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METASTATIC CARCINOMA OF PROBABLE TRANSITIONAL CELL ORIGIN IN 66 FREE-LIVING CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*), 1979 TO 1994

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ABSTRACT: Sixty-six (18%) cases of widely metastatic carcinoma of probable transitional cell origin were identified in 370 California sea lions (*Zalophus californianus*) stranded alive along the central California (USA) coast, between January 1979 and December 1994. Live animals were usually emaciated and anorectic, with perineal edema and occasionally hind-flipper paralysis or paresis. Large yellow caseous masses were observed in the sub-lumbar lymph nodes, often extending around the ureters resulting in hydroureter. Histologically, metastases were usually widespread, and the primary neoplastic focus undetectable. This is the highest reported prevalence among necropsied animals of neoplasia in a pinniped population to date.

Key words: Transitional cell carcinoma, neoplasia, California sea lion, *Zalophus californianus*, California, free-ranging, pinniped.

INTRODUCTION

Reports of neoplasia in marine mammals are uncommon, but are increasing in number. Earlier documentation of neoplasia consisted of scattered individual case reports, and tumors in pinnipeds were considered rare (Ridgeway, 1972; Sweeney, 1974; Landy, 1980). In 1974, Mawdesley-Thomas (1974) stated only seven reports of neoplasia in pinnipeds were known to exist. However, by 1983, in a survey of 1500 marine mammals, 2.5% were found to have neoplastic lesions (Howard et al., 1983). In 1994, an even higher prevalence of 50% was reported in beluga whales (*Delphinapterus leucas*) in the St. Lawrence River (eastern Canada), with some animals having multiple tumors (DeGuise et al., 1994). This increased reporting may partly be explained by the increased number of animals being examined by experienced veterinary pathologists, but may also reflect an increased tumor prevalence. Although the etiology of neoplasia in marine mammals in unknown, there may be a real increase in tumor prevalence in conjunction with increased chemical pollution of the marine environment (Howard et al., 1983). Among reported tumors, transitional cell carcinoma has been noted in California sea lions (*Zalophus californianus*) and beluga whales (Landy, 1980; Howard et al., 1983; Martineau et al., 1985; Gerber et al., 1993). These carcinomas in beluga whales may be related to environmental contaminants, particularly polycyclic aromatic hydrocarbons such as benzo[A]pyrene (Martineau et al., 1987).

In laboratory rodents, experimental exposure to such chemicals as nitrosourea compounds can induce transitional cell carcinoma of the urinary tract (Alden and Frith, 1990). In humans, transitional cell carcinomas have been associated with cigarette smoking, and employment in rubber, paint, dye, aluminum, or tar industries (Cohen and Johansson, 1992). These risk factors can be identified only by the investigation of large numbers of cases. Such large sample sizes are rarely available for marine mammal populations. We describe the epizootiology and pathology of 66 cases of carcinoma of probable transitional cell origin in California sea lions.

The California sea lion population was estimated at 145,000 individuals in 1983 (Le Boeuf et al., 1983), and since then the

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populations in California (USA) have been increasing (Reijnders et al., 1993). Animals breed off the coasts of southern and Baja California, migrating north after the breeding season to feed off central and northern California, Oregon, and Washington (USA) (Riedman, 1990). They mature sexually between four and six years of age, when they typically weigh 60 to 70 kg, and can live 15 to 24 yr (Reijnders et al., 1993). In this paper, we describe carcinoma in sea lions stranding in central California, just north of their breeding range.

MATERIALS AND METHODS

Three hundred and seventy sub-adult and adult California sea lions were necropsied at The Marine Mammal Center, Sausalito, California between 1 January 1979 and 31 December 1994. All animals died during rehabilitation following live stranding along the central California coast. Animals were considered stranded according to the criteria of Gerber et al. (1993). Sex and weight were recorded for each animal. Observation of external characteristics (size, tooth development and wear, coat color, crest development) was used to identify animals as sub-adult and adult (Mate, 1978). Nineteen animals were aged by counting annual growth layers of dentin in sagittal sections of the decalcified upper left canine tooth (Payne, 1978). Following post-mortem examination, representative samples of all tissues were fixed by immersion in 10% neutral buffered formalin. Tissues collected included brain, thyroid, lung, heart, aorta, liver, pancreas, spleen, stomach, intestine, colon, kidney, adrenal, gonad and reproductive tract, ureter, urinary bladder, urethra, skeletal muscle, lumbar spine, multiple lymph nodes, and omentum. Fixed tissues were embedded in TissuePrep (Fischer Scientific, Fairlawn, New Jersey, USA), sectioned at 5 μm, and stained with hematoxylin and eosin. Selected tissue sections were stained with Gomori's Methenamine Silver technique for spirochetes, Periodic Acid-Schiff for fungi, Masson's Trichrome technique for collagen, Brown and Brenn technique for bacteria, and Congo Red for amyloid (Luna, 1968). In selected cases, immunohistochemical staining was performed using the Streptavidin-Biotin Complex staining technique (Cartun and Pedersen, 1988). Representative sections of neoplastic lesions in these cases were stained for desmin, smooth muscle actin, vimentin, myelin basic protein, S100 protein and triplet neurofilament, to identify non-epithelial tumor types (Luna, 1968). Although previously diagnosed by various pathologists, all available histologic slides were reviewed for this publication by one pathologist. Representative slides were deposited at the Registry of Comparative Pathology, Armed Forces Institute of Pathology, Washington D.C., 20306 (accession number 1833409).

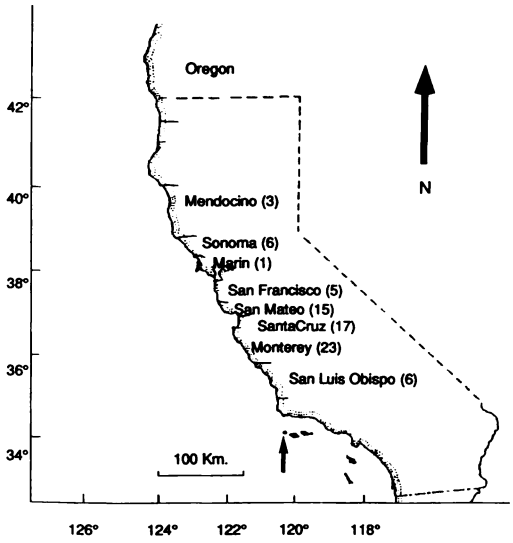


FIGURE 1. Stranding sites in central California of 76 California sea lions with gross lesions suggestive of disseminated carcinoma. San Miguel Island is indicated by the arrow. Numbers stranded at each site in parentheses.

RESULTS

Seventy-six (21%) of the 370 sub-adult and adult sea lions examined between 1 January 1979 and 31 December 1994 had similar clinical signs and gross lesions at post-mortem examination. On histologic examination, 66 (18%) had widely metastatic carcinoma of probable transitional cell origin. Two animals had spindle cell sarcomas, one an adenocarcinoma, one a renal fibroma, and one an islet cell adenoma and adrenocortical adenoma in addition to the disseminated carcinoma. Tissues were unavailable for histologic examination in five cases.

The distribution of stranding sites for these 76 animals was similar to the overall distribution of stranded sea lions transported to The Marine Mammal Center between 1979 and 1994 (Fig. 1). Most ani-

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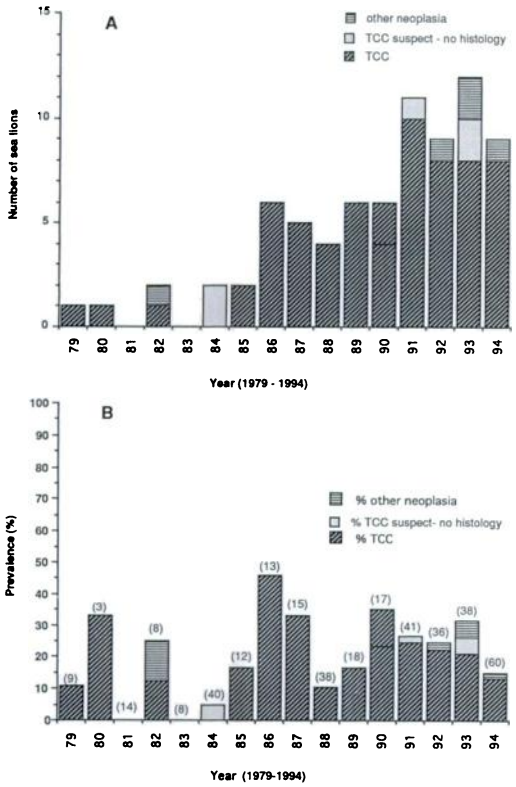


FIGURE 2. Number (a) and prevalence (b) of California sea lions with disseminated carcinoma of probable transitional cell origin (TCC), by year, 1979 to 1994. Number of sea lions examined each year in parentheses.

mals stranded in the area of Monterey, California (36°45′, 121°30′). The number of cases of disseminated carcinoma increased between 1979 and 1994, but the prevalence of this tumor (as percentage of necropsied stranded animals) did not increase with time (Fig. 2). There was no apparent seasonal pattern to stranding of disseminated carcinoma cases, and affected animals stranded in all months of the year. Thirty-two (42%) animals were female and 44 (58%) were male. All affected animals were greater than 50 kg in body weight, and were visually assessed as subadult or older. The 19 animals aged by dentin growth layers were all sexually mature young adults between 6 and 14 yr old. Two animals were 6 yr old, one was 7 yr old, two were 8 yr old, four were 9 yr old, two were 10 yr old, three were 11 yr old, two were 12 yr old, two were 13 yr old, and one was 14 yr old.

Animals with carcinoma were characterized clinically by emaciation, anorexia, and some degree of paresis or paralysis of the hind flippers. There often was abdominal distension, swelling of the hind flippers, and perineal edema, the latter associated with elevation of the tail (Fig. 3). Occasionally this was accompanied by rectal prolapse. In two cases, prolapse and necrosis of the penis occurred. In two other cases draining fistulae over the sacral region were observed.

On post-mortem examination, extensive caseous masses, 3.0 to 25.0 cm. diameter and weighing up to 2.8 kg., were present in the pelvic area (Table 1). These typically involved the sublumbar lymph nodes, extending around the ureters, and occasionally involved the adrenal glands, kidneys, and bladder (Fig. 4). Most cases had several liters of opaque yellow fluid in the peritoneal cavity. In two cases with draining fistulae, the sublumbar masses extended into the dorsal musculature, pelvis, sacrum, and lumbar vertebrae (Fig. 5). The urinary bladder usually was distended with urine, and hydroureter or hydronephrosis frequently were present. Less frequently, similar masses were present in the kidneys (Fig. 6), bladder, and reproductive tract. When present in the uterine wall, lesions usually were accompanied by severe pyometra. Multifocal necrotic yellow nodules, 0.5 to 2.0 cm. diameter typical of metastases usually were present throughout the lungs, liver, spleen, mediastinum, thoracic and abdominal lymph nodes, and omentum (Fig. 7). Nodules were less frequently seen in the adrenal glands, pancreas, pericardium, and myocardium.

In most cases, the neoplastic cells were arranged in small nests and sheets, and frequently were found within small vessels and lymphatic channels. Cells had abundant, pale eosinophilic to amphophilic cytoplasm, and markedly pleomorphic, polygonal nuclei. Nuclei varied from 8 to 15

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FIGURE 3. Perineal edema in a California sea lion associated with disseminated carcinoma. Bar = 10 cm.

μm diameter, and had a clumped to reticular chromatin pattern. Mitoses generally were infrequent; however, some tumors contained between one and three mitoses per 400 × field (Fig. 8). Several tumors had prominent squamous differentiation within the neoplastic foci, and occasionally formed cyst-like structures, filled with necrotic or keratinized debris; two tumors appeared to form acinar or duct-like structures, suggestive of adenomatous differentiation. Immunohistochemical studies using a panel of cytokine stains confirmed the neoplastic cells as epithelial in origin.

TABLE 1. Post mortem findings in 76 California sea lions (*Zalophus californianus*) with lesions typical of widely disseminated carcinoma, Marine Mammal Center, California, 1979 to 1994.

Lesion	Carcinoma (n=66)	Other neoplasia (n=5)	No histology (n=5)
Caseous mass in sub-lumbar lymph node	66 (100%)	1 (20%)	5 (100%)
Perineal edema	44 (66%)	1 (20%)	4 (80%)
Masses in lungs	41 (62%)	3 (60%)	3 (60%)
Masses over omentum	39 (60%)	1 (20%)	1 (20%)
Hydroureter and hydronephrosis	38 (58%)	1 (20%)	2 (40%)
Masses in liver	37 (56%)	2 (40%)	1 (20%)
Masses in kidney	32 (48%)	2 (40%)	2 (40%)
Masses in mediastinum	31 (46%)	2 (40%)	0
Abdominal fluid	29 (44%)	2 (40%)	5 (100%)
Nodules in multiple lymph nodes	23 (32%)	2 (40%)	1 (20%)
Masses in urinary bladder	20 (29%)	0	1 (20%)
Masses in reproductive tract	16 (25%)	0	2 (40%)

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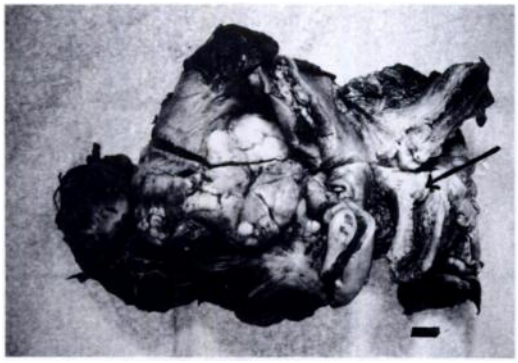


FIGURE 4. Large carcinoma, engulfing urinary and reproductive tracts of a California sea lion. The arrow points to the urinary bladder. Bar = 10 cm.

In three cases, nests of neoplastic transitional cells were found in close proximity to the mucosal surface; however clear histologic evidence of neoplastic transformation of the urothelium was not seen.

A single primary neoplastic focus was not clearly identified in any case. Metastases were often more widespread than recognized grossly, and were most frequently identified within lymph nodes, particularly sublumbar and iliac nodes (61 cases). Metastatic foci of tumor cells also were found within the lung (28 cases), omentum and mesentery (19 cases), liver (18 cases), kidney (14 cases), and spleen (11 cases). Infrequent locations of metastasis were uterus, muscle, adrenal gland,



FIGURE 6. Section of California sea lion kidney infiltrated by disseminated carcinoma, with some hydronephrosis.



FIGURE 5. Radiograph of the pelvic region of a California sea lion showing bone lysis and new bone formation associated with disseminated carcinoma. Bar = 10 cm.

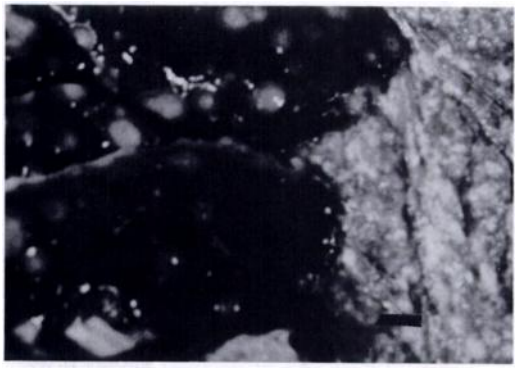


FIGURE 7. Widely disseminated carcinoma in the liver of a California sea lion. Bar = 5 cm.

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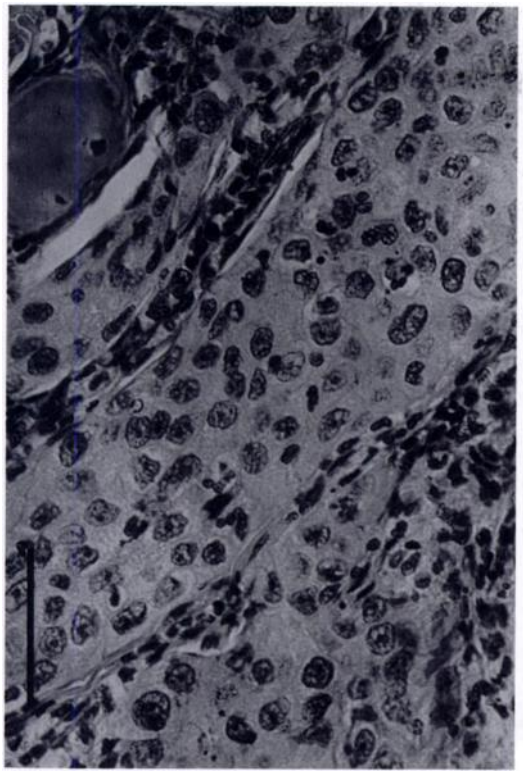


FIGURE 8. Photomicrograph of metastatic carcinoma of probable transitional cell origin, in the lung of a California sea lion. H & E. Bar = 100 μm.

ovary, testicle, heart, spinal cord, skin, and pancreas. In one case, no mass was found on post-mortem examination, but nests of neoplastic cells were found incidentally on histologic examination of a lymph node. Many tumor metastases, particularly those within lymph nodes, had necrotic centers filled with granular, eosinophilic cell debris and degenerate neutrophils.

DISCUSSION

This carcinoma was considered to be of probable transitional cell origin based on typical microscopic morphology of invasive nests and cords of pleomorphic epithelial cells, bearing strong resemblance to transitional cells (Nielsen and Moulton, 1990). In dogs and cattle, foci of necrosis, or squamous or glandular differentiation frequently occur within these tumors, as was seen in most cases (Nielsen and Moulton, 1990).

No attempt was made to grade these tumors, as the primary neoplastic focus was never identified. The inability to identify the primary neoplastic focus may be due to the advanced stage of neoplastic invasion at the time of stranding and diagnosis. Transitional cell carcinoma of the urinary bladder in dogs is often widely metastatic, and may arise from a primary tumor that is smaller than the metastatic foci at the time of diagnosis (Nielsen and Moulton, 1990). Small primary neoplasms in the bladder mucosa or ureters may have been overlooked on the initial post-mortem examination or during histopathology sample selection. In dogs and humans, epithelial hyperplasia and infiltration of epithelial cells into the submucosa without disruption of the basement membrane (carcinoma in situ) are considered to be pre-neoplastic changes (Nielsen and Moulton, 1990). In three cases described here, small nests of neoplasic cells observed below the urothelium may represent early invasion sites or carcinoma in situ. Based on the morphology of this tumor, we propose that it is a primary tumor of the urinary tract. The high proportion of cases with metastases in the sublumbar lymph nodes also is evidence of a primary origin in the pelvis or perineal tissues.

The number of carcinoma cases reported here is unusually high for a free-living species, and to date is the highest reported for a pinniped population. Although the stranded animals examined only represent a small fraction of the total wild population, we believe that this tumor is common in free-ranging California sea lions. The true prevalence of this carcinoma is difficult to obtain, as most animals that die are not examined by pathologists. The number of cases observed in the early years of this study (1979 to 1983) may be lower than the true prevalence, due to lack of detailed pathological examination of all animals during that time period. Lower prevalence (as percentage of animals examined at post

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mortem) in the years 1984, 1988 and 1994 may be related to higher mortality of sea lions due to *Leptospira* sp. infection during these years (Gerber et al., 1993).

The distribution of cases reported here reflects the distribution of stranded animals transported to The Marine Mammal Center for rehabilitation. The prevalence of the disease in other areas is unknown. A case of disseminated carcinoma in an adult female California sea lion was diagnosed by one of the authors (TRS) on San Miguel Island (Fig. 1). Therefore, the disease is not limited in distribution.

The high number of disseminated carcinoma cases reported here raises questions about the etiology of this tumor. The cause of urogenital tumors in other species is often undetermined. However, several chemical carcinogens have been incriminated in the pathogenesis of bladder tumors in rats, dogs, cattle and humans (Alden and Frith, 1990). Vesicular neoplasia has been experimentally induced in the rat and dog following administration of a number of chemicals, including aromatic hydrocarbons and nitrosamines (Alden and Frith, 1990). In humans, epidemiological studies have linked urogenital neoplasia with exposure to aluminum, chromium, insecticides and products of the aniline dye, paint, and petroleum industries (Risch et al., 1988). Transitional cell carcinoma has been reported in a beluga whale from a population of animals with a high prevalence of neoplasia and high tissue levels of organochlorines (Martineau et al., 1987; De Guise et al., 1994). De Guise et al. (1994) suggested that chemical pollutants may play a role in carcinogenesis in this species, either by directly affecting cell transformation, or indirectly by decreasing immune surveillance. Incidence of neoplasia in fish is associated with chemical contamination of the environment (Myers et al., 1991). Piscivorous species, such as sea lions, may further concentrate environmental contaminants in their tissues (Holden, 1978), and thus are potentially exposed to compounds carcinogenic to lower vertebrates.

Chemicals also may induce neoplasia by acting synergistically with viruses. Bladder carcinoma in cattle is associated with ingestion of bracken fern (*Pteridium aquilinum*) coupled with infection by a papilloma virus (Maxie, 1993). Viruses may also induce neoplasia alone. Human papilloma viruses may transform human cells in vitro, and have been identified in many cases of human transitional cell carcinoma, although their role in transitional cell carcinoma remains controversial (Anwar et al., 1992; Yu et al., 1993). Viral particles have been previously detected in a northern fur seal (*Callorhinus ursinus*) with lymphoma (Stedham et al., 1977) and in sperm whales (*Physeter catodon*) with genital papillomatosis (Lambertson et al., 1987). Herpes-like viral particles have been identified by electron microscopy in a hyperplastic plaque on the penis in one California sea lion examined by the authors (L. J. Lowenstine, unpubl.).

Other infections resulting in chronic inflammation of the urinary bladder have also been associated with urogenital cancer. In Egypt, there is a high prevalence of bladder cancer in humans associated with *Schistosoma hematobium* infection (Cohen and Johansson, 1992). Other factors that could possibly be involved in the pathogenesis of this carcinoma are genetic. Transitional cell carcinoma can be familial in humans (Greenland et al., 1993), although no individual chromosomal abnormality is consistently found in bladder cancer (Kroft and Oyasu, 1994).

Thus, based on analogy with urinary tract carcinoma in other species, several different factors could be involved in the pathogenesis of this carcinoma in California sea lions. The importance of chemical carcinogens in other species is evidence that chemical contaminants may play an important role in the etiology of this carcinoma in California sea lions. The large number of cases of neoplasia reported in this paper highlights the need to explore

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the relationship between chemical contamination of the ocean and neoplasia in marine mammals. Further studies are needed to investigate the etiology and ultrastructure of this relatively common cancer in California sea lions, and to confirm its origin in transitional uroepithelium. Qualitative and quantitative toxicologic studies are needed to determine the nature and amount of chemical contaminants these sea lions are being exposed to.

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