Acute and Chronic Gas Bubble Lesions in Cetaceans Stranded in the United Kingdom

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Abstract. The first evidence suggestive of in vivo gas bubble formation in cetacea, including eight animals stranded in the UK, has recently been reported. This article presents the pathologic findings from these eight UK-stranded cetaceans and two additional UK-stranded cetacean cases in detail. Hepatic gas-filled cavitary lesions (0.2–6.0 cm diameter) involving approximately 5–90% of the liver volume were found in four (two juvenile, two adult) Risso's dolphins (*Grampus griseus*), three (two adult, one juvenile) common dolphins (*Delphinus delphis*), an adult Blainville's beaked whale (*Mesoplodon densirostris*), and an adult harbour porpoise (*Phocoena phocoena*). Histopathologic examination of the seven dolphin cases with gross liver cavities revealed variable degrees of pericavitary fibrosis, microscopic, intrahepatic, spherical, nonstaining cavities (typically 50–750 µm in diameter) consistent with gas emboli within distended portal vessels and sinusoids and associated with hepatic tissue compression, hemorrhages, fibrin/organizing thrombi, and foci of acute hepatocellular necrosis. Two common dolphins also had multiple and bilateral gross renal cavities (2.0–9.0 mm diameter) that, microscopically, were consistent with acute (*n* = 2) and chronic (*n* = 1) arterial gas emboli–induced renal infarcts. Microscopic, bubblelike cavities were also found in mesenteric lymph node (*n* = 4), adrenal (*n* = 2), spleen (*n* = 2), pulmonary associated lymph node (*n* = 1), posterior cervical lymph node (*n* = 1), and thyroid (*n* = 1). No bacterial organisms were isolated from five of six cavitated livers and one of one cavitated kidneys. The etiology and pathogenesis of these lesions are not known, although a decompression-related mechanism involving embolism of intestinal gas or de novo gas bubble (emboli) development derived from tissues supersaturated with nitrogen is suspected.

Key words: Cetacea; decompression; gas bubble; gas emboli; hepatopathy; marine mammals; nephropathy.

Cetaceans are highly evolved for their aquatic lifestyles. A range of behavioral, anatomical, and physiologic adaptations enable some species to exploit deep-water habitats while resisting the debilitating dive-related phenomenon of decompression sickness (DCS) or nitrogen narcosis, which can occur in human divers and other experimentally dived mammals.[11,19,36,38,39] Nitrogen gas, being inert, easily accumulates in biologic tissues and is driven to higher concentrations by increasing hydrostatic pressure at depth, is generally accepted as being responsible for both conditions. However, no studies have been conducted specifically to determine whether nitrogen bubbles can form in naturally diving cetaceans, and no diagnostic methods for determining narcotic states in stranded cetaceans exist. Indeed, the question of whether in vivo bubbles could persist in animals that dive to depths where hydrostatic pressure would presumably crush bubbles or bubble nuclei remains unanswered.

A recent study reported the first pathologic evidence consistent with in vivo bubble formation and tissue trauma in a number of cetacean strandings in the Canary Islands and in the UK.[21] This was shortly followed by reports of bony lesions in sperm whales (*Physeter macrocephalus*) that may have been caused by metabolic disease, age-related degeneration, or dysbaric osteonecrosis.[32] In the former study, the Canary Islands' cases consisted of 10 beaked whales that mass stranded coincident with a naval exercise using active sonar, increasing concern that acoustic factors may play a role in bubble formation.[12,13,21] The Canaries-stranded beaked whales had acute, systemic lesions consistent with, although not diagnostic of, DCS in humans and experimental animals.[12,13,21] In this study, we describe in detail pathologic findings from the eight UK-stranded cetaceans reported by Jepson et al.[21] and additional UK-stranded Risso's dolphin (*Grampus griseus*) (*n* = 1) and common dolphin (*Delphinus delphis*) (*n* = 1) cases that had acute and chronic lesions associated with gas bubbles in the liver and other parenchymatous organs. Although the etiology of the gas bubbles remains uncertain, the report demonstrates the

Table 1. Range of tissues examined using histology from each animal with in vivo bubble lesions.

Tissue	Case No. and Species*									
	1 (G.g.)	2 (P.p.)	3 (G.g.)	4 (M.d.)	5 (G.g.)	6 (D.d.)	7 (D.d.)	8 (G.g.)	9 (D.d.)	10 (D.d.)
Adrenal		+				+	+	+		+
Bladder	+	+				+	+	+		+
Blubber		+							+	+
Brain					+				+	+
Epididymis						+				
Esophagus						+				
Intestine				+						
Kidney		+	+		+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+
Lung	+	+	+	+	+	+	+	+	+	+
Mamm. gl.							+	+		+
Mesentery								+		
Mes LN			+		+	+	+	+	+	+
Myocardium						+		+		+
PALN	+				+	+	+		+	+
PCLN							+			
Placenta								+		
Skel. muscle			+					+		
Skin			+	+						
Spinal cord									+	+
Spleen		+			+	+	+	+	+	+
Stomach				+						
Testis						+				
Thymus		+			+	+	+			+
Thyroid					+	+	+	+		+
Uterus							+			
Uterine cyst								+		

* G.g., Grampus griseus; D.d., Delphinus delphis; P.p., Phocoena phocoena; M.d., Mesoplodon densirostris; Mamm. gl., mammary gland; Mes LN, mesenteric lymph node; PALN, pulmonary associated/diaphragmatic lymph node; PCLN, posterior cervical lymph node; Skel. muscle, skeletal muscle.

first evidence that bubbles can form in vivo in cetaceans and persist through time.

Materials and Methods

Between September 1990 and March 2004, 1,970 stranded cetaceans and 406 phocid seals around the UK coasts were necropsied using standard protocols[1,29] except for great whales that were examined rudimentarily. Tissue samples were taken, depending on carcass condition, for microbiology, histology, parasitology, and other studies. Tissue samples or swabs of selected tissues were taken aseptically for bacteriologic examination. Samples were incubated for up to 14 days on Columbia sheep blood agar (Oxoid, Basingstoke, UK) at 37° C in 10% CO_2 and aerobically on MacConkey agar (Oxoid) for 48 hours (case Nos. 5 and 8) for 2–5 days in an aerobic, anaerobic, and a CO_2-enriched atmosphere (GENbox, bioMérieux, France) at 37° C using Columbia blood agar base (Oxoid, CM331) with 5% horse blood (case Nos. 6 and 7), and aerobically for 48 hours on 5% sheep blood agar and MacConkey (case No. 9). Any organisms recovered were identified using conventional methods including growth characteristics, colony morphology, staining properties, and biochemical characterization using the API identification system (bioMérieux, France). Sexual maturity was estimated from body length and gonadal appearance. Ten cases demonstrated remarkable and similar lesions that are the focus of this report. The carcass condition was considered fresh (case Nos. 2 and 8), fresh to slightly decomposed (case No. 9), slightly decomposed (case Nos. 5–7), slight to moderately decomposed (case No. 10), moderately decomposed (case Nos. 1 and 3), and moderate to advanced decomposition (case No. 4). Case No. 2 in this group of animals was stored frozen before postmortem examination.

A range of tissue samples was preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 2–6 μm, and stained with hematoxylin and eosin for histologic examination. Selected sections were also stained with Elastic van Gieson and Gomori's trichrome. Tissues examined from four Risso's dolphins (*G. griseus*) (case Nos. 1, 3, 5, and 8), four common dolphins (*D. delphis*) (case Nos. 6, 7, 9, and 10), one harbour porpoise (*Phocoena phocoena*) (case No. 2), and one Blainville's beaked whale (*Mesoplodon densirostris*) (case No. 4) in this study are shown in Table 1. Sections of formalin-fixed lung tissue from case Nos. 5–7 and 9 were processed by cryosectioning and stained by Oil-Red-O using standard methodology[2] to demonstrate fat emboli.

Table 2. Number of UK-stranded marine mammal necropsies and number and relative frequency of cases with in vivo bubble disease (by taxonomic family) (September 1990–March 2004).

Family	Number of Animals Necropsied	Number and Relative Frequency of Cases with In Vivo Bubble Disease
Phocoenidae	1,195	1 (0.1%)
Delphinidae	690	8 (1.2%)*
Ziphidae	19	1 (5.0%)†
Balaenopteridae	36	0
Physeteridae	26	0
Kogiidae	4	0
Phocidae	406	0
Total	2,376	10 (0.4%)

* These comprise 4/24 (17.0%) Risso's dolphins (*Grampus griseus*), 4/393 (1.0%) common dolphins (*Delphinus delphis*), 0/84 striped dolphins (*Stenella coeruleoalba*), 0/60 white beaked dolphins (*Lagenorhynchus albirostris*), 0/56 Atlantic white-sided dolphins (*Lagenorhynchus acutus*), 0/40 bottlenose dolphins (*Tursiops truncatus*), 0/26 pilot whales (*Globicephala melas*), 0/5 killer whales (*Orcinus orca*), and 0/2 unidentified dolphin.

† These comprise 1/1 Blainville's beaked whale (*Mesoplodon densirostris*), 0/14 Sowerby's beaked whales (*Mesoplodon bidens*), 0/3 northern bottlenose whales (*Hyperoodon ampullatus*), and 0/1 Cuvier's beaked whales (*Ziphius cavirostris*).

Immunohistochemistry for the detection of epithelial tissue, smooth muscle, and blood vessels was performed on selected tissues from two common dolphins (case Nos. 6 and 7) using standard techniques. The antibody markers (Dako, Cambridge, UK) used were anticytokeratin (AE1/AE3, diluted 1:50 in Tris-buffered Saline), anti–smooth muscle actin (SMA) (1A4, diluted 1:25 in TBS), and anti–von Willebrand factor (rabbit polyclonal, diluted 1:200 in TBS). Sections labeled with cytokeratin or von Willebrand factor were placed in preheated Target Retrieval Solution (Dako) and boiled for 20 minutes on high power in a microwave oven. The detection system used was the Vectastain Elite ABC Universal (Vector Labs., Peterborough, UK), and this was used according to the manufacturer's instructions. The final reaction was observed using 3,3-diaminobenzidine tetrahydrochloride (Dako), and the sections were counterstained with hematoxylin.

Results

Between September 1990 and March 2004 inclusive, 2,376 UK-stranded marine mammal carcasses were necropsied comprising 1,195 harbour porpoises (Phocoenidae), 690 dolphins (Delphinidae), 36 baleen whales (Balaenopteridae), 26 sperm whales (Physeteridae), 19 beaked or bottlenose whales (Ziphiidae), four pygmy sperm whales (Kogiidae), and 406 phocid seals (Phocidae) (Table 2). Of these, four out of 24 Risso's dolphins (*G. griseus*), four out of 393 common dolphins (*D. delphis*), one out of 1,195 harbour porpoises (*P. phocoena*), and one out of one Blainville's beaked whale (*M. densirostris*) had similar and remarkable (predominantly hepatic) pathologic findings (Table 2). Case Nos. 1–4 were found between October 1992 and July 1993, and case Nos. 5–10 between December 2001 and March 2004 (Table 3). These cases had a wide spatial distribution around the UK coastline, although all common dolphins (case Nos. 6, 7, 9, and 10) were found in Cornwall, England, between January 2002 and March 2004 (Table 3). Throughout the results section the case numbers, followed by the initials of the genus and species of each case, are stated in parentheses.

Gross pathology

Cavitary lesions. The liver was moderately to markedly enlarged although disproportionately light in weight, firm and resilient, or fibrous (case Nos. 1, 3, and 5 *G. griseus*; case No. 2 *P. phocoena*; case No. 4 *M. densirostris*; case Nos. 7 and 9 *D. delphis*). Total liver weights measured were 10.1 kg (case No. 3 *G. griseus*), 9.4 kg (case No. 5 *G. griseus*), 2.8 kg (case No. 7 *D. delphis*), and 2.6 kg (case No. 9 *D. delphis*). The liver surface showed areas of transparent or opaque, variably sized, subspherical, raised lesions caused by gas-filled subcapsular cavities, which gave the liver surface a ''bubble-wrap'' appearance (case Nos. 7 and 9 *D. delphis*) (Fig. 1). On sectioning, all livers contained predominantly spherical, discrete or intercommunicating, dry and smooth-walled parenchymal and subcapsular cavities containing colorless and odorless gas (Fig. 2). The diameter of these lesions ranged from 1–2 mm up to 20–30 mm in all cases, and the largest lesion diameter (60 mm) was recorded in case No. 3. The cavities occupied approximately 5% (case No. 6 *D. delphis*) to 80–90% of the total liver volume (case Nos. 3 and 5 *G. griseus*; case No. 7 *D. delphis*) (Fig. 2) and did not collapse when sectioned. The walls of some cavitary lesions were thin, translucent or slightly opaque (especially in case No. 4 *M. densirostris*; case Nos. 6 and 7 *D. delphis*), or thicker and more fibrous (especially in case Nos. 1, 3, and 5 *G. griseus*; case No. 2 *P. phocoena*). There was a sharply demarcated small area of paler beige-tan cavitated liver tissue in case No. 9 (*D. delphis*). The liver was grossly normal in case No. 10 (*D. delphis*).

In case No. 6 (*D. delphis*), approximately 50–60% of the right kidney and 5–10% of the left kidney lobules were dark red-black externally. On cut surface, these abnormal lobules were pale red-brown with darker red hemorrhagic foci, firm, and had multiple, dry, discrete or intercommunicating, distended, gas-filled cavities, typically 2–8 mm in diameter, located within the inner cortex, medulla, and corticomedullary junction (Fig. 3). In case No. 10 (*D. delphis*), the left kidney had a multilocular group of four immediately adjacent, translucent, subserosal, cystlike structures

Table 3. Details of UK-stranded cases.*

Case No. (Species)	Age Cat.	Sex	Nutritive Status	State of Decomposition	Date Found	Location Found	Macroscopic Cavities	Microscopic Cavities (Bubbles)
1 (*G. griseus*)	Adult	M	Good	Moderate	19 October 1992	Lossiemouth, Scotland	Liver (++)	Hepatic portal vessels and sinusoids (++/+++)
2 (*P. phocoena*)	Adult	M	Poor	Fresh	17 November 1992	Shetland, Scotland	Liver (++)	Liver not fully examined
3 (*G. griseus*)	Juv.	F	Poor	Moderate	26 November 1992	Borth, Wales	Liver (+++)	Hepatic portal vessels and sinusoids (+++); mes LN (++)
4 (*M. densirostris*)	Adult	F	Good?	Moderate-advanced	18 July 1993	Aberaeron, Wales	Liver (++)	Liver not fully examined/autolysed
5 (*G. griseus*)	Juv.	F	Poor	Slight	14 February 2001	Orkney, Scotland	Liver (+++)	Hepatic portal vessels and sinusoids (+++); PALN and mes LN (++); renal capsule vessels (+/++)
6 (*D. delphis*)	Adult	M	Good	Slight	18 January 2002	Cornwall, England	Liver (+), left kidney (+/++), right kidney (+++)	Hepatic portal vessels and sinusoids (+/++); renal vessels (arteries) (+++); spleen (+)
7 (*D. delphis*)	Adult	F	Good-mod	Slight	20 January 2002	Cornwall, England	Liver (+++)	Hepatic portal vessels and sinusoids (+++); adrenal (+); mes LN and posterior cervical LN (+)
8 (*G. griseus*)	Adult	F	Poor	Fresh	03 July 2002	Stromness, Scotland	Liver (+/++); periuterine/bladder serosa (++)	Hepatic portal vessels and sinusoids (++); adrenal (+/++); bladder serosa (++/+++)
9 (*D. delphis*)	Juv.	F	Poor	Fresh-slight	28 January 2003	Cornwall, England	Liver (++/+++)	Hepatic portal vessels and sinusoids (++/+++)
10 (*D. delphis*)	Adult	F	Good	Slight-moderate	22 March 2004	Cornwall, England	Left kidney (+/++), right kidney (+), periaortic bubbles (++)	Renal vessels (arteries) (+/++); mes LN (+/++); thyroid (+/+/++), spleen (+)

* +, few/mild; ++, moderate; +++, extensive/severe; Juv., juvenile; mes LN, mesenteric lymph node; PALN, pulmonary associated/diaphragmatic lymph node.
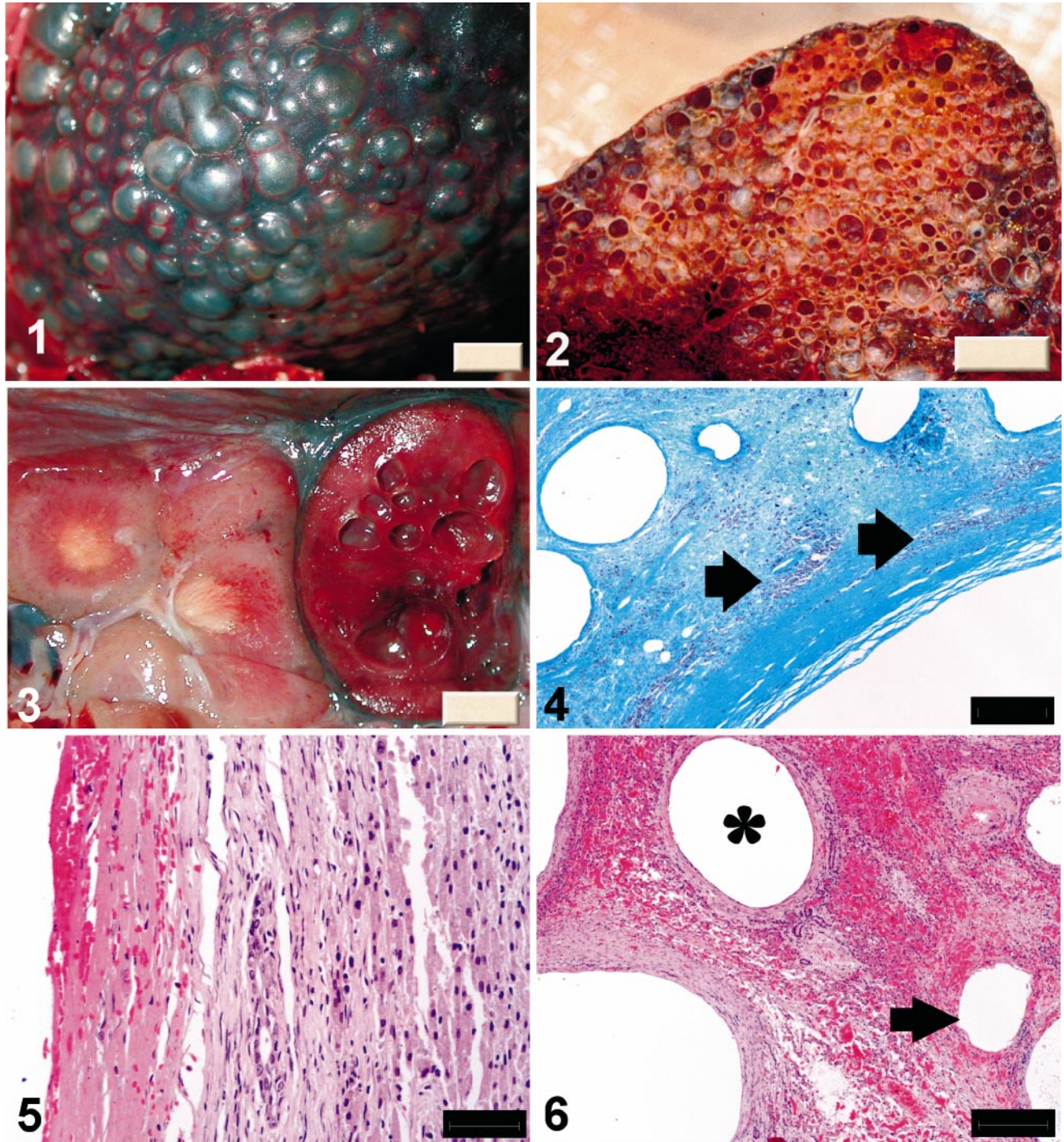
Fig. 1. Liver; common dolphin, case No. 7. Anterior surface of left liver lobe with multiple, gas-filled cavitary lesions beneath Glisson's capsule. Bar = 2 cm.

Fig. 2. Liver; Risso's dolphin, case No. 3. Sectioned liver demonstrating extensive cavitary lesions. Bar = 4 cm.

Fig. 3. Kidney; common dolphin, case No. 6. Sectioned kidney demonstrating cavitary lesions within dark red (infarcted) reniculus (right) adjacent to grossly normal reniculus (left). Bar = 6 mm.

Fig. 4. Liver; Risso's dolphin, case No. 3. Hepatic cavitary lesions and extensive hepatic/pericavitary fibrosis (blue-green stain). Note thin zones of preserved hepatocytes (arrows). Gomori's trichrome. Bar = 500 μm.

Fig. 5. Liver; common dolphin, case No. 6. Matrix of proteinaceous material (probably fibrin), active fibroblasts and erythrocytes on inner aspect of mature (fibrosed) hepatic cavitary lesion. HE. Bar = 50 μm.

Fig. 6. Liver; common dolphin, case No. 9. Large cavities (left), portal (*) and nonportal (probably sinusoidal) (arrow) bubblelike cavities in the liver. HE. Bar = 250 μm.

(12–19 mm in diameter) containing clear gas and resembling a gas-filled bunch of grapes pedunculated from the dorsal capsular surface. The gas within the lesion appeared to be pressurized (the entire lesion was preserved at 4 C for 3–4 days and became noticeably more flaccid during that period). Most of the remaining renal tissue of the left kidney was dark red apart from two enlarged, grayish/black, and firm groups of adjacent lobules that consisted of multiple, dry, discrete (gas filled) cavities (1–9 mm in diameter) involving both cortex and medulla. The right kidney had one abnormally enlarged, firm, and gray-black renal lobule containing similar dry, discrete, and distended gas-filled cavities (1.0–6.5 mm in diameter) within both the cortex and medulla. In both cases (Nos. 6 and 10 *D. delphis*), several cavities were typically present within each abnormal lobule, and the inner walls of the cavities were smooth, and the gaseous contents were colorless, odorless, and possibly pressurized because they appeared to markedly distend affected lobules. In case No. 10, numerous subserosal, bubblelike lesions (approximately 1–3 mm in diameter) were seen running alongside the thoracic aorta. The distribution and extent of all macroscopic cavitary lesions identified are summarized in Table 3.

Noncavitary lesions. Case No. 1 (*G. griseus*) had some subcutaneous bruising and skin sloughing suggestive of entanglement in fishing gear.

Case No. 2 (*P. phocoena*) had a plerocercoid cyst within the blubber of the anogenital region, a heavy burden of nematode parasites (*Anisakis simplex*) associated with a single chronic ulcer of the cardiac stomach mucosa, liver cysts containing trematode parasites (*Campula oblonga*), and adhesions between the liver capsule and the serosa of the stomach and intestine.

Case No. 3 (*G. griseus*) had generalized subblubber edema, approximately 1 liter of blood-tinged serous peritoneal fluid containing fibrinous clots, small and large intestines that appeared edematous and friable, an enlarged and hyperemic lymph node adjacent to the liver, some subcutaneous bruising, and tattoolike cutaneous lesions resembling those caused by poxvirus infection.[44]

Case No. 4 (*M. densirostris*) had multiple, peri- or postmortem fractures of both halves of the mandible and of the left ribs (numbers 6–8).

Case No. 5 (*G. griseus*) had congested lungs and blood-stained frothy fluid within the trachea and bronchi.

Case No. 6 (*D. delphis*) had a minimal volume of serosanguineous fluid and a light burden of fine white nematodes (*Halocercus delphini*) within the bronchi of both lungs, localized chronic pleural adhesions, gastric nematode parasites (*A. simplex*) within the cardiac stomach and (focally ulcerated) pyloric stomach, and trematode parasites (*Pholeter gastrophilus*) within the fundic stomach wall associated with localized and encapsulating submucosal fibrosis.

Case No. 7 (*D. delphis*) had bronchial nematodes, gastric nematodes (*A. simplex*) associated with a chronic mucosal ulcer in the cardiac stomach, and multifocal and mild pulmonary emphysema.

Case No. 8 (*G. griseus*) had generalized subblubber edema, congested/oedematous lungs, and serous pleural fluid. The fundic stomach of case No. 8 had prolapsed through the cardiac stomach and was lying in the esophagus causing esophageal distension. The intestinal mesentery was slightly thickened and opaque with brownish fibrin tags throughout the mesentery, and a large volume of clear, straw-colored peritoneal fluid was present. Multiple, gas-filled, cystlike structures were located within the serosa of the bladder and around the body of the uterus, which appeared to have torn and partially healed. A mummifying fetus (40 cm in length; 8 kg in weight) was lying separately within the anterior abdomen with thick, white fibrinous material on its surface. Another intraabdominal structure (approx. 15 cm across) (probably mummifying placenta) was covered with fibrinous material. There was a dark brown and opaque discharge from the vagina, but the cervix was closed with a large mucoid plug.

Case No. 9 (*D. delphis*) had a small burden of slender white nematodes within the fundic stomach.

Case No. 10 (*D. delphis*) had a number of findings consistent with entanglement in fishing gear (bycatch)[28] including a fractured beak, some broken teeth, a penetrating wound in the body wall, partially amputated fins, pericranial subcutaneous contusions, contusions in the thoracic rete mirabile, recently ingested prey, and good nutritive status. There were multiple, dark red and orange-brown lesions between 2 and 6 mm width (one of which was sampled for histopathologic examination) in the blubber layer. There were areas of extravasated blood in the subarachnoid space of the base of the brain, possibly because of hypostasis. The heart weighed 862 g and had a large atrial septal defect (Fig. 5) lined internally by smooth endocardium and had marked right ventricular hypertrophy. The right ventricular free wall was 14.5 mm thick, and the left ventricular free wall and ventricular septum were both 21.0 mm thick. The lungs were red/dark red, incompletely collapsed, soft, and slightly puffy. A moderate volume of serosanguinous fluid was found within the bronchi, and bloody fluid exuded from the cut lung surface. There were multiple, firm, pleural adhesions between both lungs and the parietal pleura. The uterus was fully involuted and had been previously gravid.

Histopathology

Cavitary lesions. Cavitary lesions were not detected either macroscopically or microscopically in the liver of case No. 10 (*D. delphis*). Liver tissue from case Nos. 2 (*P. phocoena*) and 4 (*M. densirostris*) was insufficiently sampled to demonstrate the structure of the gas-filled cavitary lesions seen grossly. A sample of liver from case No. 2 (*P. phocoena*) did demonstrate hepatic parasitic cysts associated with trematode eggs and localized hepatic fibrosis and mineralization. A section of the markedly autolysed liver from case No. 4 (*M. densirostris*) contained multiple, clear, nonstaining spaces, some of which also contained eosinophilic fluid, but the absence of any associated hepatic tissue reaction suggested that these cavities were probably bubbles formed after death by putrefaction.

In the other cases, the hepatic macroscopic cavitary lesions appeared microscopically as clear, nonstaining, round spaces of variable size encapsulated by minimally to markedly fibrosed walls, typically 50–1,150 µm thick (case Nos. 1, 3, and 5 *G. griseus*) (Fig. 4) or 50–250 µm thick (case Nos. 6, 7, and 9 *D. delphis*; case No. 8 *G. griseus*), although some very thin walls stretching between two adjacent cavities were as thin as 5–10 µm. Pericavitary hepatic fibrosis was exceptionally extensive and diffuse in case No. 3 (*G. griseus*), where thin zones of surviving hepatocytes only a few cells thick were commonly seen within the excessive fibrosis (Fig. 4). Some cavities appeared to have an endothelium-like lining and exhibited peripheral margination of erythrocytes, although the fibrous and nonfibrous walls of these hepatic cavities did not stain positively for elastin, indicating that they were venous or sinusoidal in origin. Preserved bile ducts were present within the pericavitary fibrous tissue of many of the large and fibrosed cavities, consistent with a portal location.

Acute lesions, including hepatocellular compression, hemorrhage, and acute hepatocellular necrosis, were seen cuffing some mature cavitary lesions (case Nos. 3 and 8 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*). In case No. 9 (*D. delphis*), there was an extensive area of liver tissue where all elements were hypereosinophilic and lacked cellular detail, but the architecture was retained and was sharply demarcated from surrounding tissue, indicating that this was probably an area of coagulative necrosis representing an hepatic infarct. In case No. 6 (*D. delphis*), some mature and fibrosed cavitary hepatic lesions exhibited acute injuries on their inner aspect, characterized by a partial scaffold of active fibroblasts within a matrix of proteinaceous material (probably fibrin) and erythrocytes (Fig. 5). Preserved islands of hepatocytes (often with attenuated cytoplasm) were also seen within the pericavitary fibrous tissue (case Nos. 3 and 5 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*). Some of the preserved hepatocytes exhibited cytoplasmic vacuolation (probable fatty change) and margination of nuclei (case No. 6 *D. delphis*). Foci of bile duct hyperplasia (case Nos. 1, 3, and 8 *G. griseus*) were seen, and some biliary epithelial cells had plump nuclei with prominent nucleoli and attenuated cytoplasm (case No. 6 *D. delphis*). The bile ducts preserved within pericavitary fibrous tissue appeared normal in case Nos. 3, 5, and 8 (*G. griseus*) and case Nos. 6 and 9 (*D. delphis*). Diffuse or periacinar hepatic congestion (case No. 5 *G. griseus*; case Nos. 7 and 9 *D. delphis*) and foci of predominantly periacinar hepatocellular degeneration and necrosis sometimes associated with weakly eosinophilic, single, round inclusions within occasional degenerate hepatocytes (case No. 5 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*) were also observed.

Large numbers of spherical, nonstaining cavities, typically up to 750 µm in diameter, were located predominantly within (often markedly) distended portal vessels and hepatic sinusoids, causing compression of surrounding hepatic tissue (case Nos. 1, 3, 5, and 8 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*) (Fig. 6). Many of these hepatic intravascular bubblelike cavities were associated with combinations of acute lesions, including hemorrhages (case Nos. 3, 5, and 8 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*) (Fig. 7), foci of acute hepatocellular necrosis (case Nos. 3, 5, and 8 *G. griseus*; case Nos. 6, 7, and 9 *D. delphis*) (Fig. 7), and fibrin or early organizing thrombi (case No. 5 *G. griseus*; case Nos. 6 and 9 *D. delphis*). Therefore, intrahepatic cavities, acute tissue injuries, and more longstanding (fibrosed) cavities of variable size tended to coexist in the same animal. The Glisson's capsule of case No. 7 (*D. delphis*) was markedly thickened because of extensive intracapsular edema.

Sections of the grossly abnormal renal lobules (case Nos. 6 and 10 *D. delphis*) contained multiple cavities approximately 0.1–6.0 mm in diameter located predominantly within the corticomedullary junction and medulla. These cavities were associated with extensive acute coagulative necrosis, and some areas also had marked congestion and hemorrhage of the necrotic cortical and medullary tissue (Fig. 8). Multiple fibrin thrombi were also seen in some (gas distended) renal blood vessels. These findings are consistent with acute gas emboli–induced renal infarcts. Elastin stains suggested that, in contrast to the hepatic lesions, at least some of these renal bubbles were intraarterial (Fig. 9).

In case No. 6 (*D. delphis*), glomerular sclerosis, mild-moderate interstitial fibrosis, and acute mineralization of medullary tubules were lesions common to both infarcted and grossly normal renal tissue, and a focus of necropurulent nephritis was seen within a
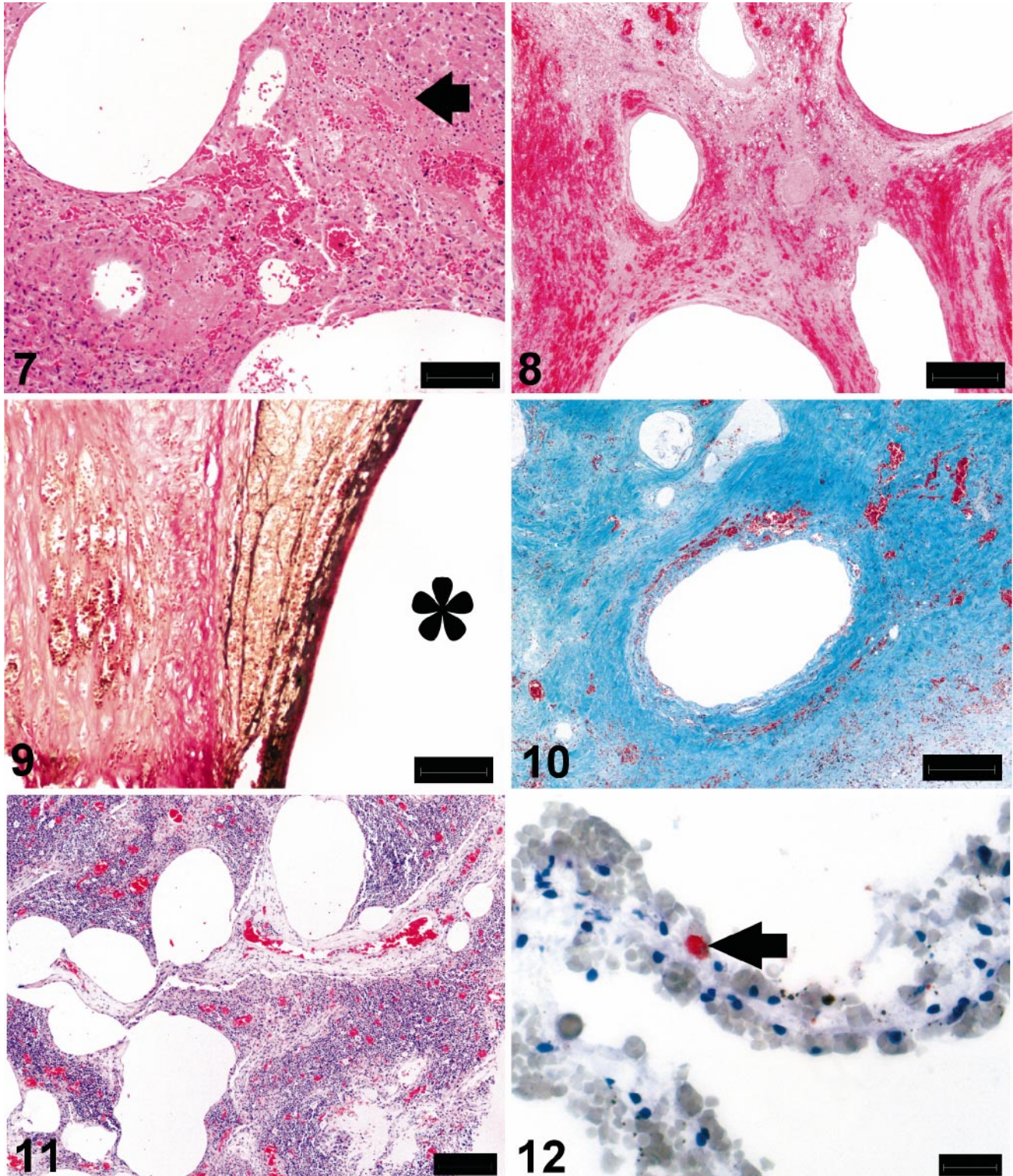
Fig. 7. Liver; Risso's dolphin, case No. 5. Intrahepatic bubblelike cavities associated with hemorrhage and hepatocellular necrosis (arrow). HE. Bar = 100 μm.

Fig. 8. Kidney; common dolphin, case No. 6. Acute coagulative necrosis and hemorrhage (consistent with a renal infarct) associated with multiple cavitary gas bubblelike lesions. HE. Bar = 500 μm.

Fig. 9. Kidney; common dolphin, case No. 6. Positive elastin-staining (brown staining) of attenuated and fragmented internal elastic lamina of gas-distended arterial blood vessel. The vessel lumen is marked (*). Elastic van Gieson. Bar =

grossly normal renal lobule. In case No. 10 (*D. delphis*), some grossly normal renal lobules were markedly congested, had multifocal-diffuse, mild-severe glomerular sclerosis and interstitial fibrosis involving both cortex and medulla, and interstitial aggregates of lymphocytes. One acutely infarcted and hemorrhagic lobule (case No. 10 (*D. delphis*) had multiple, clear cavities of variable size surrounded by mature fibrosis consistent with chronic gas bubble lesions (Fig. 10). The use of Gomori's trichrome stain confirmed the pericavitary (case No. 10 *D. delphis*) and interstitial fibrosis and glomerular sclerosis (case Nos. 6 and 10 *D. delphis*). Acute necrotic (infarcted) areas of renal tissue (case Nos. 6 and 10 *D. delphis*) stained densely for collagen, probably because of the considerable degree of pericavitary tissue compression. Some organizing and organized and recanalized arterial thrombi were seen in case No. 10 (*D. delphis*). Small, nonstaining, clear spaces (possibly gas emboli) were seen within some corticomedullary blood vessels and, in one lobule, a central, clearly demarcated, wedge-shaped area of markedly sclerotic and fibrotic renal tissue was lightly infiltrated with scattered lymphocytes, consistent with a healed infarct. In addition to the renal gas bubble lesions seen in case Nos. 6 and 10 (*D. delphis*), a few (possibly lymphatic) vessels were dilated within the renal capsule of case No. 5 (*G. griseus*).

Clear, nonstaining cavities (up to 3 mm diameter) were seen in the cortex and medulla of the mesenteric lymph node (case No. 3 *G. griseus*; case No. 10 *D. delphis*). Smaller, clear, nonstaining round spaces (gas bubbles) were also seen in trabecular or hilar lymph or blood vessels within the mesenteric lymph node (case No. 5 *G. griseus*; case No. 7 *D. delphis*). Similar nonstaining cavities (up to 350 μm diameter) were seen in venules or lymphatic vessels of a diaphragmatic/pulmonary-associated lymph node (case No. 5 *G. griseus*) (Fig. 11) and the posterior cervical lymph node (case No. 7 *D. delphis*). Multiple hemorrhagic foci were found in the cavitated mesenteric and posterior cervical lymph nodes of case No. 7 (*D. delphis*). Microscopic, round, clear, nonstaining spaces were seen in the red pulp (case No. 6 *D. delphis*) and in a muscular artery within a fibrous trabecula (case No. 10 *D. delphis*) of the spleen. Multiple, clear, nonstain-

ing, round or slightly elongated spaces (typically 25–160 μm diameter) associated with peripheral margination of erythrocytes and nuclear pyknosis and karyorrhexis of adjacent adrenocorticocytes were seen in the adrenal cortex (zona fasciculata and zona reticularis) of case No. 8 (*G. griseus*). Similar cavities were seen, associated with microhemorrhages, in the adrenal cortex of case No. 7 (*D. delphis*), although the tissue from this animal was slightly more autolysed. Some medullary vessels appeared distended with clear, nonstaining spaces in the adrenal medullae of case Nos. 7 (*D. delphis*) and 8 (*G. griseus*). Similar microscopic, nonstaining spaces were seen in the thyroid of case No. 10 (*D. delphis*), and although the thyroid tissue was autolytic, the spaces were not associated with bacteria. The distribution and extent of all microscopic bubblelike cavitary lesions are summarized in Table 3.

Noncavitary lesions. Case No. 1 (*G. griseus*) had marked pulmonary edema.

Case No. 2 (*P. phocoena*) had pulmonary nematodes and thymic cysts similar to those previously described in harbour porpoises[46] and bottlenose dolphins (*Tursiops truncatus*).[6]

Case No. 3 (*G. griseus*) had marked pulmonary edema and cutaneous lesions consistent with poxvirus infection.[44]

Case No. 4 (*M. densirostris*) had an apparent chronic eosinophilic enteritis.

Case No. 5 (*G. griseus*) had pulmonary congestion, marked pulmonary edema, perivascular edema of the cerebrum, adrenocortical hyperplasia, and hepatic extramedullary hemopoiesis.

Case No. 6 (*D. delphis*) had a thyroid adenoma, characterized an oval, 0.5 cm diameter mass within the thyroid parenchyma but separated by a distinct fibrous capsule. The mass consisted of well differentiated thyroid follicular epithelial cells arranged in enlarged follicles with reduced interstitium. Corpora amylacea and a few macrophages were seen within the colloid of the abnormal tissue. The fibrous capsule of the abnormal tissue appeared to compress the more congested normal tissue. Adenomatous hyperplasia is a possible alternative diagnosis for this lesion;[31] however, the abnormal tissue in case No. 6 (*D. delphis*) appeared as a solitary mass rather than multiple masses. Other findings were marked and multifocal hemorrhages within

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Fig. 10. Kidney; common dolphin, case No. 10. Renal pericavitary fibrosis. Fibrous tissue stained blue-green. Gomori's trichrome. Bar = 250 μm.

Fig. 11. Pulmonary associated/diaphragmatic lymph node; Risso's dolphin, case no. 5. Multiple, clear, nonstaining cavities within the node. HE. Bar = 200 μm.

Fig. 12. Lung; common dolphin, case no. 7. Orange-staining fat embolus (arrow) in alveolar capillary. Oil-Red-O (frozen section). Bar = 20 μm.

the atrophied and fatty thymic interstitium, multifocal verminous bronchitis, multifocal chronic verminous granulomatous interstitial pneumonia, mild and multifocal chronic orchitis, and a mild-moderate chronic cystitis.

Case No. 7 (*D. delphis*) had multifocal verminous bronchitis, multifocal chronic verminous granulomatous interstitial pneumonia, chronic purulent bronchopneumonia, mild pulmonary anthracosis, a small subepicardial fibrous scar, acute cortical tubular degeneration/necrosis (possibly caused by renal hypoperfusion), mild glomerular sclerosis and marked mineralization of renal medullary tubules, adrenocortical hyperplasia, and hepatic and splenic extramedullary hemopoiesis.

Case No. 8 (*G. griseus*) had a moderate number of small mononuclear cells within the endometrial interstitium and (para)uterine cysts consisting of spongy connective tissue lined by low cuboidal epithelium. One section of myometrium and cysts was heavily laden with lipofuscin pigment. Also present were numerous discrete to coalescing, apparently multiloculated, oval, clear spaces (0.5 cm diameter maximum) consistent with gas-filled cavities within the serosa of the bladder. The lesions were possibly distended blood or lymphatic vessels, but it was not possible to be conclusive. Other lesions included individual eosinophilic pyknotic fibers that were seen within the myocardium and skeletal muscle, abundant hepatic hemosiderin and lipofuscin, adrenocortical hyperplasia, mild pleural and alveolar hemorrhages, and mild anthracosis.

Case No. 9 (*D. delphis*) had focal verminous pneumonia and mild splenic extramedullary hemopoiesis.

Case No. 10 (*D. delphis*) had multifocal granulomatous panniculitis in blubber, associated with numerous multinucleate giant cells and multiple yellow and refractile (probably fat) globules, corresponding to the yellow/brown blubber lesions seen macroscopically. The cervical spinal cord had mild, multifocal hypereosinophilia and hemorrhages within the pia mater. The liver had mild-moderate and multifocal capsular, portal and periacinar fibrosis. The hepatic parenchyma exhibited microhemorrhages and mild, multifocal hepatocellular vacuolation. The myocardium (right ventricular wall) had a mature fibrous scar and acute, multifocal-diffuse myocardial degeneration (wavy fibers, apparent contraction band necrosis, variable eosinophilia, and interstitial edema). The lung sections had areas of alveolar collapse, areas of alveolar hyperinflation, and multifocal moderate-severe alveolar edema in all sections. Red blood cells and normally stained lung tissue were restricted to peribronchial and subpleural regions. The remainder of the parenchyma was hypereosinophilic and devoid of erythrocytes. There appeared to be multifocal, acute adrenocortical necro-

sis, characterized by discrete foci of hypereosinophilia and nuclear pyknosis and karyorrhexis within the zona glomerulosa. The cells of the zona fasciculata were disrupted, possibly because of autolysis. The pulmonary-associated/diaphragmatic lymph node examined histologically had multifocal, mild granulomatous lymphadenitis, multifocal microhemorrhages, scattered hemosiderin-laden macrophages, and some anthracosis. The mammary glands were mature and nonlactating, and there were multiple megakaryocytes within the spleen consistent with extramedullary hemopoiesis.

Fat stains

Oil-Red-O-stained frozen sections of formalin-fixed lung tissue were considered positive for intravascular fat emboli in case Nos. 5 (*G. griseus*) and 7 (*D. delphis*) (Fig. 12). Oil-Red-O-stained frozen sections of formalin-fixed lung tissue for fat emboli were inconclusive in case Nos. 6 and 9 (*D. delphis*). Case Nos. 1–4 and 8 were not examined for fat emboli.

Immunohistochemistry

Immunohistochemistry using cytokeratin antibody markers in liver sections of case Nos. 6 and 7 (*D. delphis*) bound specifically to the biliary epithelium but did not stain the wall or lining of hepatic cavities. Immunohistochemistry using SMA antibody markers demonstrated that a small proportion of intrahepatic gas bubbles resided within muscular blood vessels (possibly portal sphincters) in case Nos. 6 and 7 (*D. delphis*). Some intrarenal nonstaining bubble cavities resided within SMA-positive muscular blood vessels (case No. 6 *D. delphis*). A von Willebrand antibody marker was also applied to sections from case Nos. 6 and 7 (*D. delphis*) but did not bind specifically to either vascular or lymphatic endothelium.

Bacteriology

Case Nos. 1 (*G. griseus*), 2 (*P. phocoena*), and 10 (*D. delphis*) were not examined bacteriologically.

No significant pathogens were isolated from the liver, kidney, lung, or spleen of case No. 3 (*G. griseus*). *Clostridium perfringens* was cultured from the autolysed liver of case No. 4 (*M. densirostris*). In case No. 5 (*G. griseus*), a *Haemophilus* sp. was isolated from the lung and *Brevibacterium epidermis* from the brain, but cultures of the liver, spleen, mediastinal lymph node, mesenteric lymph node, intestine, and one kidney were sterile. In case No. 6 (*D. delphis*), cultures of both kidneys were sterile. In case No. 7 (*D. delphis*), cultures of lung, liver, and one kidney were sterile. In case No. 8 (*G. griseus*), a mixed bacterial growth was cultured from the lung, *Serratia marcescens* from the brain, *Pasteurella* sp. from the intestine

and mesenteric lymph node, and *Edwardsiella hoshinae* from the vaginal discharge, but samples of liver, spleen, and blood were sterile. In case No. 9 (*D. delphis*), no organisms were cultured from the liver.

Discussion

In the 13.5 years before March 2004, 1,970 stranded cetaceans and 406 phocid seals that died in UK waters were examined postmortem. Of these, 10 cetaceans had macroscopic and chronic cavitary hepatic or renal lesions unlike anything previously documented in human, domestic animal, or marine mammal pathology. The stranding and death of case Nos. 1, 3, 5 (*G. griseus*), 7, and 9 (*D. delphis*) were possibly because of liver failure caused by progressive loss of functional liver tissue or inanition due to compromised dive capability (e.g., buoyancy changes, pain). Case No. 6 (*D. delphis*) may have died because of acute renal failure induced by multiple gas emboli–induced infarcts superimposed on preexisting chronic renal disease. Because there are no known pathogens or inflammatory/infectious renal diseases known to cause extensive renal gas bubbles and emboli, the chronic renal disease (medullary mineralization, interstitial fibrosis, and multifocal chronic necropurulent nephritis) is considered an unlikely cause of the renal gas cavities in case Nos. 6 and 10 (*D. delphis*). In some of these cases, the fresh carcass condition when found, absence of scavenger damage, abrasions of leading edges of the fins and flukes, mild pulmonary hypostasis, and multifocal-diffuse periacinar congestion and hepatocellular degeneration were consistent with stranding alive.

In two of these cases, a harbour porpoise and a Blainville's beaked whale, insufficient tissue sampling precluded the microscopic characterization of the macroscopic hepatic cavitary lesions. The relatively fresh condition of seven of these carcasses, the absence of any bacterial isolates from five of seven cavitated livers or from either cavitated kidney (in case No. 6), the tissue distribution of the gas bubbles, and the fibrosed nature of many of the hepatic cavitary lesions and, in one case, renal lesions, were inconsistent with antemortem or postmortem gas production by bacteria (e.g., Clostridia).[23] Immunohistochemistry using cytokeratin and SMA antibody markers on liver sections of case Nos. 6 and 7 (*D. delphis*) demonstrated that the hepatic cavities were not epithelium-lined gaseous cysts and that some intrahepatic and intrarenal gas bubbles resided within muscular blood vessels.

The coexistence of antemortem intravascular gas bubbles (emboli) with tissue compression, hemorrhage, fibrin thrombi, and acute hepatocellular and renal necrosis (acute tissue responses) and pericavitary fibrosis (a chronic tissue response) suggests that in vivo bubble formation and embolism is the proximate etiology of this disease process, leading to the progressive encapsulation of intrahepatic and (in one case) intrarenal gas by fibrosis. A range of ''noncavitary'' lesions were also found in these cases, including parasitic lesions, poxlike cutaneous lesions, subcutaneous contusions, a benign thyroid tumor (thyroid adenoma), and common nonspecific findings such as pulmonary congestion and edema. These lesions, many of which are commonly found in cetaceans without cavitary lesions, are not documented primary causes of gas emboli or cavitary lesions in domestic terrestrial mammals,[40] or humans,[5,15] and are therefore considered unlikely to be related in etiology and pathogenesis to the macroscopic and microscopic cavitary lesions in these cetacean cases.

Although these hepatic and renal bubbles could be autochthonous in origin, their predominantly or exclusively intravascular location within hepatic portal and sinusoidal vessels and renal (arterial) blood vessels is more consistent with hematogenous transfer to these organs as gas emboli. Portal gas emboli have rarely been identified in animals but have been increasingly demonstrated in recent years in human medicine because of the wider use of ultrasound and CT imaging techniques.[35] Portal gas in humans is most frequently associated with bowel ischemia, especially mesenteric ischemia in adults and necrotizing enterocolitis in infants.[35] Other reported causes include necrotizing pancreatitis, abdominal abscess, intestinal obstruction, perforated gastric ulcer or carcinoma, diverticulitis, inflammatory bowel disease (ulcerative colitis, Crohn disease), blunt abdominal trauma, intestinal injury due to ingestion of a caustic agent, enema administration, colonoscopy, use of gastrotomy tubes, and liver transplantation.[35] The absence of any severe macroscopic or microscopic septic, inflammatory, ischemic, traumatic, or neoplastic lesions in the abdomen or intestinal tract (apart from the uterine tear and mummified fetus in case No. 8) suggests that such causes of portal gas in human medicine are unlikely causes of the portal gas and hepatic cavities in these cetacean cases. Case No. 4 (*M. densirostris*) did have an eosinophilic enteritis, but this was relatively mild. Although normally associated with systemic venous gas, portal gas emboli have also been demonstrated in decompression sickness (DCS) in humans[3] and in experimental decompression in dogs.[4] Arterial gas embolism in humans and experimental animals is most commonly associated with barotrauma[33] but can also occur in DCS because of arterialization of venous gas by way of transpulmonary passage or cardiac defects such as a patent foramen ovale or atrial septal defect.[15]

For logistical reasons, no attempt was made to capture and analyze the gaseous constituents of the liver cavities, and so their composition is not known. In

human and domestic animal medicine and pathology, air emboli have occasionally been associated with some surgical procedures,[40] rupture of uterine venous sinuses during parturition or abortion,[5] pulmonary injury associated with pneumothorax[5] and pulmonary overexpansion (barotrauma), as commonly seen in human divers.[33,41] In one Risso's dolphin (case No. 8), there were severe, chronic uterine lesions, including uterine rupture and the presence of an intraabdominal mummified fetus. In this particular case, rupture of uterine venous sinuses is a possible portal of entry for gas leading to the development of periuterine, gas-filled serosal cavities and venous gas emboli, although such uterine venous gas emboli are more likely to be transported to the lungs via the vena cava rather than to the liver within the portal veins. All other cases were sexually immature, male, or adult females with grossly normal (non-pregnant) uteri and none had any obvious lesions that could act as a portal of entry for air emboli.

Pulmonary barotrauma, a common cause of gas emboli in human divers, is considered an unlikely cause of these cetacean gas emboli and associated lesions for a number of reasons. First, cetaceans have lungs reinforced with elastic, collagen, smooth muscle, and cartilaginous tissue to withstand considerable pressure changes without injury.[44] Second, both pulmonary overpressurization and overdistension are considered necessary to induce pulmonary barotrauma in human divers,[33,42] and this would appear extremely unlikely in cetaceans that inspire air at atmospheric (not hyperbaric) pressure. Third, pulmonary barotrauma in humans usually results in arterial gas embolism,[33] whereas the gas emboli in the livers of all cetacean cases described in this study were venous or sinusoidal. Only the renal infarcts in case Nos. 6 and 10 appeared to be partly or completely induced by arterial gas emboli. Finally, arterial air embolism induced by pulmonary barotrauma,[33] including primary blast overpressure,[9,24,30] frequently causes other traumatic injuries (e.g., pneumomediastinum, pneumothorax) that were absent in these cetacean cases.

In contrast, nitrogen bubbles and emboli (within venous, arterial, portal, and lymphoid vessels) can develop in vivo in humans and experimental animals because of expansion of preexisting gas nuclei submitted to rapid decompression.[15] If bubble formation is extensive enough, the process can produce DCS. Although typically noted under compressed air dive conditions, cases of DCS have been induced in humans undertaking repetitive breath-hold diving.[14,34] The predominantly venous (including portal) distribution of the gas emboli in the cases described in this study and the association between arterial gas emboli and an atrial septal defect in case No. 10 (*D. delphis*) are con-

sistent with findings of DCS in humans.[15] Bubblelike cavities in the adrenal cortex and medulla (case Nos. 7 and 8) were similar to bubble cavities in the adrenal cortex and medullary veins of guinea pigs subjected to experimental decompression.[17] In addition, pulmonary fat emboli were found in two of the four cases that had sections of lung stained for fat. Although fat emboli are nonspecific findings, and the fat staining method used (Oil-Red-O staining of tissue cryosections) can produce processing artifacts, fat emboli have been associated with DCS including dysbaric osteonecrosis.[22] It is therefore plausible that the pulmonary fat emboli, lymphoid gas bubbles, and (portal, sinusoidal, and arterial) gas emboli and associated lesions in these cetacean cases could have a pathogenesis similar to DCS. Potential sources of bubbles include decompression-related embolism of intestinal gas and nitrogen bubble evolution from gas-supersaturated tissues. The latter process may occur through static means, as in typical human DCS, but alternative acoustically mediated mechanisms of bubble formation have also been proposed (e.g., rectified diffusion).[7]

Nitrogen bubble formation during ascent from a dive predominantly occurs in fatty tissues (e.g., adipose tissue, bone marrow, white matter of the CNS), because of the increased solubility of nitrogen in lipid,[8,15] and is suspected of forming through cavitation in sites providing mechanical stress to biologic fluids (e.g., articulating joints). The lipid-rich brain and the skeleton are logical tissues for investigation if nitrogen bubble formation is suspected in stranding cases. For logistical reasons, the brain was only examined in three cases and the cervical spinal cord in two cases, and a detailed examination of the articular skeleton was not conducted in any case. It was therefore not possible to comprehensively determine the presence or absence of acute or chronic CNS or bony lesions (i.e., dysbaric osteonecrosis) that have been associated with decompression illness in humans.[8,15] Although fatty tissues (e.g., bone marrow, blubber, mandibular fat channels, brain, or spinal cord) were not examined in detail in most of these cases, multiple hemorrhages were seen histologically in the adipose tissue of the thymic interstitium in case No. 6 (*D. delphis*).

A number of anatomical, physiologic, and behavioral adaptations are proposed to mitigate against in vivo nitrogen bubble formation in marine mammals[11,19,25,26,36] and, until recently, no evidence of in vivo bubble formation, or lesions consistent with DCS in humans and laboratory animals, had been reported in marine mammals. However, empirical evidence of nitrogen tissue supersaturation has been demonstrated in bottlenose dolphins[38] and some deep-diving cetacean species, such as the northern bottlenose whale

(*Hyperoodon ampullatus*), have been predicted to acquire levels of nitrogen tissue supersaturation in excess of 300% over a short sequence of repetitive breath-hold dives.[20] In addition, the acute lesions in 10 beaked whales that mass stranded in the Canary Islands in 2002 are also considered consistent with the theory of bubble formation due to DCS.[12,13,21]

The hepatic location and extent of the fibrosed cavitary lesions described in this study are inconsistent with lesions induced by bubble formation in all known causes of gas embolism (including DCS) in human[5,15] and domestic animal pathology.[40] However, cetaceans differ from humans behaviorally (as obligate, repetitive breath-hold divers), physiologically (e.g., profound diving reflex, hypocoagulable blood),[37] and anatomically (e.g., retia mirabilia, large epidural venous spaces and portal veins, portal and diaphragmatic sphincters, lipid predominantly stored as blubber).[19,44] Cetacean cardiovascular adaptations, such as the double capillary network in the lung alveoli,[44] may help prevent transpulmonary passage (arterialization) of venous gas emboli and the extensive meshwork of small arteries (the retia mirabilia) that perfuse the entire CNS[45] might efficiently filter any arterialized gas emboli.[39] The large epidural venous spaces[19] and the lack of Hageman and other clotting factors and more potent heparin in cetacean blood[37] might also reduce the risk of cetacean spinal cord injury due to in vivo bubble formation because autochthonous and venous gas bubbles, and epidural venous thrombosis, have been proposed as mechanisms of spinal DCS lesions in humans.[15,18] Whether or not these adaptations evolved to mitigate deleterious nitrogen bubble formation, it is feasible that at least some could provide a CNS-protective effect from autochthonous bubble formation or venous and arterial gas embolism whatever the initiating mechanism of bubble formation. Given these adaptations, it is perhaps too simplistic to assume that the distribution, severity, and chronicity of gas emboli-induced lesions, whatever their cause, will be identical in both humans and free-living cetaceans.

Of the cetaceans examined in this study, there was a higher prevalence of bubble lesions in deep-diving species, such as Risso's dolphins[27] and beaked whales (Table 2), which is consistent with modeled predictions of nitrogen tissue saturation and risk of nitrogen bubble formation by static or rectified diffusion.[20] Although there was an absence of cases in sperm whales (*P. macrocephalus*), another deep-diving species, most of the 26 carcasses examined were markedly decomposed and only partially examined. No evidence of gas bubble lesions has ever been described in pinnipeds, and none was found in 406 phocid seal necropsies in this study. Unlike cetaceans, most phocid seals studied are thought to dive on expiration,[11,36] and complete lung collapse occurs at relatively shallower depths, such as 25–50 m in Weddell seals,[11] resulting in reduced tissue nitrogen uptake compared with cetaceans. Finally, in some of the cetacean cases, progressive hepatic gaseous cavitation (involving up to 80–90% liver volume) could significantly increase the animal's buoyancy, dive profile characteristics, and resultant nitrogen tissue loading and supersaturation, possibly leading to an increased risk of subsequent bubble development.[20]

In conclusion, these findings provide convincing evidence of in vivo gas bubble formation and gas embolism associated with acute and chronic tissue injury in several cetacean species. The etiology and pathogenesis of bubble development is not known. However, given that there is increasing concern regarding the effect of anthropogenic sound on marine mammals,[10,16,43] a theoretical framework for acoustically mediated nitrogen bubble formation has been proposed,[7,20] and recent strandings of beaked whales coincident with active sonar use demonstrate trauma arguably consistent with nitrogen bubble formation,[10,12,13,21] further research into in vivo bubble formation in cetaceans is warranted. Such research should investigate the incidence, etiology, and pathogenesis of bubble lesions and undertake retrospective and prospective examination of cases for gas emboli and associated lesions, which may have been historically underreported in marine mammal pathology. Future studies should also document whether and how nitrogen bubble formation can occur in diving cetaceans, including the characterization and quantification of any contributory factors (e.g., dive behavior, depth of complete lung collapse, critical levels of nitrogen tissue supersaturation, acoustic exposure) to bubble development.

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