of kidney elements has been detected when compared with terrestrial mammals, including man.

Examining the Atlantic bottlenosed dolphin, Simpson and Gardner (1972) illustrated a section of urinary bladder consisting of a transitional epithelium, loose connective tissue and concentric smooth muscle layers. They further mentioned one instance of acute necrotizing cystitis in this species, in which the epithelial lining was denuded, beeing the site of an acute inflammatory reaction. However, there appear to be no literature accounts of papillomatous growths of urinary bladder epithelium, as found in the present specimen. Although the grade of growth was slight in this case, a remarkable epithelial swelling with numerous intercellular bridges constructed from prominent desmosomes has recently been described in an aquarium-reared Acipenser (Honma et al., 1999).

Barbosa (1914) first described bronchiole sphincters, peculiar to dolphins. Subsequently, Fiebiger (1916), Nayres and Laurie (1937), Belanger (1940), Wislocki (1942), Kikkawa (1944), Murata (1951), Engel (1966), Ito et al. (1967), Simpson and Gardner (1972) and Honma et al. (1992) reported microscopic observations on cetacean lungs.

Among others, Wislocki (1942) and Honma et al. (1992) described a specialized system of myoelastic sphincters constituting "valvular segments" such involving even the smallest bronchiolar passages in the lungs. They noted also that such a characteristic was not present in larger cetaceans. As indicated in the results, in small odontocetous whales, which have a relatively short diving time, the myoelastic sphincters connecting the cartilage fragments, which surround and support the tubular wall of the bronchiule, are well developed and extended down to the respiratory bronchiule, at the beginning of the alveolar duct.

On the other hand, Lacoste and Baudrimont (1938), Goudappel and Slijper (1958) and Harrison and Tomlinson (1963) pointed out that bronchiolar sphincters were absent in larger whales, which are characterized by deep and prolonged diving, and relatively small lung capacity.

Although Simpson and Gardner (1972) described the cardiovascular system of marine mammals, the present study could examine only a small block of cardiac muscle and detailed comparisons cannot be made at this time.

As described above, the histological examination gave no insight into the factors leading up to the death of the present harbor porpoise. More fresh material of marine mammals stranded on the coast of Niigata District, Sea of Japan, are necessary for elucidating the cause of death during the winter season and for the accumulation of knowledge on the comparative histology of marine mammals.
References


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Fig. 1. Section of liver from a harbor porpoise, *Phocoena phocoena*, showing polyhedral hepatic cell cords running parallel with sinusoidal cords (s). Note granular cytoplasm and distinct nucleolus (arrow) in hepatic cell. Masson Goldner associated with AF (MG-AF) stain. $\times 400$

Fig. 2. Section of liver showing considerable fat components (f). Note round nucleus (arrow) in granular hepatic cells. MG-AF. $\times 400$

Fig. 3. Section of pancreas consisting of granular (zymogen) cells (z) and granule-exhausted light cells (l). a: Note several masses of islets of Langerhans (arrow head). Lower magnification. MA-AF. $\times 100$, b: Higher magnification. MA-AF. $\times 400$

Fig. 4. Part of islet of Langerhans in exocrine pancreatic tissue exhibiting three types of endocrine cells: AF strongly positive B (B) cells, phoxine stained A (A) cells and light green faintly stained D (D) cells. Note two types of acinar cells (z, l). MG-AF. $\times 400$
Fig. 5. Part of spleen showing small lymphocytes (l), red blood cells (r) and macrophages (m). Hematoxylin-eosin (HE) stain. ×400

Fig. 6. Medullary portion of loose lacunal spleen, showing slightly active macrophages (m), some of which were contaminated with lipofuscin (arrow). MG-AF. ×400

Fig. 7. Part of glomerular capillaries (G) and macula densa (md). HE. ×400

Fig. 8. Glomerulus (G), proximal (p) and collecting (c) convoluted tubules in the renal cortex. Apices of proximal tubule cells equipped with cilia brush border. Note juxtaglomerular apparatus comprising Goormaghtigh cells (arrow head) and juxtaglomerular cells (arrow). HE. × 400
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Fig. 9. Part of the transitional epithelium of urinary bladder, showing papillary elevation. Note polyhedral cell with a distinct nucleus (1) and cell membrane equipped with an intercellular bridge (arrow head). MG-AF. ×400

Fig. 10. Papillomatous growth of transitional epithelium. Note mitotic figures absent from nuclei. HE. ×400

Fig. 11. Endothelial cells of alveolus (a), a cartilage fragment (c) and part of the myoelastic sphincter. (veins, v). HE. ×400

Fig. 12. Circular mucous glands between the myoelastic sphincter and vein. HE. ×400

Fig. 13. Part of cardiac muscle showing anastomosis of branching muscle fibers. Note intercalated discs (arrow). MG-AF. ×400

Fig. 14. Cross-section of cardiac muscle fibers showing so-called Cohnheim fields. Note nuclei (n) and pale portions without myofibrils (arrow) located in central portion of fibers. HE. ×400
新潟県西山海岸へ漂着したネズミイルカ諸器官の組織学的観察

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2001年2月26日に、新潟県西山海岸へ漂着した若い♀ネズミイルカ（Honma et al., 2002）の諸器官を組織学的に観察し、漂着理由の一端を解明することを試みた。肝小葉は不明瞭で、多角状の肝細胞は、ほとんどが正常で、一部に脂肪蓄積や出血ならびに壊死が認められた。脾臓内分泌部は、酵素原顆粒を含有するものと、放出したと思われる腺房細胞との両者および腺房中心細胞から成る。内分泌部（ランゲルハンス小島）は内分泌組織中に多数散在し、A F陽性のB（インシュリン産生）、フロキシン陽性的A（グルカゴン産生）およびライトグリーン淡染のD（ソマトスタチン産生）細胞が識別できた。脾柱が発達した脾皮質部の白脾髄と赤脾髄の区別はあまり明瞭でなかったが、髄質部には大食細胞やリポフェストが目だった。腎臓は腎分体の集合より成るが、ごく少数の腎小体が壊死を起こしていた以外に、尿細管には異常が認められなかった。膀胱の移行上皮には、軽度の乳頭腫が発生していた。肺葉の肺拡張性活性約束は、大半を軟骨片を取り巻き、イルカ類の特徴を備えていた。心筋は、不随性横紋筋繊維が吻合し合い、繊維境界を成す介在板が観察された。一方、横断面では筋原纖維束がサーケンハイム野として現れた。しかし、死因となるような像はみられなかった。