

Morphology of the lymphoid organs of the bottlenose dolphin, *Tursiops truncatus*

DANIEL F. COWAN^{1,3} AND TOBY L. SMITH^{1,3}

¹ Department of Pathology and ² The Marine Biomedical Institute, University of Texas Medical Branch, and ³ Texas Marine Mammal Stranding Network, Galveston, Texas, USA

(Accepted 12 January 1999)

ABSTRACT

The anatomy of the lymphoid organs was studied during the course of detailed dissections of 50 beach-stranded bottlenose dolphins, *Tursiops truncatus*. Constant lymph nodes occur in 4 groups, based on their location and structure. These groups are *somatic*, including nodes of the cervical region and pelvic recess; *lung-associated*, included marginal, diaphragmatic and hilar nodes; *visceral*, including the mesenteric, pancreatic, pericolic and porta hepatis nodes; and *aortic arch* nodes. Lymphatic drainage of the lung is primarily to the marginal and diaphragmatic nodes. The mesenteric node mass is well-endowed with capsular and trabecular smooth muscle, and a network of muscle fascicles within the organ implies an important contractile function in the circulation of lymph. In addition to constant nodes, occasionally nodes are found in relation to the thoracic aorta, the kidney, and under the scapula. Gut-associated structures include dorsal and ventral oropharyngeal tonsils, mucosal aggregates in the straight segment of the intestine (colon) and anal tonsils; this gut-associated lymphoid tissue tends to involute with age, being greatly reduced by puberty. Formed lymphoid organs include the thymus and the spleen, the latter being relatively small in relation to body size. None of these structures is unique among cetaceans, but the anal tonsils are particularly well developed in *T. truncatus*. The lymphoid aggregates in the colon resemble the arrangement in the vermiform appendix, which is lacking in most cetaceans, and may have functions analogous to that organ.

Key words: Cetaceans; lymphatic system; lymph nodes; lymphoepithelial organ; anal canal; anal tonsil; spleen; thymus.

INTRODUCTION

The lymphatic system of mammals includes (1) the lymph nodes, precisely structured masses of lymphoreticular tissue occurring at intervals along the lymphatic vessels; (2) diffuse and organised masses of lymphocytes associated with mucosal surfaces, including tonsils and appendix, as well as associated with connective tissues (to include bone marrow, blood, and lymph); (3) the spleen; (4) the thymus. For many terrestrial species, this system has been studied in great detail and therefore its development is known to vary among mammals. In some species, such as ruminants, the system is fully developed at birth, while in rodents, for example, full development appears to require exposure to environmental antigens (Banks, 1982). Some lymphoid organs (e.g. spleen) appear to

be active throughout life, while others (e.g. thymus) involute relatively early in life (Burkitt et al. 1993).

For most cetaceans, reports in the literature about the development and structure of the lymphoid system are scanty, fragmented, and frequently old. Notable exceptions include the study by Romano et al. (1993) who conducted an extensive microscopic examination on the lymphoid organs in belugas, *Delphinapterus leucas*, detailing the morphological architecture of the immune system. Simpson & Gardner (1972) also examined lymphoid organs in selected marine mammal species, providing general histological information. However, no other comprehensive studies have been performed and published on this system in any cetacean species of which we are aware.

In the course of examinations of cetaceans stranded along the Texas Gulf coast, we have had the

opportunity to examine more than 50 bottlenose dolphins (*Tursiops truncatus*) of both sexes and a range of ages. Particular attention has been given to the lymphoid system as a potential target organ for noxious agents in the environment (Lahvis et al. 1993). Our intention here is to report the organisation and histology of the lymphoid system of *T. truncatus*, in particular to describe typical features and changes that might be attributed to maturation and ageing. Recognising the volume of information that could be presented in such a study, this report concentrates primarily on the general organisation and morphology of the lymphoid system, rather than the cytological details of any particular element.

MATERIALS AND METHODS

Source of animals

The dolphins included in this study were collected by the Texas Marine Mammal Stranding Network, under the auspices of the National Marine Fisheries Service. The Network is a group of volunteers who recover beach-stranded marine mammals from the Texas coast. The collection area ranges from Brownsville at the Mexican border to Sabine Pass at the Louisiana border, i.e. the entire Texas Gulf coast. An occasional animal is retrieved from adjacent Louisiana.

Age estimation

Age estimation for young animals by size and general conformation has been shown to be reasonably accurate when confirmed by tooth age (Hohn et al. 1989). Sexual maturity, which is known to take place in a related population of *T. truncatus* at about 8–12 dentinal growth layer groups (GLG) in females and 10–15 GLG in males, is easily determined by examination of the gonads. Males and females appear to grow at about the same rate while young, but in the adult animal, males tend to be considerably larger than females of the same age. Therefore, age estimation by size alone is not very reliable in mature animals. However, since the important changes in the lymphoid system seem to take place around or before sexual maturity, precision in age estimation of mature animals, while desirable, was not essential for the purpose of this study.

Selection and sampling

Well preserved animals were brought to a central laboratory at Texas A&M University at Galveston for necropsy, which included gross examination, weighing, and systematic histological sampling of all

organs. Typically, animals were dead 6–20 h before necropsy. Preservation ranged from good to excellent, except for the glandular parts of mucosae, which were usually somewhat autolysed. Dissection was complete, with examination of all organs. During the course of necropsy, all viscera were removed, as well as all soft tissues from the skeleton. Tissues were collected in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin and eosin (H&E), or haematoxylin, phloxine and saffron (HPS), a trichrome method used to differentiate collagen from muscle.

Commercial immunocytochemical reagents used to classify lymphocytes into subcategories are largely nonreactive in *Tursiops* (Kumar & Cowan, 1994). Lymphocytes, plasma cells and the cells associated with the lymphatic system were recognised on the basis of their characteristic morphology using conventional stains. Commercial monoclonal antibodies against cytokeratin (AE1/3, Boehringer Mannheim Biochemicals, Indianapolis, IN) and smooth muscle actin (A4, Dako, Carpinteria, CA) were used to support identification or display the architecture of particular tissues. The antibodies were labelled with diaminobenzidine, which imparts a brown colour to reactive constituents. Standard techniques were employed throughout (Kumar & Cowan, 1994).

Causes of stranding

The causes of stranding of Cetacea must be distinguished from the causes of death. Stranding alone is sufficient to cause the death of the animal due to heart failure from hyperthermia and/or respiratory difficulties (Cowan et al. 1986). Conditions associated with stranding may not have, by themselves, been adequate to cause death, but may merely reflect impairment. Thus a stranded animal may not show signs of chronic disease. About 1/3 of the animals in this study died as a result of trauma. Some animals had pneumonitis, some pleural inflammation and some skin infections. For lack of data, it is not clear how the physiological or pathological conditions associated with stranding affect a lymphoid organ.

RESULTS

Lymph nodes

Lymph nodes occur in well defined groups in certain areas. Some of these node groups have colloquial names and some appear to have equivalents listed in the *Nomina Anatomica Veterinaria* (NAV), while others do not. Anatomical features of the dolphin,

such as lack of ischial and pubic bones, and the very short neck, do not permit direct comparisons of anatomical locations. The names we have elected are descriptive of location, reflecting analogy with the names given to groups in land animals. When we believe that the node or group is represented in the NAV, we have used that designation. We have identified structural differences that allow nodes found in relation to viscera to be distinguished from those primarily associated with somatic tissues. A striking feature of the node groups of *T. truncatus* is the variability of definition of the individual nodes. In some animals nodes within a group may be closely applied to each other, but remain distinct, while in other animals the nodes fuse to form an irregular, lobular mass.

Cervical lymph nodes (Lymphonoduli cervicale superficialis et profunda). Cervical nodes occur consistently in the neck area, in front of and behind (ventral and dorsal to) the middle of the diagonal mastohumeral muscle (Ridgway et al. 1974). They are apparent immediately beneath the fascia, and the upper group may extend deeply between muscles, for 4–6 cm. The nodes in this region are usually discrete, but may occasionally be fused. The dorsal group is usually the larger of the 2, consisting of 6–8 ovoid nodes, each measuring 2–3 cm in length. This group has a constant relation to a large nerve. The ventral group may contain from 2 to 6 nodes. These nodes are called by some the *prescapular nodes*; however, they are consistently 6–10 cm cranial to the anterior edge of the scapula, and seem to be truly cervical nodes.

Pelvic nodes. Pelvic nodes reside outside the peritoneum, deep in the pelvic recess, and under the innominate bones. Ischial and pubic bones are absent. The nodes are very low (caudal), and while closely associated with the body wall, lie next to the rectum and anal canal. They form 2 discrete lateral groups of 4–8 nodes each. While usually small (each node measuring 1.5–2 cm), they can become quite large in animals with active infections, weighing in aggregate over 100 g. No precisely equivalent group can be found in the NAV.

Nodes associated with the respiratory tract. Three groups of lymph nodes are constantly associated with the respiratory tract: the *hilar nodes*, the *node of the anterior free margin*, and the *diaphragmatic node mass*. We have recently recognised a complex lymphoglandular structure in the larynx of *T. truncatus*, which is present in every animal. This structure, which occurs in many species of cetacea, seems analogous to the adenoid (Cowan et al. unpublished observations).

Hilar nodes (lymphonodi tracheobronchiales crani-

ales). These are associated with the main bronchus at its insertion into the lung. One node is commonly present above (cephalad to) the bronchus on the left and between the main and an accessory bronchus on the right. A group of 2 or 3 nodes occasionally reside below (caudal to) the bronchus on each side. None of these nodes are very prominent and are covered by the pleura of the lung. They may either protrude slightly above the lung surface, or be flush with it, and are therefore easily overlooked.

Marginal node of the lung. This is a large bilateral triangular mass that occurs on the anterior (ventral) free border of each lung where it intersects with the diaphragmatic surface. Owing to the acute angle of the diaphragm, this junction is nearly in front of the heart. The size of the node relates reasonably well to the size of the lung. In an adult, they measure about $5 \times 2 \times 2$ cm. A series of very prominent lymphatic vessels converge over the surface of the lung to the marginal node, which is always present and solitary. Sometimes these nodes and the edge of the lung are free, but most often they are adherent to each other across the midline, or to the parietal pleura of the sternum. We can find no precise NAV equivalent.

Diaphragmatic node group. This is a flat, U-shaped mass of lymphoid tissue attached to the diaphragmatic surface of the lungs and to the diaphragm, situated behind the pericardium, centred around the oesophagus and diaphragmatic hiatus. This mass extends both laterally, occasionally between the pericardium and the diaphragm, and ventrally, in some individuals almost joining the marginal node anteriorly (ventrally). Usually the nodes of both sides form a single mass, which in the adult may measure $10 \times 20 \times 1$ cm. This group is normally flat and plate-like, appearing as a cluster of partly fused nodes, but when reacting to an inflammatory process, it becomes thick and nodular. Owing to its location, it is easily overlooked. We find no NAV equivalent.

Nodes of the aortic arch region (Lymphocentrum mediastinale). Several nodes are always associated with the thymus and the thyroid, usually distinguished from both these organs by the gross appearance of the nodes on sectioning. As many as 10 nodes can be found in this area, although 6–8 are more frequent counts. The thyroid, thymus, and nodes, together with the blood and lymphatic vessels, are invested within a delicate fascial plane in the mediastinum. These organs and their associated nodes, which in some species reside in the neck, are found in the mediastinum close to the aortic arch.

Nodes associated with the gastrointestinal tract. Four lymph nodes or groups of nodes are constantly

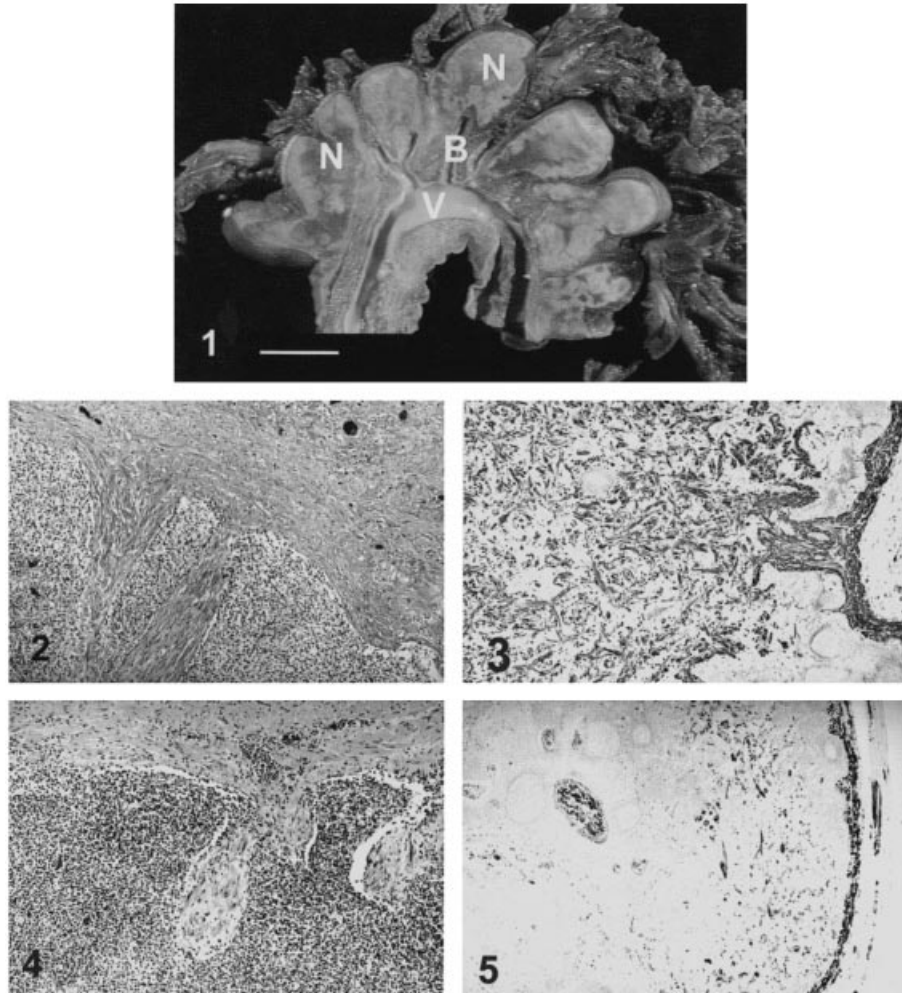


Fig. 1. Section through the mesenteric lymph nodes and artery illustrating the close relationship between these structures. V, mesenteric artery; B, representative branch artery; N, representative nodes. Bar, 2 cm.

Fig. 2. Section of a mesenteric lymph node illustrating the thick muscular capsule (top) and trabeculae. Haematoxylin, phloxine and saffron (HPS) stain, $\times 100$.

Fig. 3. Section of a mesenteric lymph node, reacted with a diaminobenzidine labelled antibody for smooth muscle actin. Staining shows smooth muscle fibres in the capsule (right) and in the stroma. Note the great density of interlaced fibres in the interior of the node, $\times 20$.

Fig. 4. Section of a lung marginal lymph node illustrating the thick capsule (top), and a broad blunt trabeculation. HPS stain, $\times 100$.

Fig. 5. Adjacent section of a marginal lymph node reacted with a diaminobenzidine-labelled antibody for smooth muscle actin. Staining shows smooth muscle fibres in the capsule (right) and stroma. Compare with Fig. 3 for density of muscle fibres within the stroma. In the marginal node muscle is present, but very sparse in comparison with the mesenteric node, $\times 20$.

associated with the gastrointestinal tract: the *mesenteric nodes*, the *pancreatic node*, the *mesocolic node*, and the *node of the porta hepatis*.

Mesenteric lymph nodes are a prominent lymphoid mass often called the ‘pseudopancreas’ (Pilleri & Arvy, 1971). This mass is a string of discrete, but occasionally joined nodes that follow the mesenteric artery for about 25–30 cm. Occasionally this structure is very large. In some animals, we have observed these nodes as a fused, thickly-encapsulated, and elongated mass nearly 15 cm long and 4 cm in diameter, while in others it occurs as a dispersed cluster of 15–20 discrete nodes (Fig. 1). We can find no precise NAV equivalent.

Pancreatic node. There is always at least 1 large node closely associated with, and usually within, the capsule of the pancreas. Its exact location in relation to the organisation of the pancreas is difficult to specify, owing to the folded, rounded configuration of that organ, although it lies on the intestinal, rather than the hepatic, aspect of the pancreas. In this region several nodes are often found, especially when they are reactive. Some have the architecture typical of the mesenteric node mass and are considered to belong to that group. The constant pancreatic node architecture is different from that of the mesenteric nodes, being less dense and containing less smooth muscle (see Histology below).

Mesocolic nodes (Lymphonodi colici). One and sometimes 2 nodes are consistently present in the mesentery of the straight segment of the intestine (colon) about 10–15 cm above the portion of the anal canal lined by stratified squamous epithelium (see below). They are typically closely applied to the wall of the intestine.

Hepatic hilar node (Lymphonodi hepatici portales). There is always at least 1 large node at the hilus of the liver, although its relations are difficult to determine, as the organs in the area are very densely packed in together.

All the nodes described above are constant, while other nodes are variable and only occasionally found. Lymph nodes are occasionally found near the angle of the jaw, under the scapula, along the thoracic aorta, and along the abdominal aorta near the kidney. As the renal artery enters the anterior pole of the kidney, the latter node, while situated several centimetres away from the kidney, may represent a renal hilar node.

Histology of the lymph nodes. The somatic nodes (cervical, pelvic) have a relatively simple architecture, while the visceral nodes all contain variable amounts of smooth muscle, depending on their location. The mesenteric nodes are the most muscular of all the node groups. This muscle occurs as a component of the thick capsule, extending into the node as part of the trabeculae (Fig. 2). In addition, fascicles of smooth muscle occur in a loosely interwoven or basket-weave pattern in the mesenteric nodes (Fig. 3). The other visceral nodes all have lesser amounts of smooth muscle in the capsules and trabeculae. For example, the nodes of the respiratory tract all have thick capsules, with small amounts of smooth muscle (Figs 4, 5). The most distinctive feature of these nodes is the antler-like branching of the blunt collagenous trabeculae, a pattern which occurs within a short distance of the capsule. Both somatic and visceral node groups have the general structure of sinuses and cords, and in responding to antigenic challenge will produce prominent follicles with germinal centres. Marginal sinuses are incomplete.

Mucosa-associated lymphoid tissue of the intestine (MALT)

The intestine of *Tursiops truncatus* is typical of cetaceans, being very long, slender, and lacking a caecum, appendix, and external features that readily allow distinction between a 'small intestine' and a 'colon'. Nearly all the intestine is suspended on a fan-

shaped mesentery, within which is the large mesenteric lymph node mass. The distal-most segment, however, is straight and suspended by a short mesentery attached to the midline of the dorsal aspect of the body cavity. This segment extends from the anus to the level of the spleen, where it abruptly angles to join the part of the intestine suspended from the fan-shaped mesentery. The anal canal is the squamous-lined terminal segment of the intestinal tract, extending in continuity from the anal skin inwards for a variable distance, usually 50–75 mm (Cowan & Smith, 1995). In young animals, the straight segment contains a layer of lymphoid structures in the lamina propria of the mucosa, extending from the angulation, or splenic flexure, to about 8–10 cm above the squamous-lined anal canal. This distal 8–10 cm segment of glandular mucosa, however, does not contain abundant lymphoid tissue, even in very young animals. The mucosa containing the lymphoid tissue has longitudinal folds, while the segment of mucosa without them has a pebbly or pigskin appearance (Fig. 6). This lymphoid tissue is very abundant in neonates and nurslings, forming a continuous layer in which are set closely approximated germinal centres (Figs 7, 8). As the animal increases in length (age), the lymphoid tissue is gradually depleted and in the adult is represented only by the occasional aggregation of lymphocytes.

Anal tonsils

The anal tonsil complex is a constant structure in *T. truncatus* (Cowan & Smith, 1995). Briefly, this complex is a circumferential cluster of discrete, tonsil-like aggregations of lymphoid tissues, together with epithelial ducts ('crypts') and occasional mucous secretory units in the extreme lower portion of the intestinal tract. They occur almost exclusively in the intestinal segment lined by stratified squamous epithelium (anal canal) and extend for a variable distance cranially from the anal aperture. More than a hundred pinpoint to 0.5 mm openings occur in 5–6 parallel linear arrays (Fig. 9). Occasionally, a few openings are found in the glandular mucosa of the intestine, though still in close relation to the anal canal.

The anal tonsils have the typical features of branched ramifications of the squamous epithelium of the surface mucosa penetrating into the lamina propria. The crypts are sheathed by dense collections of lymphocytes, which may be organised as germinal centres and permeate the epithelium of the lower portion of the crypts (Figs 10, 11). These tonsils are typical of the organisation of the palatine tonsil (Burkitt et al. 1993).

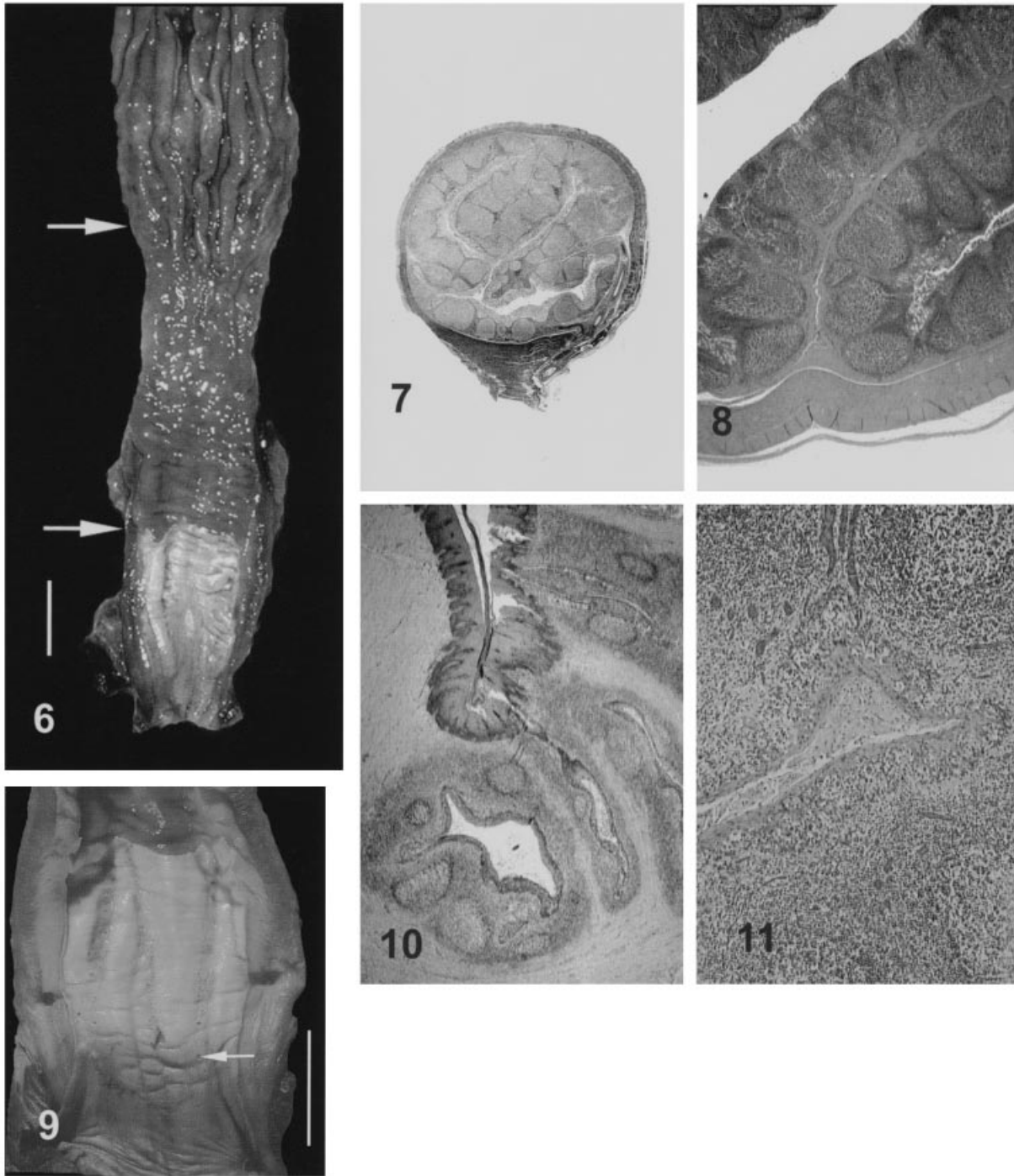


Fig. 6. Segment of distal intestine from a very young *Tursiops* opened longitudinally. Squamous-lined anal canal is at bottom. Lower arrow indicates squamocolumnar junction; upper arrow indicates junction between longitudinally-folded mucosa containing lymphoid aggregates (upper), and segment containing no aggregates (lower). Bar, 2 cm.

Fig. 7. Cross-section of the straight segment of the intestine of a young *Tursiops* (Fig. 6), showing a lamina propria filled with lymphoid aggregates. The lumen is compressed by the thick mucosa. Actual diameter of the segment is 13 mm. HPS stain.

Fig. 8. Enlargement of one of the mucosal folds from Fig. 7 showing densely packed lymphoid follicles occupying the mucosa and submucosa. HPS stain, $\times 20$.

Fig. 9. Squamous mucosa-lined anal canal. Arrow indicates one of many linearly arranged openings of crypts of the anal tonsils. Bar, 2 cm.

Fig. 10. Section through a crypt and the underlying lymphoepithelial complex. In this specimen, involution has resulted in enlargement of the deep portion of the crypt. HPS stain, $\times 20$.

Fig. 11. Deep portion of the crypt channel, with marked permeation of the squamous epithelium by lymphocytes. HPS stain, $\times 400$.

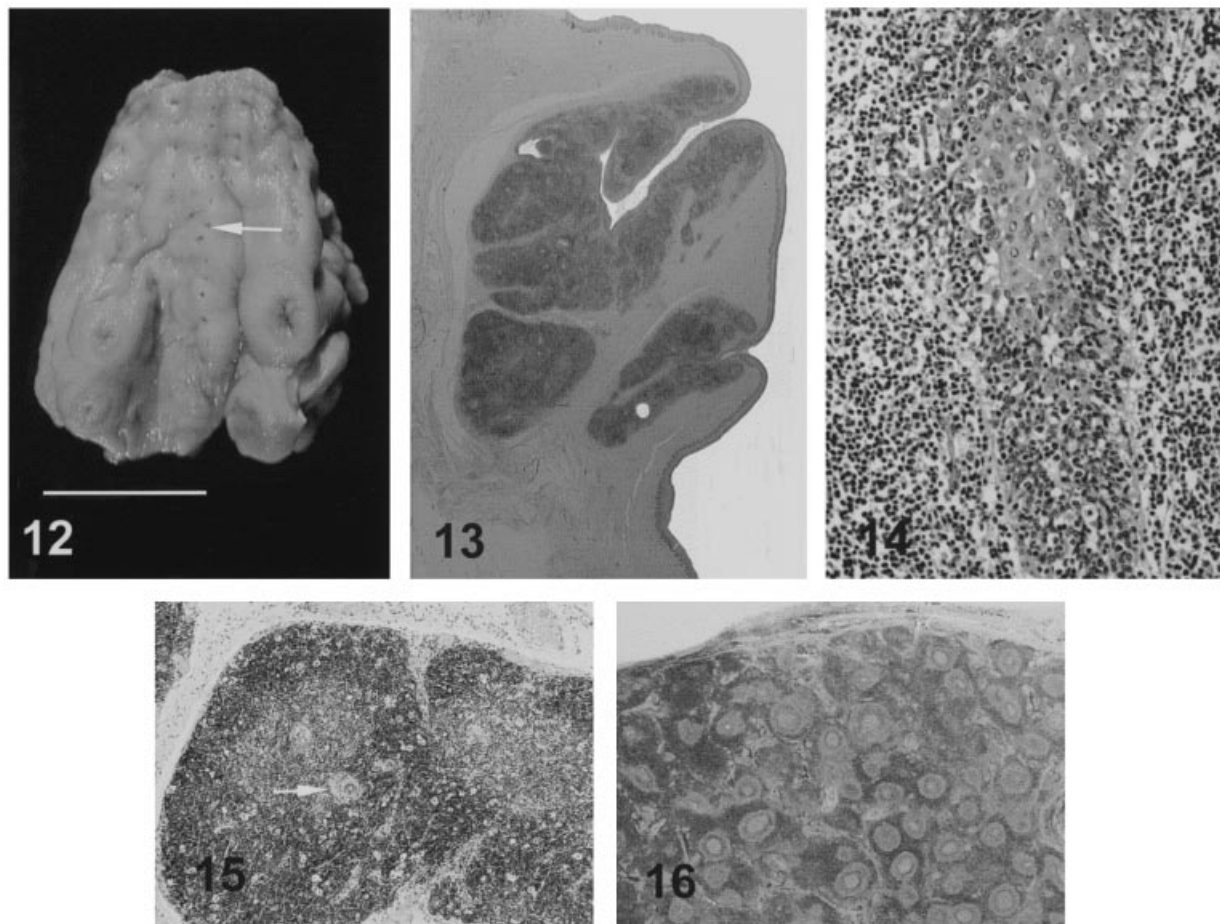


Fig. 12. Portion of the dorsal pharyngeal mucosa, collected from just anterior to the nasal opening. The tonsils are evident as the paired mamillations, just below the arrow, which indicates the opening of a small mucous gland. Bar, 2 cm.

Fig. 13. Section through a tonsil showing the crypt and dense lymphoid aggregation around it. HPS stain, $\times 4$.

Fig. 14. Deep portion of the crypt showing the squamous mucosa to be thoroughly permeated by lymphocytes, typical of a lymphoepithelial organ. HPS stain, $\times 400$.

Fig. 15. Single lobule of the thymus of a neonate *Tursiops truncatus*. The dark cortex, lighter medulla and a Hassall body (arrow) are evident. HPS stain, $\times 100$.

Fig. 16. Section of spleen, showing prominent follicular activation. This degree of activation presents grossly as tiny white granules on the cut surface. In this illustration, the target-like bodies are the germinal centres, surrounded by shells of small lymphocytes, than larger lymphocytes. The small arteries are not discernable at this magnification. HPS stain, $\times 10$.

Oropharyngeal tonsils

Two pairs of oropharyngeal tonsils occur in *Tursiops*. One pair is dorsal, in the mucosa of the palate about 3–4 cm in front of the nasal opening and 2 cm apart. They vary markedly in size, being larger in younger animals. They are easily recognised, as they form palpable masses 1.5–2 cm in length and have 2–6 obvious crypt openings per tonsil (Fig. 12). They have the typical structure of palatine (dorsal oropharyngeal) tonsils (Burkitt et al. 1993) (Figs 13, 14). The second, much smaller pair occurs, one in each pyriform fossa, just lateral to and at the extreme anterior extent of the larynx ('goose beak'). These differ from the dorsal pair in that the tonsillar elements appear as part of a complex also containing mucous

Table 1. *Thymus weights from 8 Tursiops truncatus*

Animal	Sex	Body length (cm)	Body weight (kg)	Thymus weight (g)
GA 668	IF*	145	45.9	165.7
PA 355	IF	165	51.2	53.0
GA 426	IF	174	68.6	38.9
LA 038	IM	200	87.0	28.0
LA 040	IM	215	103.4	67.0
SP 189	F	233	151.1	53.6
PA 361	F	243	164.8	27.5
PA 397	F	265	225.0	40.7

* I = Sexually immature animal.

glands. The tonsillar pairs seem to be equivalent of Waldeyer's ring. Occasionally, scattered very small tonsil-like structures are identified in the ventral

Table 2. Spleen weights from 44 *Tursiops truncatus*

Animal	Sex	Body length (cm)	Body weight (kg)	Spleen weight (g)	Spleen index
GA 769	NF	115	ND**	6.0	ND
GA 668	IF*	145	45.9	74.7	0.16
GA 426	IF	174	68.6	127.4	0.19
PA 355	IF	175	51.2	45.2	0.09
PA 375	IM	187	52.9	53.1	0.10
GA 286	IM	189	ND	48.1	ND
PO 249	IF	190	61.6	44.9	0.07
GA 460	IM	195	89.2	98.4	0.11
PA 381	IM	199	117.5	136.3	0.12
LA 038	IM	200	87.0	70.9	0.08
GA 539	IF	205	101.9	72.4	0.07
GA 407	IF	206	ND	92.8	ND
GA 705	IM	210	96.8	42.2	0.04
LA 040	IM	215	103.4	61.1	0.06
GA 535	IF	217	117.8	124.7	0.11
GA 484	IF	219	ND	72.8	ND
PO 275	F	225	80.6	57.7	0.07
GA 476	M	226	112.4	51.8	0.05
PA 236	F	230	107.0	152.3	0.14
PA 387	F	230	144.7	88.5	0.06
SP 189	F	233	151.1	122.6	0.08
GA 406	F	236	ND	35.4	ND
SP 179	F	236	ND	31.3	ND
SP 153	F	237	169.0	61.3	0.04
GA 710	M	238	186.5	98.4	0.05
GA 775	F	240	ND	60.7	ND
GA 458	F	241	ND	33.3	ND
CC 110	F	242	139.6	90.6	0.06
PA 361	F	243	164.8	123.2	0.07
GA 466	F	244	ND	99.2	ND
GA 699	F	245	140.7	101.8	0.07
GA 675	F	245	218.2	125.5	0.06
PA 229	F	247	148.0	70.3	0.05
GA 440	M	247	208.7	227.0	0.11
GA 664	F	255	130.4	53.8	0.04
PA 342	M	255	168.2	137.3	0.08
GA 436	F	255	202.7	110.4	0.05
GA 740	M	255	ND	175.8	ND
GA 803	M	256	ND	91.2	ND
PA 397	M	265	225.3	45.8	0.02
PA 224	M	270	ND	135.0	ND
PA 292	M	271	202.0	156.4	0.08
PO 256	M	272	258.3	33.3	0.01
PO 331	M	294	225.9	72.4	0.03

* I = Sexually immature animal; **ND = data not determined.

mucosa. No structure with the histological features of the adenoid was found in the naso- or oropharynx, but a complex lymphoglandular structure which may be analogous to the adenoid is constantly present inside the larynx.

Thymus

The thymus of *T. truncatus* has all the typical features of the mammalian thymus, with cortex, medulla, Hassall's corpuscles (Fig. 15) and epithelial reticulum.

In the youngest animals, it extends from the arch of the aorta, where it invests the brachiocephalic vessels and partly or completely overlies the thyroid gland. Because of its colour, soft texture, and lobular architecture, the thymus is easily mistaken for adipose tissue. The thymus loses lymphocytes progressively with increasing age (involutes) and has a striking propensity to develop cysts derived from the epithelial reticulum, which may completely replace it (Cowan, 1994). The weights of the thymus in relation to body size are presented in Table 1.

Spleen

The spleen of *T. truncatus* is a slightly flattened globe, most often greyish-blue, but occasionally covered in part with whitish patches, representing fibrous thickening of the capsule. The weight of the spleen in relation to body size ($n = 44$) is shown in Table 2. The average spleen weight of the 10 smallest animals, all of whom were immature, was 76.5 g, and of the 10 largest animals, 103.2 g. Thus there does not appear to be an absolute decline of spleen weights in the oldest animals. However, the calculated spleen index (spleen weight as a proportion of body weight) of the 10 smallest animals was 0.10, while of the largest 10 animals was 0.05. Thus the weight of the spleen appears to increase after puberty, but not at a rate in keeping with body size.

The general architecture of the spleen of *T. truncatus* is similar to other cetaceans. The capsule is double-layered, with a fibrous outer layer, and a fibromuscular inner layer. Depending on its state of reactivity, the cut surface of the spleen may display white, pinpoint nodules easily visible to the unaided eye, which represent activated germinal centres (follicles) formed in periarterial lymphoid sheaths (Fig. 16). Most spleens we examined had prominent reactive germinal centres, visible microscopically, probably owing to a condition of the animal that related to its stranding. All spleens contained cells morphologically identical to megakaryocytes, the number of which varied greatly, from infrequent, to 1 or 2 per $\times 400$ microscopic field. Of the 50 *T. truncatus* included in this study, 8 (16%) had accessory spleens, 4 of which were embedded in the pancreas, covered by the same serous membrane as the pancreas.

DISCUSSION

It is not clear how representative the lymphoid system of *T. truncatus* is of cetaceans in general. Published information on the lymphoid system of cetaceans is sparse.

Lymph nodes

In cetacean lymph nodes, germinal centres are frequently absent, the nodes are mainly sinus and paracortical tissue and marginal sinuses are incomplete (Romano et al. 1993). Germinal centres, if present, are frequently found deep in the nodes, and

some have likened this arrangement to an inverted architecture (Moskov et al. 1969). A large mass of lymph nodes, Aselli's 'pseudopancreas', is consistently present in the mesentery (Pilleri & Arvy, 1971). A node is present in the region of the porta hepatis and in the vicinity of the stomach and pancreas in the white-sided dolphin (Bespalova, 1975). A mass of lymphoid tissue on the free edge of the lung is present in a number of cetacean species (Arvy, 1976).

Thymus

The cetacean thymus follows the typical mammalian plan with a cortex, medulla, and Hassall's corpuscles (Cave, 1980; Romano et al. 1993; Cowan, 1994). In *T. truncatus*, an epithelial reticulum has been demonstrated using a labelled monoclonal antibody against cytokeratin (Cowan, 1994). It is clear that lymphocyte depletion ('involution') occurs over time, but the age or rate of progression at which this occurs is not defined for any Cetacean species, as far as we can determine.

Spleen

The relative size of the cetacean spleen is small compared with land mammals (Slijper, 1958; Blessing et al. 1972; Bryden, 1972), approximating 0.2% of the animal's total body weight (Slijper, 1958), as we have found for *T. truncatus*. Bryden (1972) has observed that, as in most mammals, the cetacean spleen reaches maximum size with the onset of puberty, and subsequently decreases in relative and absolute weight with increased age. In general, the cetacean spleen is a single organ, but accessory spleens are common, found in 21% of *Delphinus delphis* and 18% of *Stenella coeruleoalba* (Arvy & Pilleri, 1970). Retterer & Neuville (1916) described lobulation and accessory spleens in a number of species, but made no mention of *Tursiops truncatus*. As in land animals, the cetacean spleen is composed of white pulp, consisting of lymphoid nodules developed at arterial terminals, evenly distributed throughout the red pulp. Periarterial lymphatic sheaths are characteristically prominent. Lymphoid nodules are composed of small to medium-sized lymphocytes (Romano et al. 1993). Germinal centres may be identified as white granules on gross inspection, but as they reflect a reactive state, they are inconstant and often absent (Simpson & Gardner, 1972; Cave, 1980; Nakamine et al. 1992). In many cetacean species, the splenic capsule has 2

layers, an outer fibrous and an inner muscular layer. Trabeculae extend from the capsule into the parenchyma, each bearing arteries and veins. (Zwillenberg, 1958, 1959; Blessing et al. 1972; Cave, 1980).

Oropharyngeal tonsils

Pharyngeal tonsils, with crypts and lymphoid follicles are described in a specimen of *Tursiops truncatus* (Cave, 1979) and *Delphinapterus* (Romano et al. 1993).

Anal tonsils

The so-called 'anal tonsils' have been observed in several species of cetacean, including the California grey whale, *Eschrichtius robustus* (Cowan & Brownell, 1974), the Ganges River dolphin, *Platanista gangetica* (Yamasaki et al. 1977), and the striped dolphin *Stenella coeruleoalba* (Komatsu, 1979), as well as the bottlenose dolphin, *T. truncatus* (Cowan & Smith, 1995). They have also been found in the rough-toothed dolphin, *Steno bredanensis*, and Fraser's dolphin, *Lagenodelphis hosei*, but not in the single infant beaked whale, *Mesoplodon sp.* examined (Cowan & Smith, unpublished observations). They may occur in the sperm whale, *Physeter catodon* (Uys & Best, 1966). It is not clear therefore, that anal tonsils are universal in cetaceans. Ortmann (1960) does not include cetaceans in his broad survey of anal tonsils in a large number of mammalian species, and they are not mentioned by Cave (1979) in his study of tonsillar formations in a *Tursiops*, nor by Romano et al. (1993) in a detailed study of the lymphoid organs of the beluga, in which gut-associated lymphoid tissue, but not anal tonsils, are discussed. Yamasaki et al. (1975, 1977) say that they do not occur in the boto, *Inia geoffrensis*, or in the franciscana, *Pontoporia blainvillei*.

Mucosa-associated lymphoid tissue

Mucosa-associated lymphoid tissue (MALT) occurs in the beluga as scattered aggregations of lymphocytes in the submucosa and lamina propria of the intestine, without the formation of Peyer's patches (Romano et al. 1993). Peyer's patches are said to occur in several species according to Arvy (1976), citing very old sources. Simpson & Gardner (1972) referred to gut-associated lymphoid tissue in cetaceans, but did not give any histological detail or mention species. Cave

(1980) found abundant lymphoid tissue in the large intestine of a *Tursiops*.

Appendix

No reports of an organ equivalent to the vermiform appendix have been published for any cetacean. Only Mysticetes and the Gangetic dolphin have a caecum, the structure with which the appendix is normally associated (Slijper, 1979).

The lymphoid organs of Tursiops truncatus

However generally representative of cetaceans they may be, the lymphoid organs of *T. truncatus* follow the typical mammalian pattern in organisation and distribution, with notable exceptions, such as the lack of an appendix, the presence of the marginal node and diaphragmatic node mass of the lung, and the well-formed anal tonsil complex.

Architecture of the lymph nodes of *T. truncatus* varies depending on location (somatic vs visceral). The major differences among the groups involve the amount of muscle in the capsule and the trabeculae. The somatic nodes have very little smooth muscle in these locations. Smooth muscle is readily discernable in the lung-associated nodes, and quite prominent in the visceral nodes, reaching a maximum in the mesenteric node mass. In this location, muscle effectively encapsulates the nodes and in addition to extending along the trabeculae, forms an interlacing network throughout the node. Assuming that this constant tissue has a purpose, the clear implication is that the visceral nodes are contractile organs, having an important, active role in moving as well as filtering lymph. We agree with Romano et al. (1993) that the term 'pseudo-pancreas' serves no useful purpose, and should be abandoned in favour of the more useful and accurate designation 'mesenteric lymphoid mass', or 'mesenteric lymph nodes'.

The location of the groups of lymph nodes associated with the respiratory tract is unusual. It appears that the primary lymphatic drainage of the lung is not to the hilar nodes, which are usually small and inconspicuous, but rather to the marginal and diaphragmatic nodes, based on the reaction of the latter nodes in animals with pneumonitis. The diaphragm of the dolphin is very oblique, so that the dorsal surface of the lung is roughly twice as long as the ventral. The marginal and diaphragmatic nodes are therefore positioned relatively central in the drainage field, which probably accounts for their

prominence. The hilar nodes are more peripheral to the mass of the dolphin lung, rather than central as in man.

Anal tonsils are very well developed in *T. truncatus*. According to Ortman (1960), the idea of the 'anal tonsil' was introduced by Hebrant in 1899, and Zimmermann in 1904, based on studies of structures in the anal canal of dogs. Bouvier in 1892 may have been the first to see anal tonsils in a cetacean (*Hyperoodon rostratus*) without recognising them for what they were (Arvy, 1976). While the term 'tonsil' has been loosely applied to any macroscopic lymphoid formation associated with the alimentary canal (Cave 1979), the term correctly applies only to complex organs involving the intimate association of epithelium and lymphocyte aggregates, usually consisting of a squamous-lined branching crypt, and epithelium infiltrated by lymphocytes. Lymphoid structures in the intestinal tract lacking these specific features would in aggregate be designated 'mucosa-associated lymphoid tissues', or MALT, of which the tonsils form a subset (Burkitt et al. 1993).

It is likely that anal tonsils occur in most species of marine Cetacea, although we have examined one infant beaked whale, *Mesoplodon* sp., in which we were unable to identify them despite specific search, including histological examination. Whether or not they are recognised depends very much on the dissection technique used to examine the intestine. No observation about their presence or absence in any given species can be taken as valid if examination does not include opening the full length of the intestine, through the anus to the perianal skin. The function of the anal tonsil is unknown, but in *T. truncatus*, tonsils are present in every animal examined. They appear to be most active, if that judgement is based on the amount of lymphoid tissue present, in young animals. Depletion of lymphocytes and cystic enlargement of the crypts, probably representing functional as well as morphological involution, is a consistent feature of older animals. This involution suggests that their relative importance in the function of the immune system as a whole diminishes over time, consistent with observations in other species regarding diminution in immune functions in the tonsils and appendix (Burkitt et al. 1993). Assuming that such a constant structure has an important purpose, we believe that the anal tonsil is involved in the presentation of foreign antigen to the immune system. The anus of the dolphin is small (no more than a centimetre in diameter), short, relatively stiff, smoothly lined and the faeces are fluid. By our observation of dolphins in captivity, it is not rare for flatus to be

discharged. If the distal intestine contained compressible gas, then influx of water during diving through a structure poorly designed to prevent it is possible. Refluxed water would be the source of the foreign antigen first encountered at the distal end of the intestine.

In juvenile *T. truncatus*, the lamina propria of the mucosa and the submucosa of the straight segment of the intestine bears a continuous sheet of lymphoid tissue, including well organised germinal centres. This segment is progressively depleted of lymphocytes which are largely absent from this location in full-grown animals. This lymphoid tissue might be interpreted as confluent Peyer's patches, which are normally found in the mammalian distal small intestine. Patches were not found in extensively-sampled small intestine (intestine proximal to the splenic flexure) of even young animals. The histological appearance of the distal intestine, or colon, in juvenile animals is strikingly similar to the appendix, and it may be that this segment of the intestine is analogous to that structure.

The function of the cetacean spleen has been the object of some controversy, mainly whether it is primarily a blood storage organ ('Speichermilz'), or a metabolic organ ('Stoffwechsellmilz'), according to the classification of Herrath (1935, 1938). In general, 'storage' spleens are relatively large in size and weight, and include the spleens of larger terrestrial mammals. These spleens have well-developed trabeculae with abundant smooth muscles and nominal lymphatic tissue. In contrast, 'metabolic' spleens are relatively small, and include the spleens of more small to moderate-sized land mammals, including man. The trabeculae have less smooth muscle, but lymphoid tissue is better developed than in spleens of the storage type.

In studies of both Odontocete and Mysticete spleens, Zwillenberg (1958, 1959, 1960) argued that the cetacean spleen strictly conformed to neither of Herrath's categories. Herrath (1963) later agreed that the cetacean spleen was atypical, and hypothesised that it was similar to that of a fetal or cold-blooded vertebrate, functioning haematopoietically. Arvy & Pilleri (1970) have also argued against a reservoir function of the cetacean spleen, attributing such a function to the cetacean retia mirabilia network. The typically globular, smooth-surfaced configuration of the spleen of the bottlenose dolphin does not suggest that it is designed to accommodate large changes in blood volume. Simpson & Gardner (1972) did not find evidence of extramedullary haematopoiesis in the spleens of several species of marine mammals studied.

We, however, have found megakaryocytes in essentially all *T. truncatus* spleens, suggesting at least that component of haematopoiesis takes place in the spleen of this species. Nakamine et al. (1992) have proposed that the cetacean spleen is of a primitive mammalian type. The weight of the spleen of *T. truncatus* varies widely at all body lengths and weights, the actual weight of the spleen and the splenic index seeming to be influenced more by degree of reactivity, as expressed in follicle activation, than by age, as expressed by body length and gonad status.

Finally, if morphology is a reliable guide, we can say that the immune system of *Tursiops truncatus* is analogous to that of ruminants, in that it appears to be fully developed at birth.

ACKNOWLEDGEMENTS

This work was supported in part by grant NA16-RGO457-01 from the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration through the National Sea Grant College Program, and in part by grant MX822147-01-0 from the Environmental Protection Agency Gulf of Mexico Program. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA, the EPA, or any of their subagencies. This work would not have been possible without the enthusiastic participation and support of the volunteers of the Texas Marine Mammal Stranding Network. We are grateful to Dr Tracy Romano for a critical and helpful reading of the manuscript.

REFERENCES

- ARVY L (1976) The unknowns of the lymphatic system in Cetacea. In *Investigations on Cetacea*, vol. 7 (ed. Pilleri G), pp. 169–177. Berne, Switzerland: 'Der Bund.'
- ARVY L, PILLERI G (1970) Some characteristics of the cetacean spleen. In *Investigations on Cetacea*, vol. 2 (ed. Pilleri G), pp. 165–167. Berne, Switzerland: Benteliag.
- BANKS KL (1982) Host defense in the newborn animal. *Journal of the American Veterinary Medical Association* **181**, 1053–1056.
- BESPALOVA LS (1975) Regional lymphatic nodules of organs of the gastrointestinal tract of the white-sided dolphin. In *Marine Mammals*. Part I. *Proceedings of the All-Union Meeting, Kiev, October 1975* (ed. Agarkov GB), pp. 32–35. Kiev: Naukova Dumka Publishers.
- BLESSING MH, LIGENSA K, WINNER R (1972) Zur Morphologie der Milz einiger im Wasser lebender Säugetier. *Zeitschrift für wissenschaftliche Zoologie* **184**, 164–204.
- BRYDEN MM (1972) Growth and development of marine mammals. In *Functional Anatomy of Marine Mammals*, vol. 1 (ed. Harrison RJ), pp. 1–79. New York: Academic Press.
- BURKITT HG, YOUNG B, HEATH JW (1993) *Wheater's Functional Histology*, 3rd edn, pp. 211–213. New York: Churchill Livingstone.
- CAVE AJE (1979) Tonsillar formations in the bottle-nosed dolphin (*Tursiops truncatus*). In *Investigations on Cetacea*, vol. 10 (ed. Pilleri G), pp. 229–243. Finland: Vammalan Kirjapaino.
- CAVE AJE (1980) Note on *Tursiops* visceral histology. In *Investigations on Cetacea*, vol. 11 (ed. Pilleri G), pp. 111–113. Berne, Switzerland: 'Der Bund.'
- COWAN DF (1994) Involution and cystic transformation of the thymus of the bottlenose dolphin, *Tursiops truncatus*. *Veterinary Pathology* **31**, 648–653.
- COWAN DF, BROWNELL RL JR. (1974) Gut-associated lympho-epithelial organ ('anal tonsil') in the gray whale. In *Functional Anatomy of Marine Mammals*, vol. 2 (ed. Harrison RJ), pp. 321–327. New York: Academic Press.
- COWAN DF, WALKER WA, BROWNELL RL JR. (1986) Pathology of small cetaceans stranded along southern California beaches. In *Research on Dolphins* (ed. Bryden MM, Harrison RJ), pp. 323–367. Oxford: Oxford University Press.
- COWAN DF, SMITH TL (1995) Morphology of complex lymphoepithelial organs of the anal canal ('anal tonsil') in the bottlenose dolphin, *Tursiops truncatus*. *Journal of Morphology* **223**, 263–268.
- HERRATH E (1935) Bau und Funktion der Milz. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **23**, 375–430.
- HERRATH E (1938) Zur vergleichenden Anatomie der Säugermilz und ihrer Speicher- und Abwehraufgaben. Zugleich ein Beitrag zur Typologie der Milz und zum Problem der artlich und individuell verschiedenen Milzgröße. *Medizinische Klinik* **41**, 1355–1359.
- HERRATH E (1963) Zur Frage der Typisierung der Milz. *Anatomischer Anzeiger* **112**, 140–149.
- HOHN AA, SCOTT MD, WELLS RS, SWEENEY JS, IRVINE AB (1989) Growth layers in teeth from known age, free-ranging bottlenose dolphins. *Marine Mammal Science* **5**, 315–342.
- KOMATSU S (1979) The anal tonsil in the striped dolphin, *Stenella coeruleoalba*. *Journal of Liberal Arts and Sciences, Sapporo Medical College* **20**, 53–56.
- KUMAR D, COWAN DF (1994) Cross-reactivity of antibodies to human antigens with tissues of the bottlenose dolphin, *Tursiops truncatus*, using immunoperoxidase techniques. *Marine Mammal Science* **10**, 188–194.
- LAHVIS GP, WELLS RS, CASPER D, VIA CS (1993) *In vitro* lymphocyte response of the bottlenose dolphin, *Tursiops truncatus*. Mitogen-induced proliferation. *Marine Environment Research* **35**, 115–119.
- MOSKOV M, SCHIWATSCHEWA T, BONEV S (1969) Vergleichshistologische Untersuchung der Lymphknoten der Säuger. Die Lymphknoten des Delphins. *Anatomischer Anzeiger* **124**, 49–67.
- NAKAMINE H, NAGATA S, YONEZAWA M, TANAKA Y (1992) The whale (Odontoceti) spleen: a type of primitive mammalian spleen. *Acta Anatomica Nippon* **67**, 69–81.
- ORTMANN R (1960) Über den lymphatischen Apparat der Analregion und die sogenannte 'Analtonsille' bei Säugetieren. *Zeitschrift für Anatomie und Entwicklungsgeschichte* **121**, 459–477.
- PILLERI G, ARVY L (1971) Aselli's pseudopancreas (nodi lymphatici mesenterici) in two delphinids: *Delphinus delphis* and *Stenella coeruleoalba*. In *Investigations on Cetacea*, vol. 3 (ed. Pilleri G), pp. 189–193. Berne, Switzerland: 'Der Bund.'
- RETTNERER E, NEUVILLE H (1916) De la morphologie de la rate des Cétacés. *Comptes Rendus des Séances de la Société de Biologie* **79**, 60–64.
- RIDGWAY SH, McCORMICK JG, WEVER EG (1974) Surgical approach to the dolphin's ear. *Journal of Experimental Zoology* **188**, 265–276.
- ROMANO TA, FELTEN SY, OLSCHOWKA JA, FELTEN DL (1993) A microscopic investigation of the lymphoid organs of the beluga, *Delphinapterus leucas*. *Journal of Morphology* **215**, 261–287.
- SIMPSON JG, GARDNER MB (1972) Comparative microscopic anatomy of selected marine mammals. In *Mammals of the Sea: Biology and Medicine* (ed. Ridgway SH), pp. 298–418. Springfield, Illinois: Charles C. Thomas.

- SLIJPER EJ (1958) Organ weights and symmetry problems in porpoises and seals. *Archives Néerlandaises de Zoologie* **13**, 97–113.
- SLIJPER EJ (1979) *Whales*, 2nd edn, pp. 289–290. Ithaca, New York: Cornell University Press.
- UYS CJ, BEST PB (1966) Pathology of lesions observed in whales flensed at Saldanha Bay, South Africa. *Journal of Comparative Pathology* **76**, 407–412.
- YAMASAKI F, TAKAHASHI K, KAMIYA T (1975) Digestive tract of La Plata Dolphin, *Pontoporia blainvillei* II. Small and large intestines. *Okajimas Folia Anatomica Japonica* **52**, 1–26.
- YAMASAKI F, KOMATSU S, KAMIYA T (1977) A comparative morphology of anal tonsils in Platanistidae. *Scientific Reports of the Whales Research Institute, Tokyo* **29**, 95–100.
- ZWILLENBERG HHL (1958) Die mikroskopische Anatomie der Milz der Furchenwale. *Acta Anatomica (Basel)* **32**, 24–39.
- ZWILLENBERG HHL (1959) Über die Milz des Braunfisches (*Phocaena phocaena*, L.). *Zeitschrift für Anatomie und Entwicklungsgeschichte* **121**, 9–18.
- ZWILLENBERG HHL (1960) Die mikroskopische Anatomie der Milz der Furchenwale. *Archives Néerlandaises de Zoologie* **13**, 595–597.