Transitional Cell Carcinoma of the Urinary Bladder in a Beluga Whale (*Delphinapterus leucas*)

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ABSTRACT

A transitional cell carcinoma of the urinary bladder was found in a beluga whale stranded in the St. Lawrence middle estuary. Various organs of this animal were submitted to high resolution gas chromatography coupled with mass spectrometry analysis. High frequency of urinary bladder cancer in the human population of the same area and the presence of carcinogenic compounds in the marine environment of this animal are discussed.

Concurrent isolation of *Edwardsiella tarda* from various organs of this whale is also reported.

Key words: Cetaceans, urinary bladder, transitional cell carcinoma, carcinogens, polycyclic aromatic hydrocarbons, *Edwardsiella tarda*.

RÉSUMÉ

Épithélioma des cellules de l'épithélium de transition de la vessie, chez un béluga (*Delphinapterus leucas*)

Un épithélioma des cellules transitionnelles de la vessie est décrit chez un beluga de l'estuaire moyen du fleuve Saint- Laurent. Divers tissus de cet animal furent analysés par la chromatographie en phase gazeuse couplée avec la spectrométrie de masse.

Les auteurs signalent que la population humaine de cette région est affectée par un taux élevé de cancer de la vessie et rapportent la présence de produits cancérigènes dans l'environnement de ces animaux.

Edwardsiella tarda fut isolée de plusieurs organes de cet animal.

Mots clés: cétacés, vessie, épithélioma des cellules transitionnelles; carcinogènes, hydrocarbures polycycliques aromatiques, *Edwardsiella tarda*.

INTRODUCTION

Neoplasms have been rarely reported in cetaceans: only 36 have been described histologically (1). Of these, five were malignant and only one occurred in the urinary tract (a renal adenoma in an Atlantic bottle-nosed dolphin (*Tursiops truncatus* (2)). In the present paper, a carcinoma of the bladder found in a beluga whale, stranded in the St. Lawrence River middle estuary, is reported. This area, centered around the Saguenay river mouth is inhabited by a small indigenous population of this species composed of approximately 500 individuals (3).

The possible role of carcinogenic pollutants in the etiology of marine mammal neoplasms was discussed by Howard (1) and Landy (4). The presence of high levels of polycyclic aromatic hydrocarbons (PAH) which are strong carcinogenic compounds (5) has been reported in the marine environment of this beluga (6).

MATERIALS AND METHODS

A male beluga whale was found dead, stranded in the Matane area, Province of Quebec, approximately 420 km northeast of Quebec city, on the St. Lawrence south shore on November 30, 1983. It was 3.57 m long and its dorsal blubber was 8.5 cm thick. The presence of 33 concentric dentine layers indicated that it was a young adult animal (7). The carcass was examined at the College of Veterinary Medicine of Saint-Hyacinthe, 600 km southwestward.

Tissues from selected organs were fixed in buffered 10% formalin for histological examination. They were stained with a hematoxylin-phloxin-saffron stain and the lungs with Von Kossa, PAS, Verhoeff, Alcian blue, Mayer's Mucicarmin and Masson's Trichrome. Various tissues were cultured on blood agar, incubated at 37°C in a 10% CO_2 atmosphere and examined after 48 hours. Identification was done with an Analytical Profile Index diagnostic kit (Analytab products, Ayerst Laboratories, Plainview, New York).

Blubber, kidney, liver and lung samples were wrapped in aluminium paper previously rinsed with acetone, placed in plastic bags and kept on ice for two hours. Then, they were frozen at -20°C until analyzed.

Analyses were carried out to determine the presence of 2,2-bis (p-chlorophenyl) -1, 1-dichloroethylene (p. p' — DDE), polychlorinated biphenyls (PCB's) and benzo[a]pyrene (B[a]P). These tissues were homogenized in methanol and 10 mL KOH 10 N were added. The resulting mixture was saponified at 100°C for two hours and extracted three times with 75 mL of hexane. The organic phases

were combined and evaporated at 40°C under reduced pressure.

Extracts were then eluted on an activated alumina column (1 x 17 cm) at ambient temperature with hexane-dichloromethane (5:1 v/v) at a flow rate of 1 mL/min. Ten mL fractions were collected. The chromatographic retentions of the compounds of inter-est were determined using Aroclor 1254, p. p′ — DDE, benzo[a]pyrene (B[a]P) as reference compounds. The chlorinated compounds and B[a]P eluted in the first and the second fractions respectively.

The quantitative determinations were performed on a Perkin Elmer Sigma 2 gas chromatograph equipped with a 30 m DB-5 fused silica capillary column and a flame ionization detector. The identity and the degree of chlorination of the compounds were verified by gas chromatography-mass spectrometry analysis (GC/MS) on a Kratos MS-25 instrument.

Operating conditions for identification and quantitation of PCB's and DDE were as follows: for GC and GC/MS analysis, column temperature was programmed from 60°C at 10°C/min to 140°C and up to 315°C at 2°C/min. Masses 50 to 500 were scanned in the electron impact mode at 1 sec per decade.

The compounds were measured by digital integration of peak areas. The PCB's were estimated by comparing total area of the peaks with those of Aroclor 1254a and 1260 (Monsanto Co., St. Louis, Missouri). The DDE concentrations were determined by reference of p.p′ — DDE peak areas to a standard curve obtained by plotting the peak areas of p.p′ — DDE vs its concentration.

RESULTS

At postmortem examination multiple slit-like invaginations of epidermis, 1 to 5 cm long and 1 to 2 cm deep were found on the dorsolateral aspects of the body. Gross evidence of inflammation did not accompany these lesions.

The right ventricle of the heart was compressed by a large ovoid mass weighing 660 g. The lungs had numerous nodular foci, 1 to 8 mm long (Figure 1), throughout the parenchyma: these foci were white, with a shiny surface and a firm consistency. Many less well demarcated pale areas, with
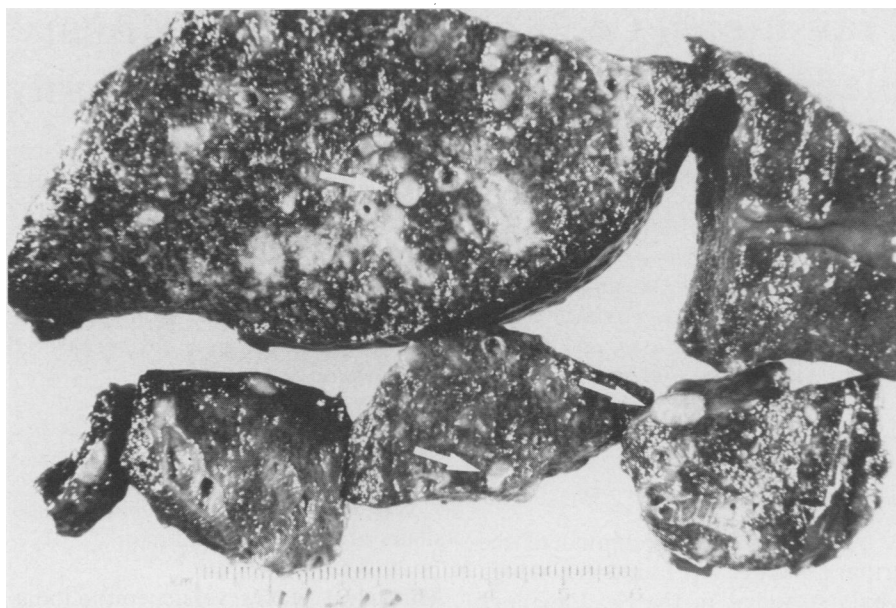

FIGURE 1. Lungs. White, shiny nodules are scattered throughout the parenchyma (arrows). The less well demarcated and pale areas represent fibrosis.

irregular shape and size were also scattered in the pulmonary tissue. Some nematodes about 3 cm long were found in the bronchioles.

Many white circular patches were seen on the aortic intima, at the level of the origin of the renal arteries. A mesenteric artery branch was nearly occluded by an intimal proliferation showing three firm yellow nodules upon incision. The gastric compartments were empty. A circular ulcer, 1.5 cm in diameter and 1 mm deep was observed in the second gastric compartment halfway between the orifices leading to the first and third compart-

FIGURE 2. Urinary bladder. An ulcer, surrounded by petechial hemorrhages is seen at apex (arrow).

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ments. The duodenal ampulla contained about fifty trematodes two to six centimeters long. An oval mass, 2 by 1 mm, composed of a thin capsule surrounding a white caseous center was observed on the serosal surface of the proximal intestine.

A circular ulcer, 2 mm in diameter with a hemorrhagic center and surrounded by petechial hemorrhages was observed at the apex of the urinary bladder on a slightly elevated mucosal patch (Figure 2). A 5 mm long nodule protruding from the left ureteral wall was located in the middle of a shallow, longitudinally oriented, dark red 2 cm long furrow.

About forty and ninety nematodes were found in the peribullar spaces of the left and right middle ears, respectively. The cranial end of the left zygomatic process of the temporal bone was partly replaced by an abundant yellow caseous material.

A dorsoventral radiograph of the head showed that the left supraorbital process of the frontal bone and the zygomatic process of the temporal bone were affected by a predominantly lytic reaction with multiple irregularly defined areas of increased bone density resulting in a granular appearance.

Microscopically, the skin invaginations consisted of crypts formed by the dermis and epidermis and evidence of inflammation was not observed. Autolysis prevented identification of the mass compressing the heart. Many foci of dense mature fibrous tissue were scattered in the lungs. The pulmonary nodules observed macroscopically were made of many conglomerates of cancerous cells. These cells had an acidophilic and finely granular cytoplasm with an irregular outline. Their large vesicular and pleomorphic nuclei had clumped and often marginated chromatin. They showed one or two prominent acidophilic nucleoli. A few multinucleated giant cells were also seen (Figure 4). Occasional mitotic figures were observed.

Most of the nodules were surrounded by a thick fibrous capsule. The largest nodules seemed to be concentrated in the subpleural space and their centers were often necrotic. Atelectasis and thickened interalveolar septa were seen in the surrounding tissue (Figure 3). Moderate lymphocytic infiltration was seen in the
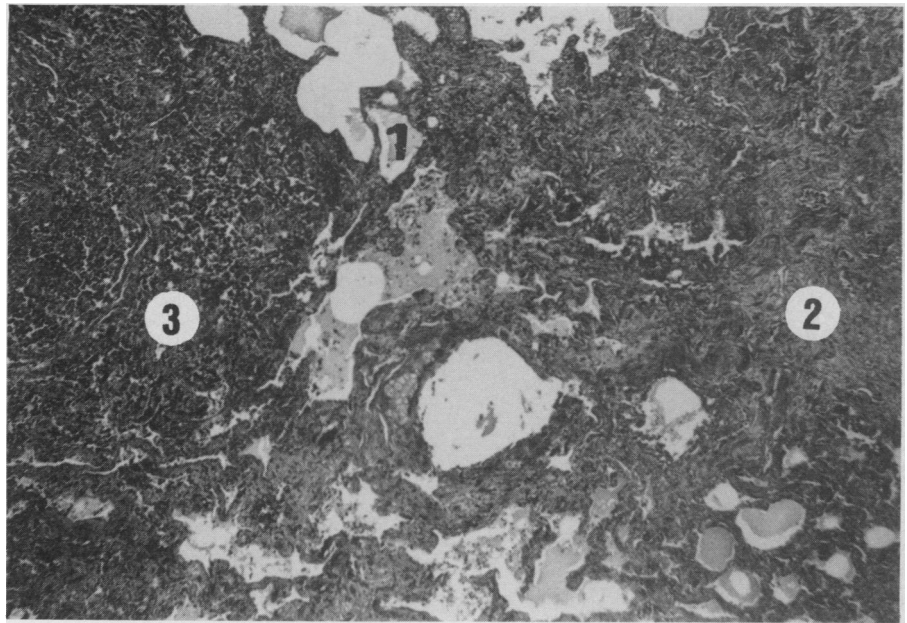

FIGURE 3. Lungs. Low magnification. A moderate quantity of acidophilic and homogeneous liquid is seen in the alveolar spaces (1). An area of dense mature and poorly vascularized fibrous tissue replaces the normal pulmonary architecture (2). A highly cellular metastasis is seen on the left (3). Masson's trichrome. X90.

nodules and in the pulmonary parenchyma.

The cancerous cells had invaded the walls of a few medium-sized vessels partly occluded by well organized thrombi containing the same pleomorphic cells (Figure 5). The presence of adult female nematodes filled with larvae, and identified as *Pharurus pallasii* (8), elicited mild focal mononuclear cell infiltrations. Intimal proliferative fibrosis was seen in many small arterioles and extended into the medium-sized pulmonary arteries
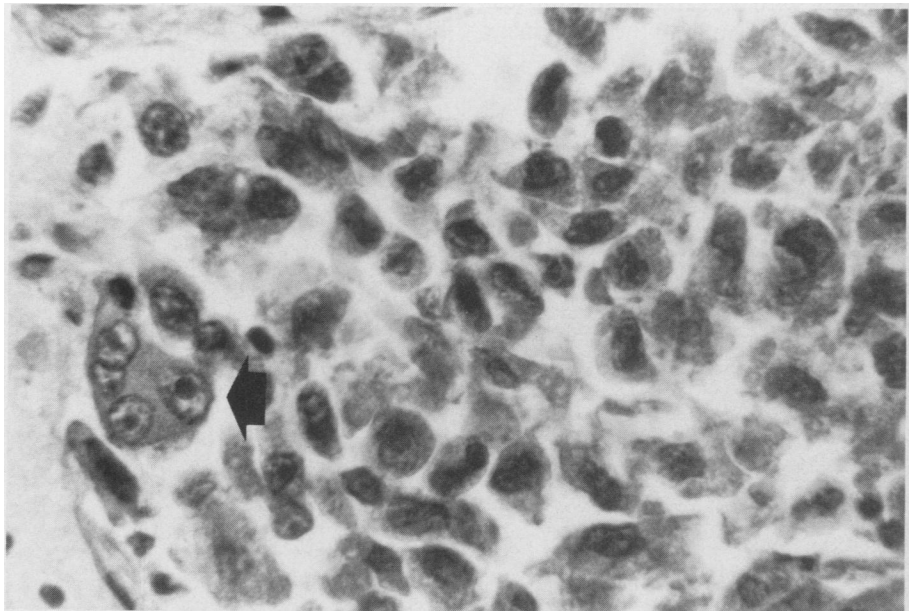

FIGURE 4. Lungs. Highly pleomorphic and poorly differentiated cells and giant multinucleated cells are seen in a metastatic focus (arrow). Note the prominent nucleoli in the giant cell nuclei. HPS. X900.
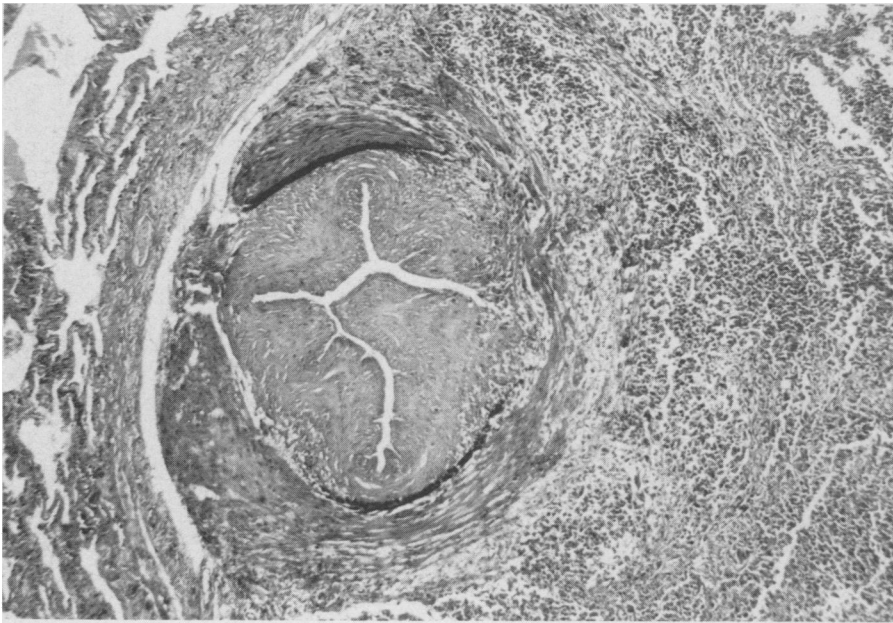
FIGURE 5. Lungs. Well organized and recanalized thrombus filling the lumen of a medium sized pulmonary artery. The wall is partly destroyed by a metastatic invasion. HPS. X90.

(200-500 μm in total diameter). The intimal proliferation was often papilliform.

The urinary bladder ulcer extended deep into the muscularis where it radiated smaller branches; these branches were surrounded by a thick capsule of dense connective tissue (Figure 6a). In the walls of the crypt and of its branches, there were nests of densely packed and highly pleomorphic cells with the same anaplastic features as those found in the pulmonary nodules (Figure 6b). In addition, many goblet-type cells with unique, irregularly sized vacuoles containing a few large mucicarmine positive granules were seen. Occasional mitotic figures were also observed in the cellular nests of the crypt.

The ureteral nodule was in the lamina propria and in the muscularis. It was sharply demarcated from the surrounding tissues and subdivided by a fine fibrous stroma supporting small blood vessels. The nodule was slightly infiltrated by lymphocytes and histiocytes and had essentially the same cellular composition as the pulmonary nodules (Figure 7). The subserosal nodule seen on the intestine had the same structure and composition as that of the ureteral one.

Mature, dense and well vascularized fibrous tissue composed the atheromatous plaque lying in the aortic intima. The underlying elastic lamellae were fragmented.

In the kidney, diffuse and slight thickening of the glomerular Bowman's capsule was seen. A few glomerular tufts were replaced or compressed by dense clumps of anaplastic cells (Figures 8a and 8b). Autolysis prevented interpretation of the mesenteric artery branch lesions and unfortunately, loss of the skull also prevented further histopathological diagnosis of the osseous lesions. The epithelium lining the peribullar space of the middle ear showed a dense mononuclear predominantly plasmocytic cellular infiltration.

The gastric ulcer consisted of an area of coagulation necrosis showing no evidence of inflammation.

Edwardsiella tarda was isolated in pure culture from the skull lesion and in large colonies from the spleen, mesenteric lymph node, urinary bladder and intestine. It was isolated in smaller colonies from kidneys, brain and gingiva.

Polychlorinated biphenyls and p.p′ — DDE were found in the tissues
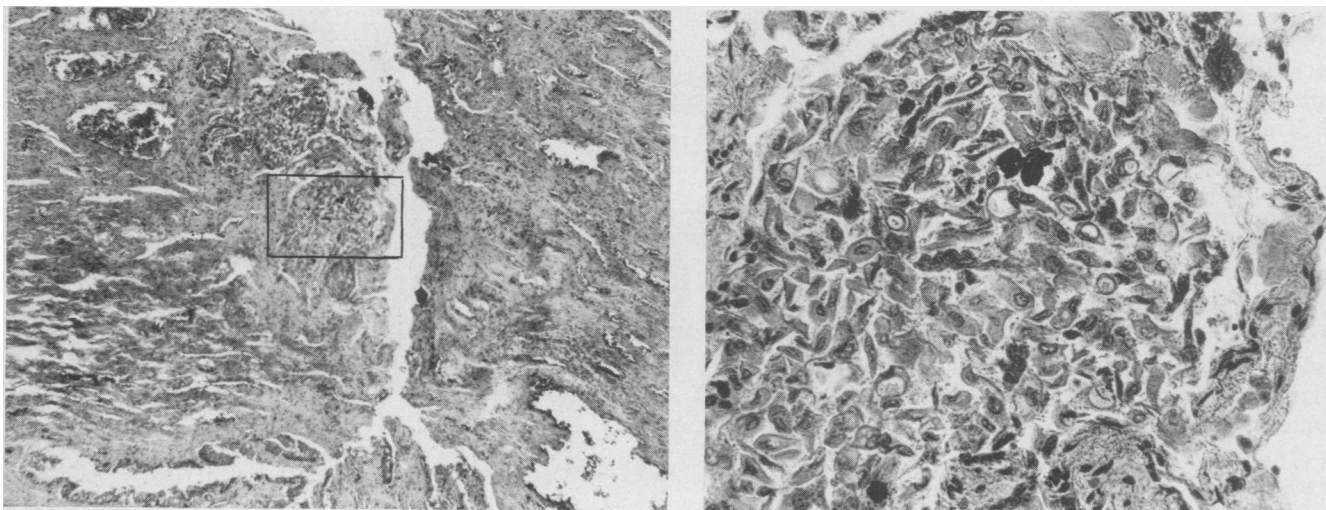

FIGURE 6. Bladder a) A crypt extends down into the muscularis. Solid nests of highly pleomorphic and poorly differentiated cells are lodged in the walls of the crypt. HPS. X90. b) Higher magnification of the framed area in a): solid nest of anaplastic cells. HPS. X571.

FIGURE 7. Ureteral metastasis. Note nuclear and cytoplasmic pleomorphism, prominent nucleoli and high nucleus:cytoplasm ratio. HPS. X900.

submitted to analysis. The results will be discussed in another paper. The research for B[a]P gave negative results.

DISCUSSION

Grossly this bladder tumor was non-papillary, infiltrating and had meta-stasized extensively. Histologically, it showed severe cellular anaplasia. According to Pamukcu's classification of urothelial tumors (9), this tumor was designated as a transitional cell carcinoma grade III. Tumors of the urinary bladder are rare in animals (9).

For instance, they account for less than 0.5% of dog tumors (10). How-ever they are frequent in cattle, in dif-ferent parts of the world, where they are attributed to the ingestion of bracken fern (Pteridium aquilinum). In the bovine species, which are phy-logenetically related to cetaceans (11), epithelial tumors are the most com-mon of all bladder tumors and transi-tional cell carcinomas occur most fre-quently. Infiltrating transitional cell carcinomas account for 21% of all primary carcinomas of the bladder in cattle (9).

An epidemiological study done by

Theriault et al (12) showed that the unusually high incidence of bladder neoplasm, seen in humans in the Chi-coutimi area, at the origin of the Saguenay river, was associated with employment in an aluminium reduc-tion plant. In a further study by the same workers (13) they concluded that exposure to polycyclic aromatic hydrocarbons estimated by the envi-ronmental benzo[a]pyrene concentra-tions seemed to be the causative factor.

On the other hand, Picard-Bérubé et al (6) found that blue oysters (Mytilus edulis L.) transplanted into the Sague-nay river from a nonpolluted area had a posttransplantation tissular B[a]P con-centration 200 times higher than their pretransplantation concentration.

Palmork et al (14) estimated that the European production of aluminium (3 millions tons a year) releases 150 tons of benzopyrene into the environment. Based on this estimation and on the annual production of aluminium of the Saguenay, Lac St-Jean area alumi-nium plants, Picard-Bérubé et al esti-mated the annual discharge of ben-zopyrene into this environment by these plants to be 20 tons (6). The total amount of anthropogenic PAH depos-ited in the sediments of the Saguenay river inner basin was estimated at 36 metric tons by Cossa (15, D. Cossa, personal communication, Institut francais pour l'exploitation de la mer, B.P. 1049, rue de l'Ile d'Yeu, 44037 Nantes Cedex, France). These figures were confirmed in a recent work (16) in which sediments of the Saguenay river were sampled at different depths and
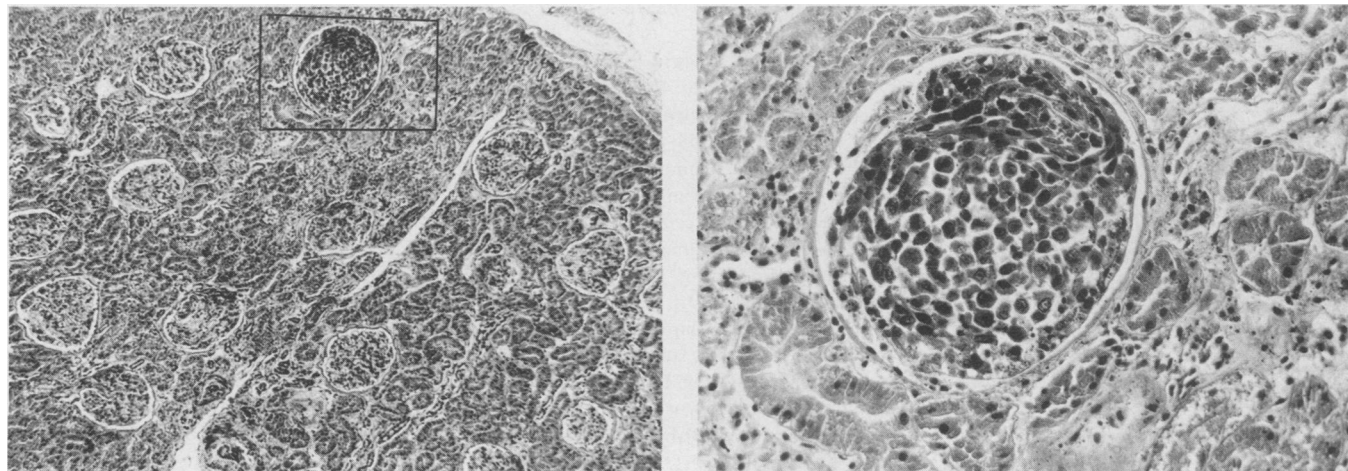

FIGURE 8. Kidney a) A glomerular tuft is replaced by metastatic cells. HPS. X143. b) Higher magnification of the affected glomerulus. HPS. X571.

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locations and analysed for PAH: they revealed an increasing gradient of PAH concentrations towards the plants and an approximative annual deposition of PAH since 1940 (year the aluminium production started) of 0.5 to 1.5 tons, depending on the year.

Belugas feed partly on bottom invertebrates (17) and dive comfortably down to 400 meters (18). Consequently, PAH contamination is inevitable. However no PAH were found in various tissues of this beluga whale by mass spectrometry analysis and the failure to detect B[a]P can be rationalized on the basis of metabolic considerations:PAH have the capacity to induce the activity of the microsomal monooxygenase enzymes, namely hydrocarbon hydroxylase (19). Polycyclic aromatic hydrocarbons are also extensively metabolized by bacteria, fungi and algae (20). Their metabolic patterns in marine organisms are very similar to those reported in terrestrial species (21). Polycyclic aromatic hydrocarbons are relatively inert compounds which are activated by microsomal enzymes to carcinogenic and very short lived metabolites (22). So their rapid transformation into unstable metabolites by activated (or nonactivated) enzymes and postmortem bacterial proliferation and consecutive bacterial metabolism could explain our failure to detect PAH in the tissues of this whale.

Benzo[a]pyrene exposure has been consistently associated with higher risk of cancer in humans based on epidemiological studies and environmental contamination (23). The very few studies which determined tissular concentrations (24) required sophisticated technology such as high pressure liquid chromatography equipped with fluorescence detector since the detected amounts were extremely low (11 to 32 parts per trillion). The technique we used (GC/MS) would not have detected such minute amounts.

We cannot establish any relation between the high frequency of urinary bladder carcinoma in the human population of that area and this isolated case: more cases and an accurate determination of tissular PAH metabolites will be needed to attempt any conclusion. Even with determination of PAH levels, no comparison of such levels will be possible since B[a]P concentrations in humans of that area have not been determined.

Edwardsiella tarda causes enteritis in aquatic birds and hemorrhagic disease in fishes (25). It has been isolated also in healthy (26) and diseased (27) marine mammals where it was considered as an opportunistic invader. We believe this was the case in the present animal judging by the presence of a highly malignant transitional cell carcinoma.

ACKNOWLEDGMENTS

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