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Authors: Lair, S., Béland, P., De Guise, S., and Martineau, D.

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S. Lair,[1,4] P. Béland,[2] S. De Guise,[3,5] and D. Martineau[1]

[1] Canadian Cooperative Wildlife Health Center, Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, Quebec, Canada J2S 7C5

[2] St. Lawrence National Institute of Ecotoxicology, Montreal, Quebec, Canada, H2Y 1B4

[3] Université du Québec à Montréal, Montreal, Quebec, Canada H3C 3P8

[4] Current address: Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada M1E 4R5; Metropolitan Toronto Zoo, Scarborough, Ontario, Canada M1B 5K7

[5] Current address: Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California Davis, Davis, California, 95616, USA

ABSTRACT: Thirty stranded beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary (Quebec, Canada) population and five animals from the Hudson Bay aboriginal hunt (Northwest Territories, Canada) were examined. Twenty one animals from the St. Lawrence Estuary had mild to severe adrenal lesions and four whales from the Hudson Bay population were affected by minimal adrenal changes. Cortical hyperplasia was observed in 24 adult beluga whales all from the St. Lawrence Estuary. Bilateral cortical cysts and cellular vacuolar degeneration were observed in the adrenal glands of 19 beluga whales from both populations. The cysts, filled with a cortisol-rich liquid, were present in both sexes. Beluga whales with adrenal cysts were significantly older than animals without cysts, and the severity of the lesions increased with age. Nodular hyperplasia of the medulla was observed in seven of the beluga whales, all from the St. Lawrence Estuary population. All lesions could be part of a normal aging process. The adrenocortical lesions might be due to stress or adrenocorticolytic xenobiotics, while the medullary hyperplasia might be caused by hypoxia or exposure to estrogenic xenobiotics.

Key words: Adrenal gland, beluga whale (*Delphinapterus leucas*), cetaceans, endocrinology, environmental contamination, marine mammals, pathology, stress, xenobiotics.

INTRODUCTION

Anomalies of the adrenal glands have been described sporadically in marine mammals (Howard, 1983). Adrenal hyperplastic lesions, that may be etiologically related to adrenocorticolytic xenobiotics have been observed in Baltic seals (Bergman and Olsson, 1985). De Guise et al. (1995) reported hyperplastic nodules and serous cysts in the adrenal glands of beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary (Canada). Since this small isolated and endangered population (Pippard, 1985) is exposed to and contaminated by high levels of xenobiotics (Martineau et al., 1994), the degenerative adrenal lesions observed might be related to adrenocorticolytic compounds. The objectives of the present study was to characterize the adrenal lesions observed in beluga whales from St. Lawrence Estuary necropsied during 1987 to 1993 and to determine the potential risk factors associated with these endocrine anomalies.

MATERIAL AND METHODS

Adrenal glands from 35 beluga whales (15 males and 20 females) were examined from 1987 to 1993 as part of standard postmortem examinations of the Canadian Cooperative Wildlife Health Center, (Quebec, Canada). Fifteen of these animals, of which nine had adrenal anomalies, have been previously listed in De Guise et al. (1995). In addition, we examined the adrenal glands from 15 beluga whales found stranded along the St. Lawrence Estuary (Quebec, Canada; 47°N, 70°W, 50°N, 66°W), and five killed for subsistence by Inuit hunters at Arviat (Western Hudson Bay, Northwest Territories, Canada; 61°06′N, 93°59′W).

A complete standard postmortem examination was performed on each animal. Both adrenal glands were removed from each whale and examined. Cysts from three glands were punctured and cortisol measurement were performed on their content by radioimmunoassay (Coat-A-Count® Cortisol, Los Angeles, California, USA). Adrenal glands were fixed in 10% neutral buffered formalin and sections of fixed tissues (5 mm) were made in order to detect any macroscopic anomaly. Transverse sections of macroscopic lesions and of the adrenal body, were embedded in paraffin, cut into 5 to 7 μm thick sections and stained with hematoxylin,

430

TABLE 1. Adrenal anomalies observed in male and female beluga whales (*Delphinapterus leucas*) examined between 1987 and 1993.

| Sex | Total examined | Cortical cysts | | Cortical hyperplasia (%) | Medullar hyperplasia (%) |
		Total[a] (%)	Macroscopic (%)		
Female	20	12 (60)	4 (27)	16 (80)	4 (20)
Male	15	7 (47)	5 (25)	8 (53)	3 (20)
Total	35	19 (54)	9 (26)	24 (69)	7 (20)

[a] Macroscopic and microscopic.

TABLE 2. Age distribution in adrenal anomalies observed in beluga whales (*Delphinapterus leucas*) examined between 1987 and 1993.

| Age (years) | Total examined | Cortical cysts | | Cortical hyperplasia (%) | Medullar hyperplasia (%) |
		Total[a] (%)	Macroscopic (%)		
≤5	6	0	0	0	0
6 to 16	9	5 (56)	0	5 (56)	2 (22)
≥17	20	14 (70)	9 (45)	19 (95)	5 (25)
Total	35	19 (54)	9 (26)	24 (69)	7 (20)

[a] Macroscopic and microscopic.

phloxine and saffron (HPS) (Luna, 1968). The thickness of the adrenal cortex and medulla were measured on the widest point of the gland, on a transverse section. All adrenal glands, including these from De Guise et al. (1995) were handled using the same protocol.

Whale ages were determined by counting dentine growth layers on longitudinal sections of teeth (Sergeant, 1973), adopting the standard of two growth layer groups per year (Brodie, 1982). A logistic regression, using whale age as a continuous independent variable of interest, was calculated to measure the statistical significance of the age as a factor in the probability to have adrenal cysts. A Pearson correlation coefficient (r) was calculated between the age and the cortical thickness. The level of significance of all tests was set at $P \leq 0.05$. Statistical analyses were performed using the SAS software (SAS Institute Inc., Cary, North Carolina, USA).

RESULTS

Examined beluga whales were < 1-yr-old to 32-yr-old (mean ± SE = 16 ± 1.6 yr). Adrenal glands of immature beluga whales (≤ 5 yr old) had a convoluted pattern, and were separated into pseudolobules by projections of dense, poorly cellular, connective tissue originating from the capsule. This pseudolobulation also was noted in adult animals but the convoluted pattern was usually far less pronounced and the fibrous projections were thicker. The cortex/medulla ratio of the gland was highly variable between animals, ranging from 0.9 to 11.5 (3.7 ± 0.6). The demarcation between the three cortical layers (*zona glomerulosa*, *zona fasciculata* and *zona reticularis*) was distinct only in well preserved tissue from immature animals.

Three types of adrenal anomalies were highly prevalent in beluga whales. These included cortical cysts, cortical enlargement with hyperplastic nodules and medullary hyperplastic nodules (Tables 1 and 2).

Cysts of various sizes (diameter ≤ 15 mm) were seen in one or both adrenal glands of 19 whales (54%). In ten cases, these cysts were microscopic, while in nine cases they were bilateral (47%). Only mature animals were affected by adrenal cysts, and both males (7 cases) and females (12 cases) displayed lesions. Belugas with adrenal cysts (19.1 ± 2.0 yr) were significantly ($P = 0.023$) older than belugas without cysts (11.5 ± 2.2 yr). Animals with macroscopic cysts (25.8 ± 1.5 yr) were significantly ($P = 0.005$) older than animals with microscopic cysts (15.6 ± 2.1 yr). Cystic lesions were found in both St. Lawrence Estuary and Hudson Bay populations in 50% and in 80% of the examined whales, respectively. In the latter, composed of younger adults, only microscopic cysts were observed.

The cysts were often multiple, contained a clear serous fluid, occupied the three cortical layers, and usually compressed the adjacent medulla and cortex (Fig. 1). They were lined by simple squamous cells, contained an acidophilic fluid of variable density and were surrounded by connective tissue capsules of variable thickness (Fig. 2). Small cysts, often coalescent, were frequently subdivided into
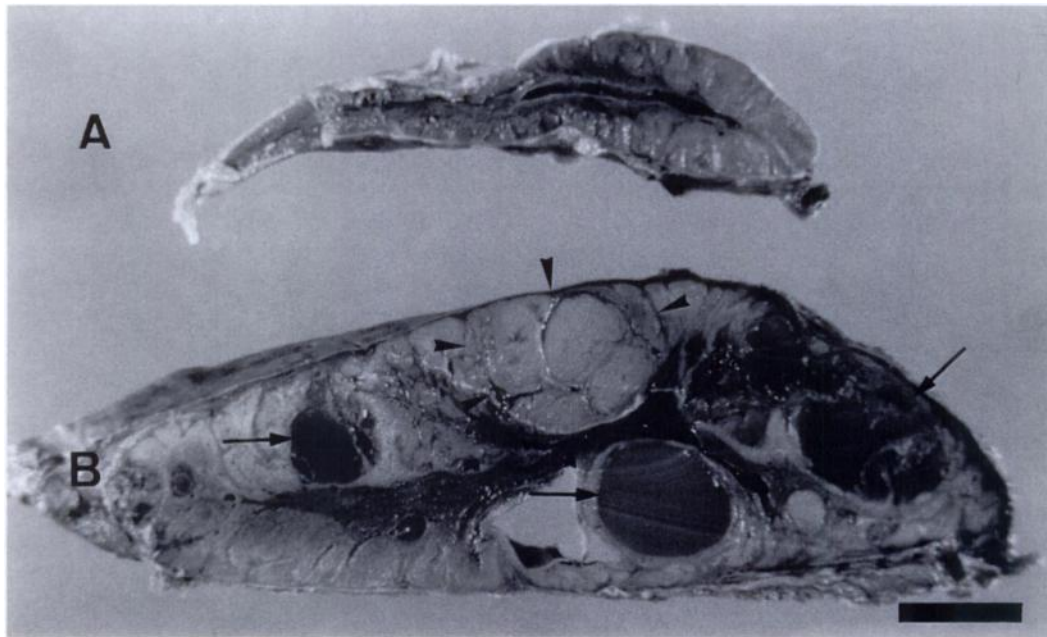
FIGURE 1.   Transverse sections of formalin fixed adrenal glands showing (A) normal glands of an adult male beluga whale and (B) cystic and hyperplastic adrenal gland of an adult female beluga whale. Note the marked nodular cortical hyperplasia (arrowheads) and the multifocal cystic structures (arrows). Bar = 1 cm.
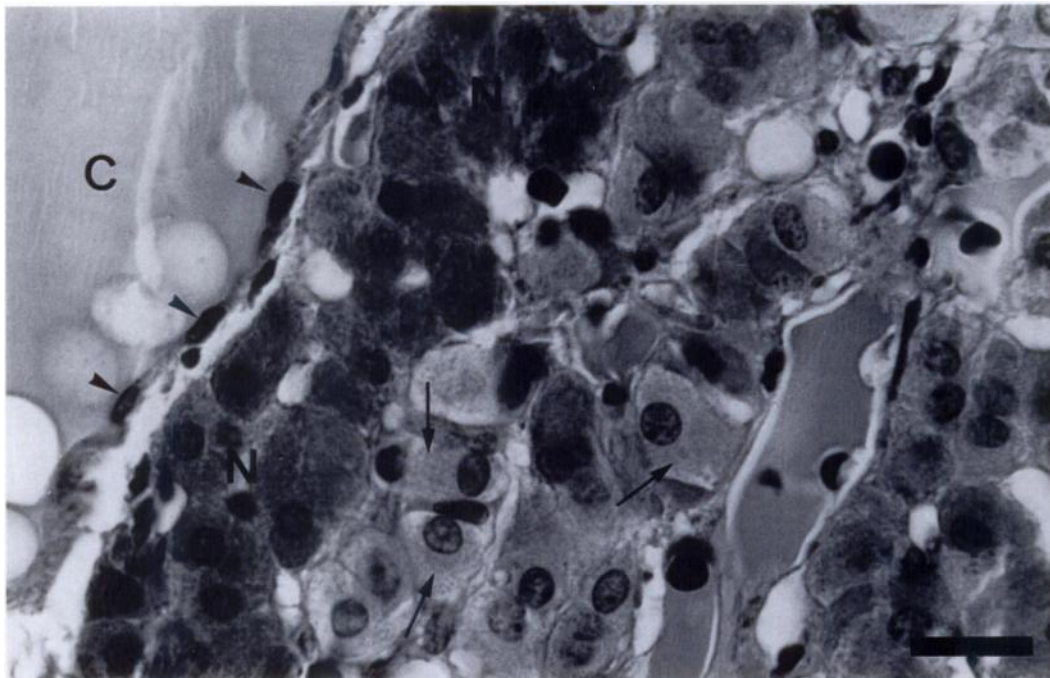

FIGURE 2.   Adrenal gland from an adult female beluga whale showing minimal vacuolar degeneration of adrenocortical cells. Compare normal cells (N) with the pale swollen cells (arrows). A portion of a cyst (C) is lined by endothelial-like flattened cells (arrowheads). Hematoxylin, phloxine and saffron. Bar = 80 μm.
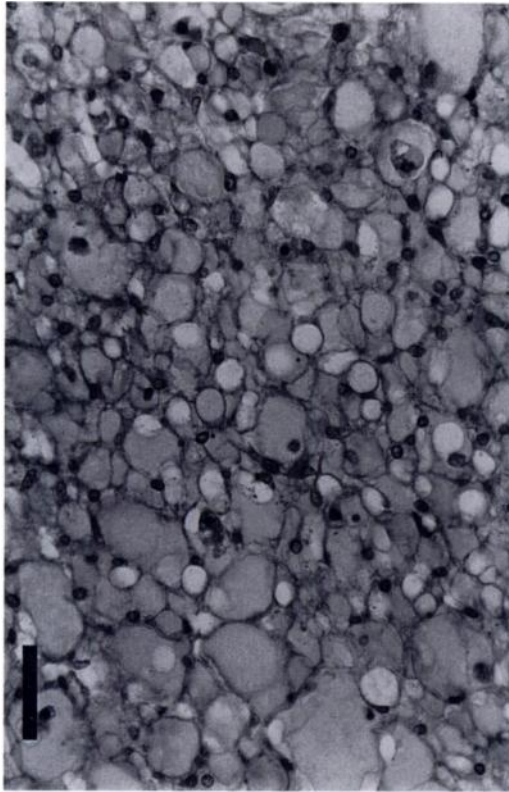
FIGURE 3. Adrenal gland of an adult female beluga whale showing severe vacuolar degeneration of adrenocortical cells and large, single or multiple vacuoles filled with pale acidophilic homogeneous fluid dilating the cytoplasm. Hematoxylin, phloxine and saffron. Bar = 40 µm.

smaller cavities by thin bands of fibrous tissue. Most of the adrenal glands with cysts also had areas of variable size in which cortical cells were swollen, pale and vacuolated (Fig. 2). The cytoplasm of the most severely affected cells was moderately to severely dilated by large, single, or multiple vacuoles filled with pale acidophilic homogeneous fluid (Fig. 3). The rupture of the cytoplasmic membranes of adjacent degenerated cells and the persistence of the connective stroma conferred a spider web pattern to some cortical areas (Fig. 4). Cyst fluid sampled from three animals from the St. Lawrence had cortisol concentrations of 663, 3034 and 4020 nmol/l, respectively.

Mild to severe diffuse cortical hyperpla-

sia, which caused the loss of the convoluted pattern, was observed in 24 of 29 adult beluga whales examined (83% of the animals > 6-yr-old). A significant positive correlation ($r = 0.65$, $P = 0.0001$) was found between the maximum adrenal cortex thickness and age of the animal.

Hyperplastic cortical nodules were present in ten adult animals (all > 15-yr-old) from the St. Lawrence Estuary. These variably sized nodules (≤ 2 cm) were often circumscribed by a fibrous capsule, usually compressed the adjacent parenchyma, and were observed in both sexes (Table 1).

Nodules were observed in the medulla of seven beluga whales from the St. Lawrence population. Three females and four males were affected, and all but one animal were > 21-yr-old. These nodules were of variable size, unencapsulated, and usually protruded into the cortex. They consisted of clusters of lightly basophilic pheochromocytes which did not compress the adjacent tissue (Fig. 5). Five of the seven whales affected by hyperplastic nodules in the medulla also displayed cortical hyperplasia.

DISCUSSION

In immature beluga whales (≤ 5-yr-old) adrenal morphology was similar to the description in other species of cetaceans (Simpson and Gardner, 1972), and the cellular morphology of the different layers was similar to the description given for large ruminants by Dellmann (1976).

A high prevalence of adrenal lesions, the severity of which increased with age, was observed in beluga whales from two Canadian populations. Adrenal lesions have been rarely reported in other marine mammals. Adrenocortical vacuolar degeneration was described in stranded marine mammals by Howard (1983) who associated it with stress, disease or trauma. These lesions are similar to the vacuolar degeneration described here. However, in beluga whales the lesions involved all three cortical layers, mainly the *zona fasciculata*, whereas only the *zona glomeru-*
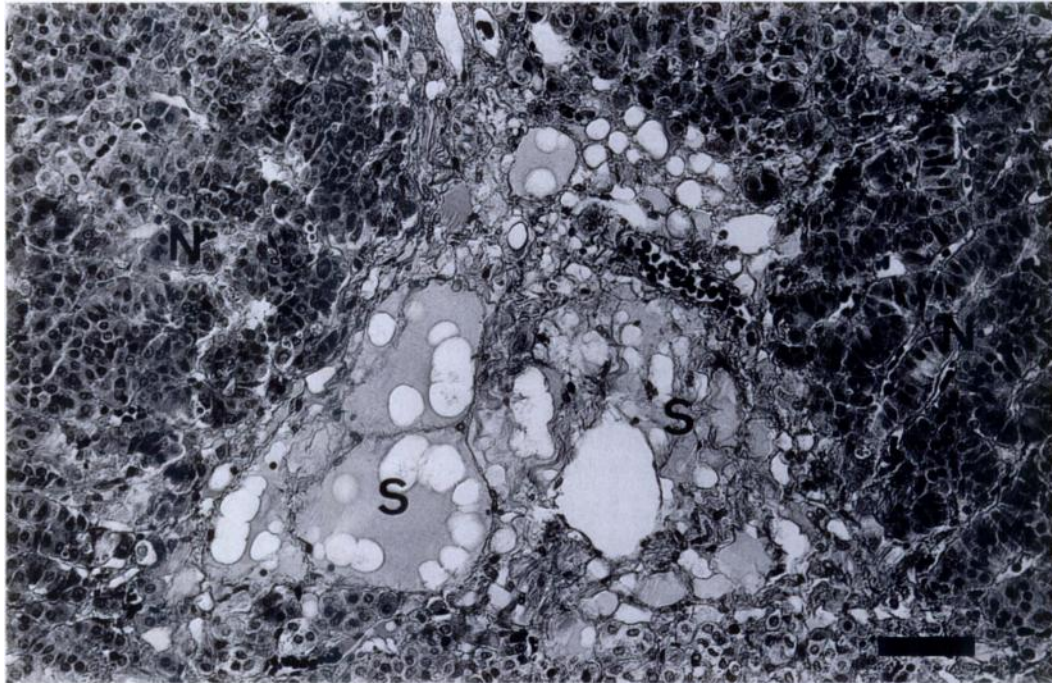
FIGURE 4.   Adrenal gland of an adult female beluga whale. Degenerate cortical areas show typical spider web pattern (S) surrounded by normal cortical cells (N). Multiple cavities formed by the fusion of ruptured swollen cells are separated by delicate strands of connective tissue. Hematoxylin, phloxine and saffron. Bar = 70 μm.

losa was affected in the animals reported by Howard (1983).

Based on our microscopic observations, the cortical cysts seemed to originate from the coalescence of microcavities resulting from the rupture of degenerated cells. The capsule, most apparent in larger cysts, was probably formed by the collapse of the adjacent collagen stroma.

Adrenocortical cysts also have been reported in a common dolphin (*Delphinus delphis*) (Cartee et al., 1995), and in stranded adult Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Geraci et al., 1978). In Atlantic white-sided dolphins, adrenal cysts were mainly observed in females and large cysts were present only in the left gland. This distribution differs from our findings where cysts were frequently bilateral and present in both males and females. Geraci et al. (1978) postulated that these cystic lesions were caused by sinusoidal blockage or hyperse-

cretion and that they were probably stress related. The very high cortisol concentration found in beluga whales adrenal cyst suggests a similar etiology.

Adrenal cysts are rare in terrestrial mammals. In humans, they are associated with organization of hemmorhage or lymphatic malformation (Page et al., 1986), which both differ morphologically from the lesions found in our study. Since the severity of cysts seems to increase with age in beluga whales, these changes could also be part of a normal aging process. In old female laboratory rats, similar lesions are considered to be a nonspecific change related to aging (Hamlin and Banas, 1990). However, these lesions have not been described in other aging mammals, and thus other etiologies might be envisaged.

The metabolites of various xenobiotics, such as 7,12-dimethylbenz(a)anthracene (DMBA), 2-(2-chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethane (p,p'-DDD),
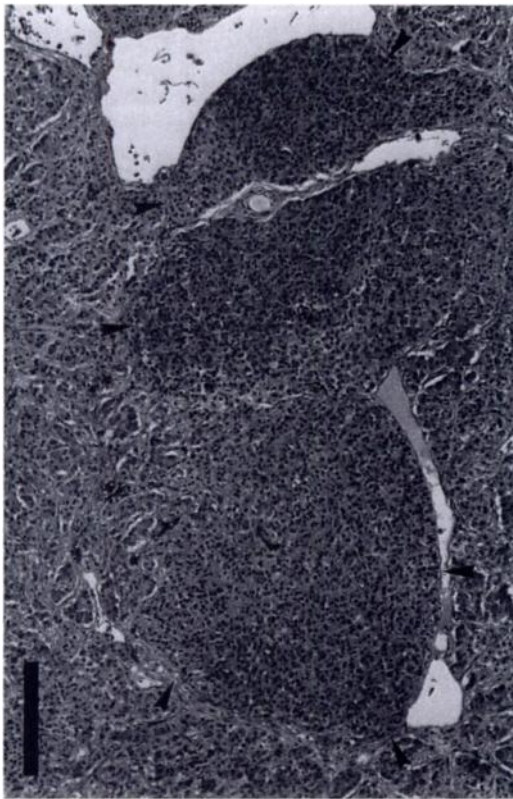
FIGURE 5.   Adrenal gland of an adult female be-luga whale. Mild nodular hyperplasia of the medulla is present. Note the demarcation between hyperplas-tic nodules and the normal medulla (arrowheads). Hematoxylin, phloxine and saffron. Bar = 200 μm.

and 3-methylsuplphonyl-2,2-bis(4-chloro-phenyl)-1,1-dichloroethene (MeSo₂-DDE), induce the degeneration of the adrenocor-tical *zonae fasciculata* and *reticularis* in various species (Brandt et al., 1992; Hall-berg, 1990). High levels of such xenobiot-ics have been demonstrated in the blubber of stranded beluga whales from the St. Lawrence Estuary (Massé et al., 1986). Consequently, adrenocorticolytic metabo-lites might be present in adrenal glands of these animals, and cause cortical degen-eration. The contamination of the Arctic beluga population by DDT and its metab-olites (Muir et al., 1990) also might explain the presence of degenerative lesions in the Hudson Bay beluga whales.

The adrenal cortex of adult belugas was diffusely and focally enlarged when com-pared to that of immature belugas and other cetaceans. Adrenocortical hyperpla-sia has been reported in harbor porpoises (*Phocoena phocoena*) that were chronically sick, and chronic stress was suggested as the causal agent (Kuiken et al., 1993). Chronic stress induces bilateral adrenal cortex hyperplasia in domestic mammals (Anderson and Capen, 1978). Since stranded beluga whales are frequently af-fected by debilitating and stressful dis-eases, disease-related stress might be re-sponsible for the cortical enlargement that we observed.

Adrenocortical hyperplasia has also been observed in seals from the Baltic sea, and MeSo₂-DDE, or other adrenocorti-colytic xenobiotics have been proposed as a possible etiology (Bergman and Olsson, 1985). However, Kuiken et al. (1993) found no correlation between the relative volume of the adrenal cortex and levels of chlorinated hydrocarbons in harbor por-poises. Also, Bergman et al. (1994) did not find any MeSo₂-DDE, in the blubber or liver of a female beluga whale from the St. Lawrence, affected by cortical hyperplasia and cortical cysts.

Adrenocortical nodular hyperplasia is common in old domestic animals (Ander-son and Capen, 1978) and humans (Bondy, 1954). Likewise, this lesion could be part of a normal aging process in beluga whales. It could also be a regenerative re-sponse to degenerative lesions such as the vacuolar degeneration that we observed (Dobbie, 1969).

Apparently, medullary hyperplastic nod-ules have not been reported in cetaceans. In laboratory rat, the incidence of nodular hyperplasia of the medulla increased with age (Hamlin and Banas, 1990). Since nod-ular hyperplasia of the medulla was ob-served mainly in older beluga whales, it can be proposed that this proliferative le-sion could be associated with aging. How-ever, a similar aging process has not been described in the adrenal medulla of do-mestic species (Capen, 1993). Nodular hy-perplasia of the medulla and cortical hy-

perplasia have been produced experimentally by prolonged hypoxia (Gosney, 1985). Hyperplasia of the adrenal cortex was observed in five of the seven whales with medullary hyperplastic nodules. Four beluga whales with medullary nodular hyperplasia had pneumonia and one had endocarditis. Thus, this adrenal lesion could be related to hypoxia caused by lung or cardiac diseases or by prolonged agony.

Proliferative lesions of the adrenal medulla have been described in bulls and humans affected by multiple endocrine adenopathies. These inherited syndromes are characterized by multiple proliferative lesions of the endocrine tissues, including medullary carcinoma of the thyroid gland (domestic bulls and humans), insulinoma (humans), and pituitary adenoma (humans) (Greene et al., 1983; Jubb and McEntee, 1959). None of these endocrine anomalies were observed in the whales examined in our study. In laboratory rats, proliferative lesions of the medulla have been caused by exposure to various agents including estrogens, nicotine, growth hormone, and reserpine (Tischler and De-Lellis, 1988). Thus, the high levels of estrogenic xenobiotics, detected in the tissues of beluga whales from the St. Lawrence population (Martineau et al., 1987), could potentially be associated with the proliferative lesions observed in the adrenal medulla. Finally, we stress the need to increase our knowledge of the endocrine physiology and pathology of free-ranging cetaceans to better define the respective effects of aging, stress, diseases, and xenobiotics on the endocrine homeostasis of these animals.

ACKNOWLEDGMENTS

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