gressive behavior, positive FA for rabies virus antigen, and the histologic lesion consisting of a lymphocytic meningoencephalitis concurrent with a spongiform change, similar in distribution to that previously reported in rabies-infected skunks. The source of the rabies in this heifer was not established. The skunk is the most likely source of rabies virus infection of domestic animals in Illinois (Illinois Department of Public Health, personal communication).

Many of the articles describing lesions of BSE come from the United Kingdom, so rabies is typically not a differential diagnosis in these cases. In the United States and other countries where rabies virus is endemic, cattle with histories of neurologic signs may be rabid. Additionally, the degree of inflammation or presence of Negri bodies is not consistent in all cases of rabies. Thus, the distinction between spongiform changes induced by BSE and those induced by rabies virus may not always be clear. Neurologic cases in cattle should be monitored for the occurrence of BSE. Rabies in cattle should also be considered as a possible differential diagnosis for spongiform changes in cattle and other domestic species.

References

Request reprints from Dr. G. L. Foley, Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, IL 61801 (USA).

Amyloidosis in the Bottlenose Dolphin, Tursiops truncatus

D. F. COWAN

Abstract. Four cases of amyloidosis were recognized in a study population of 21 (19%) bottlenose dolphins (Tursiops truncatus) examined as part of an investigation of the causes of cetacean strandings along the Texas Gulf Coast. Amyloid deposition was mainly and most prominently in the corticomedullary regions of the kidneys and less consistently in the vessels of the spleen, lung, and heart and around acini of the palatine salivary gland and the thyroid gland. Pretreatment of sections with permanganate and sulfuric acid greatly diminished Congo red staining, suggesting the dolphin amyloid is of the AA variety.

Key words: Amyloidosis; cetaceans; dolphins; stranding; Tursiops truncatus.

Amyloidosis is a disease characterized by the tissue deposition of autologous extracellular fibrillar proteins with particular tincorial and ultrastructural properties and is well recognized in a wide variety of animals, including birds, reptiles, and mammals. "Amyloid" is the term applied to a group of chemically diverse proteins that display remarkable morphologic and histochemical uniformity. Although amyloid is generally easily recognized using hematoxylin and eosin (HE) and conventional light microscopy, certain histochemical methods enhance recognition, define the amyloid, and help to categorize it. Congo red is the most commonly used stain for amyloid. However, although its reaction is typical and reliable, it is not completely specific because it also stains elastic fibers and, depending on the care applied to staining, collagen bundles. When viewed using polarizing microscopy, amyloid stained with Congo red displays a particular apple green color, which can be resolved into blue and yellow by rotation of the plane of polarization. This color
phenomenon, dichroism, is thought to be a specific, pathognomonic histochemical test for amyloid.\(^6\) Amyloid stains metachromatically with toluidine blue. Fluorochrome thioflavin T is another widely used although not absolutely specific stain for amyloid.\(^9\) Together, these stains allow amyloid to be diagnosed with a high level of confidence.

Amyloid fibrils appear to be made of nonglycosylated polypeptide chains that lie in the long axis of the fibril, in a cross-β-pleated sheet configuration.\(^7\) Although the chemical composition of the protein subunits of amyloid varies, the ultrastructure of the various forms of amyloid is quite consistent. Electron microscopy reveals amyloid to be composed of randomly oriented fibrils of indeterminate length ranging from 70 to 100 nm in diameter.

Despite its morphologic uniformity, at least two major classes of amyloid have been identified.\(^4\) One, immunoamyloid, is composed of immunoglobulin light chains called amyloid light chains (AL). The other is made of a nonimmunoglobulin amyloid-associated protein (AA). AL amyloid is associated with B-lymphocyte abnormalities, including myeloma. AA amyloid, or secondary amyloid, is associated with chronic infection. AA is differentiated from AL amyloid by its loss of affinity for Congo red after pretreatment with potassium permanganate and dilute sulfuric acid. AL is unchanged by this procedure.\(^9\)

Between April 1991 and April 1993, 21 bottlenose dolphins were examined at necropsy, and hyaline material depositions typical of amyloid was found in four of them (19%).

The dolphins included in this study were collected by the Texas Marine Mammal Stranding Network, under auspices of the National Marine Fisheries Service. The collection area ranges from Brownsville, Texas, at the Mexican border to Sabine Pass, Texas, at the Louisiana border, i.e., the entire Texas Gulf Coast. Three of the animals were fully mature, and one was approaching sexual maturity.\(^7\) Well-preserved animals were brought to a central laboratory at Texas A&M University at Galveston for necropsy, which included gross examination and systematic histologic sampling of all organs. Tissues were routinely collected in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm and stained with HE. Selected tissues were stained with toluidine blue, thioflavin T, and Congo red. Selected tissues from particularly well-preserved specimens were collected in a 2% glutaraldehyde–2% paraformaldehyde mixture for electron microscopy. Amyloid was recognized by its typical pink hyaline appearance when stained with HE and confirmed by a typical affinity for Congo red, displaying apple green birefringence on examination under polarized light with resolution into yellow and blue on rotation of the plane of polarization. The material stained metachromatically with toluidine blue and bound thioflavin T. Light microscopic sections were stained with Congo red after treatment with potassium permanganate and sulfuric acid following a previously described method\(^6\) to determine whether the amyloid was composed of AA or AL protein.
In dolphin No. 1, amyloid was found in the kidney, both in the interstitium and in walls of small arteries and muscular arterioles, and in the lungs, in which it occurred in small vessels associated with the bronchial mucosa. Grossly, the general conformation of the kidney was unchanged, but the cortical surfaces had taken on a coarsely granular “pigskin” texture. No gross alterations associated with amyloid deposition were recognized in the lungs.

In dolphin No. 2, amyloid was found massively surrounding the acini of the palatal salivary gland. Grossly, the palatal gland was thickened with dilated acini, prompting an initial suspicion of cystadenoma. Light microscopy revealed no suggestion of neoplasms, but did reveal amyloid deposition and mucus retention and cystic atrophy of acini (Fig. 1). Electron microscopy showed the amyloid material to be composed of a random array of nonbranched fibrils of about 100 nm (Fig. 2). Amyloid was also found in plaquelike arrays of submucosal bronchial arterioles (Fig. 3), many intramural arteries of the cardiac ventricles, most vessels of the adrenal capsules, a few small arteries in the spleen, pancreas, hilar lymph nodes, liver, and kidneys, and surrounding a few distal renal tubules. Massive renal deposits were absent.

In dolphin No. 3, amyloid was found massively in the interstitium of the corticomedullary regions of the kidneys (Fig. 4), rimming the acini of the palatal salivary gland, as patchy depositions in the mucosa of the second chamber of the stomach (the first glandular compartment of the four chamber stomach), and in the small arteries of the spleen, small arteries of the mesenteric lymph node, and the thyroid gland. In this dolphin, there were no gross alterations in the salivary gland, because the volume of the deposits was less than that in dolphin No. 2. The renal cortical surfaces were coarsely granular, as in dolphin No. 1.

Dolphin No. 4 was forming gas in tissues, and in anticipation of autolysis, sampling was limited to the heart, lungs, and kidneys. Massive deposits of amyloid were found in the kidneys, in the region of the cortico medullary junctions, and in small renal arteries and arterioles.

The amyloid in these four dolphins all had the same histochemical reactions. All showed avidity for Congo red, with typical response in polarized light, that was abolished by pretreatment with permanganate and sulfite acid, metachromasia with toluidine blue, and fluorescence with thiazolin. Tissues from all four dolphins responded the same way to the histochemical stains used, indicating that the amyloid has approximately the same composition in each instance. The exact nature of the amyloid fibrils is not clear. The reaction to pretreatment with permanganate and sulfite acid suggests that dolphin amyloid is the AA variety. With the exception of the salivary gland and the massive renal
deposits, amyloidosis in the bottlenose dolphin is primarily found in the blood vessels. The distribution of amyloid in vessels among the various organs in the dolphin is unusual; in these four animals, deposition was most marked in the lungs.

Considering that amyloidosis has not previously been described in the dolphin, an incidence of 19% seems extraordinary. Amyloidosis is not difficult to recognize histologically and should not have been overlooked by a trained observer. Increasingly, beach-stranded dolphins are being examined both grossly and histologically by trained pathologists. No amyloid was found during a study of 51 stranded dolphins (primarily *Delphinus delphis* [common dolphin]) in southern California, or 68 individuals of two species of *Stenella* (*S. attenuata* [spotted dolphin] and *S. longirostris* [spinner dolphin]) killed incidentally in the open Pacific, or in 55 pilot whales (*Globicephala melaena*) killed in Newfoundland. *Tursiops truncatus* is a common species, and many individuals have been examined. Thus, there may be something unusual about the local population of these dolphins or they may be exposed to some unusual pathogenetically related agent. The dolphins with amyloidosis were not distinguishable by age distribution or disease pattern from the larger group in which they appeared. AA amyloid is thought to be a group of substances, closely related but differing among the various animals species in which it has been recognized. The general class of AA amyloids is associated with chronic inflammation, virus infections, a variety of malignancies, and (in humans) familial Mediterranean fever and is the form induced in the standard experimental models of amyloidosis. Essentially, all beached dolphins have some sort of acute and chronic inflammatory foci, usually in the lungs and often associated with lungworms. These dolphins may also have a variety of skin ulcers, which are usually at least superficially infected. Because inflammation is found in all dolphins under these circumstances, it is currently not possible to identify any particular or special association between amyloidosis and inflammation.

**Acknowledgement**

This work was supported in part by grant NA16RG0457-01 from the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration through the National Sea Grant College Program. The views expressed herein are those of the author and do not necessarily reflect the views of NOAA or any of its subagencies.

**References**


Request reprints from Dr. D. F. Cowan, Division of Surgical Pathology, E 88, University of Texas Medical Branch, Galveston, TX 77555-0588 (USA).