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PREVALENCE OF IDIOPATHIC LIVER LESIONS IN ENGLISH SOLE AND EPIDERMAL ABNORMALITIES IN FLATFISH FROM VANCOUVER HARBOUR, BRITISH COLUMBIA, 1986

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By

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REVIEW NOTICE

This report documents part of a larger study being conducted in Vancouver Harbour by Environmental Protection. Field collections and observations were undertaken by Environmental Protection personnel; histological analysis by D. Brand (1987), University of Victoria (DSS Contract KE603-6-0848). Any inquiries regarding this report should be directed to:

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ABSTRACT

Histopathological conditions in a number of demersal fish species have been previously linked (by association) with exposure to xenobiotic chemicals found in sediments and the water column. Since Vancouver Harbour receives a variety of urban and industrial waste discharges which potentially can contain toxic and carcinogenic chemicals, English sole (Parophrys vetulus) were collected from seven locations within the harbour in 1986 and examined for liver tissue abnormalities. High prevalences (58.8%) of idiopathic liver lesions were found in English sole from Port Moody Arm, an area receiving petroleum refinery waste water and other pollutants; moderate frequencies (20.0 to 30.0%) along the shoreline of the main harbour and lower frequencies (8.3%-13.3%) in the central and outer portions. The type and frequency of idiopathic liver lesions in English sole can provide useful measurements on the effects of anthropogenic chemical exposure in Vancouver Harbour, and comparison with other coastal harbours. In addition, over 10,000 flatfish representing sixteen species were examined for epidermal abnormalities from May 1985 to September 1986.

RESUME

On a déjà associé l'état histopathologique chez un bon nombre d'espèces démersales de poissons à l'exposition à des produits chimiques xénobiotiques contenus dans les sédiments et dans l'eau. Comme différents effluents industriels et urbains qui sont peut-être toxiques et cancérigènes, sont déversés dans le port de Vancouver, des soles anglaises (Parophrys vetulus) ont été prélevées en 1986 en sept endroits dans le port afin de voir si elles avaient des anomalies hépatiques. Il y avait prévalence accentuée (58.8%) de lésions hépatiques idiopathiques dans les soles du bras de Port Moody, un secteur où sont déversées des eaux usées d'une raffinerie de pétrole et d'autres polluants. Par comparaison, on a observé chez la sole des lésions en fréquence modérée (20.0 à 30.0%) près du rivage dans la partie principale du port et en faible fréquence (8.3% - 13.3%) dans la partie centrale et la partie du côté du large. Le type et la fréquence des lésions hépatiques idiopathiques chez la Sole anglaise constituent une mesure utile des effets de l'exposition à des produits chimiques anthropiques; la comparaison avec d'autres ports de la côte se révèle également utile. En outre, plus de 10,000 poissons plats de 16 espèces ont été examinés pour voir s'ils avaient des anomalies au niveau de l'épiderme, entre Mai 1985 et Septembre 1986.

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SUMMARY

1. Idiopathic (non-parasitic, non-infectious) liver lesions were found in medium (mean = 27 cm) to large (mean = 33 cm) sized English sole from all sampling locations in Vancouver Harbour.

2. The types of idiopathic lesions were: nonspecific necrosis, megalocytic hepatosis, hepatocellular steatosis, foci of cellular alteration (clear cell foci, eosinophilic foci, basophilic foci) and liver cell adenoma.

3. From a total of 66 liver samples, the overall prevalence of idiopathic liver lesions in English Sole from Vancouver Harbour was 30.3%, with 20% of the livers affected by preneoplastic lesions and 9% by neoplastic lesions.

4. The prevalence of one or more types of idiopathic lesions varied according to sampling location: Port Moody Arm (Ioco) showed the highest prevalence at 58.8%; north and south shore of the inner harbour (Burrard Yarrows and Coal Harbour) with moderate prevalences of 30% and 20%, respectively; and the outer harbour (Pacific Environment Institute) and centre of the inner harbour (Centre Channel) with the lowest percentages of 13.3% and 8.3%, respectively.

5. Most types of idiopathic liver lesions found in flatfish from Vancouver Harbour have been linked with exposure to sediment-associated contaminants.

6. Chemical studies of the bottom sediments in Vancouver Harbour conducted concurrently with the liver histopathology, have shown elevated levels of petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and various trace metals. Studies in Puget Sound and elsewhere have established a positive correlation between PAHs and prevalence of idiopathic liver lesions in English sole.

7. Prevalence of idiopathic liver lesions in English sole from Port Moody Arm, an area with poor water circulation and elevated organic chemical and trace metal levels, was significantly greater ($p<0.05$) than other sampling locations in the harbour.

8. Frequency of epidermal papillomas observed in English sole and rex sole from Vancouver Harbour was relatively low (less than 5%). Statistical correlations among sample sites were not attempted due to limited sample size and uncertainties with cause/effect relationships to chemical exposure and environmental conditions.

9. Further studies into the prevalance of idiopathic liver lesions in fish populations within Vancouver Harbour, fish behavioural patterns and exposure to chemical contaminants are warranted. Studies should be coupled with positive measures to reduce sources of chemical input to the harbour.

1 INTRODUCTION

Marine environments near industrial and urban centres are exposed to a wide spectrum of chemicals, some of which have carcinogenic potential. Chemicals can originate from a variety of point and non-point sources such as spills, dumping operations, urban runoff, municipal and industrial waste discharges. These may consist of materials such as petroleum hydrocarbons or by-products, polychlorinated biphenyls (PCBs) and other chlorinated compounds, certain pesticides and metals (Malins et al., 1984). Some of these compounds are subject to chemical and biological transformation in the environment potentially resulting in more toxic compounds (Malins et al., 1981). For example, polycyclic aromatic hydrocarbons (PAHs) originate from the pyrolysis of organic materials and it is the subsequent transformation of the parent compounds to intermediates (metabolites) in the host liver which produces a carcinogenic effect (Neff, 1979).

Research has shown a positive link (by association) between histopathological conditions in demersal fish and xenobiotic chemicals in sediments and seawater (Myers et al., 1987). High tumor prevalences have been identified in wild fish from areas receiving industrial and domestic effluents. Significant relationships have also been identified between sediment-associated PAHs and prevalence of neoplasms and other idiopathic (non-parasitic, non-infectious) liver lesions in English sole (Parophrys vetulus).

While the identification of carcinogenic compounds in the environment implies a certain carcinogenic risk, tumor induction in all exposed resident fish may not necessarily follow. Antioxidants such as selenium, can be effective inhibitors of PAH-induced tumor development. Some PAH compounds may inhibit tumor development, others may be highly carcinogenic, co-carcinogenic, or promoters of carcinogenesis (EPA, 1980).

Considering the complex biological and chemical interactions in the environment, in addition to the cost and complexity of PAH analysis, demonstration of a definitive biological response that is consistant with a response to pollutant exposure is a more efficient measure of ecological impact than chemical analysis alone. One means of determining the factors

which potentially lead to carcinogenic or toxic effects, is to investigate sensitive marine organisms for certain pathological conditions found either exclusively or at statistically higher prevalences in organisms resident in polluted areas. With the association between certain histopathological conditions in some demersal fish species and exposure to xenobiotic chemicals, monitoring for neoplasia and related cell disorders in fish has obvious merit.

Earlier studies have shown high prevalence of epidermal lesions in English sole from urban areas (Stich et al., 1977). In the Vancouver area (north arm jetty) prevelance was observed as high as 56.8%. Despite an apparent relationship between pollutants in urban areas and epidermal lesions the mechanisms involved are not well established (Mix, 1986). Studies conducted over a wide geographical area have shown no consistent distributional pattern for flatfish with different types of skin lesions and varying levels of contamination (Mearns and Sherwood, 1977). Biological agents, such as viruses and protozoans, have also been implicated in the development of skin tumors (Patton and Couch, 1984). Fin erosion shows a consistent pattern of increased prevalence in areas contiguous to urban development (Murchelano and Wolke, 1985). However, fin erosion occurs primarily in young fish resulting in a shortened life expectancy, and thus is not an effective monitor of the effects of prolonged chemical exposure.

Liver lesions, on the other hand, have been shown to occur in bottom dwelling fish from highly contaminated areas (Malins et al., 1984). These occur in older fish exhibiting the effects of exposure to a broad range of pollutants over a long period of time. Although general observations of the external appearance (skin lesions, colouration, parasites) of all flatfish species were recorded for fish between May 1985 and September 1986, the primary emphasis in this study was on liver lesions because of the uncertainties in relating skin lesions to chemical pollutants.

English sole are relatively ubiquitous along the Pacific northwest coast and are particularly sensitive to the development of idiopathic liver lesions, including neoplasms (Myers et al., 1987). Popham (1984) in an earlier pilot study had shown the presence of liver lesions in English sole from Burrard Inlet. English sole was therefore the primary target species for this study. The types and prevalences of histologically detectable

pathologic conditions with emphasis on idiopathic liver lesions, will be described for the following areas within Vancouver Harbour:

a) Outer harbour (Pacific Environment Institute) which is under the influence of outflows from Vancouver Harbour, the Fraser River, and Howe Sound,
b) four areas within the inner harbour (Burrard Yarrows, Coal Harbour, Sterling, and Centre Channel) and,
c) two areas in Port Moody Arm (Port Moody and Ioco) which has a long history of petroleum refining operations, bulk loading facilities and light industry.

The current study is part of a larger study being conducted by Environmental Protection to determine benthic environmental quality in Vancouver Harbour. Concurrent with the histopathological studies, benthic sediment and tissue samples have been taken for trace metal and organic contaminants analysis. These will be the subject of future reports. Relative abundances and species distribution patterns found during the study are reported by Goyette and Thomas (1987).

2 STUDY AREA

The study area extends from West Vancouver to Port Moody Arm (Figure 1). Hydrographically, the area can be divided into four regions: the outer harbour between Point Atkinson and First Narrows, the inner harbour between First and Second Narrows, the central portion between Second Narrows and the entrances to Indian Arm and Port Moody Arm, and the eastern portion formed by Indian Arm and Port Moody Arm. Names given to sampling sites are arbitrary hydrographic chart names given by the researchers and do not necessarily reflect the principal source of chemical pollution.

Tides in Vancouver Harbour range from -0.2 to 5.6 metres with an average of 3.3 metres (Canadian Hydrographic Service). Current velocities through First and Second Narrows can exceed six knots during ebb and flood tides. With the exception of Port Moody Arm, strong tides and twice daily tidal exchanges result in a fairly high degree of mixing throughout much of the harbour.

2.1 Outer Harbour

The shoreline of the outer harbour is largely a mixture of residential areas and parkland. The Lions Gate Sewage Treatment Plant located near First Narrows, is the largest point source discharge into the outer harbour. The outer harbour, under certain flow conditions, can be affected by water from the Fraser River and tidal outflow from the inner harbour and Howe Sound. One sampling site (Pacific Environment Institute) was chosen to represent the outer harbour.

2.2 Inner Harbour

The inner harbour is the most active region, with both shores having a variety of industrial and urban activities. Bulk loading terminals, shipping docks, shipyards, sawmills, light industry, numerous marinas and a variety of combined and sanitary municipal sewer overflows and stormwater discharges are located within the inner harbour. Four locations (Burrard Yarrows, Coal Harbour, Centre Channel and Sterling) were selected in the inner harbour.

2.3 Central Harbour

The central portion is a mixture of industrial and residential areas. The majority of the area is affected by the strong tidal currents from Second Narrows. Industrial activities include a sodium chlorate plant and chlor-alkali plant near Second Narrows, tank farms, two petroleum refineries and shipyards. Process effluent from both petroleum refineries in the central portion discharge to the regional sewer system; stormwater from the tank farms and refinery areas is treated and discharged to the harbour.

2.4 Port Moody Arm

In the eastern portion, the survey was confined to Port Moody Arm. Indian Arm is largely undeveloped and primarily used for recreation. Industrial activities in Port Moody Arm consist of two petroleum refineries, (one of which discharges both treated process effluent and stormwater into Burrard Inlet, the other only stormwater), a thermal generating plant, bulk loading terminal, several chemical plants (phenolic resins, alum), a sawmill and several marinas. Two locations were sampled in Port Moody Arm (Ioco and Port Moody) to represent the eastern section of the harbour.

3 MATERIALS AND METHODS

3.1 Field Collection

Flatfish were collected from each site in Vancouver Harbour (Figure 1) aboard the survey vessel C.S.S. Vector using a small otter trawl with a 5.8 metre throat and a 3.8 cm mesh net. Trawls were towed with a 2.5:1 scope (wire length vs depth) at a speed of approximately 1.5 knots for a distance of 0.9 km (or 0.5 nautical miles). Trawls were conducted in May and October 1985, January, May and September 1986. In January 1986, six specimens (5 - English sole, 1 - rex sole) showing skin lesions were collected and fixed in buffered formalin for histological examination. In September 1986, sole from the first trawl at each location were sorted into small (mean = 22 cm), medium (mean = 27 cm), and large (mean = 33 cm) size classes. Large and medium size classes were kept in holding tanks with running seawater for a maximum of one hour. English sole from the large size class were randomly selected and whole livers excised. If the sample size was too small, fish from the medium size class were included. Liver samples were fixed in buffered formalin and shipped to the University of Victoria for histological analysis. Out of a total of 106 English sole caught during the first trawl at each location in the harbour, 66 liver samples or 62% were selected for histological analysis.

Along with the random selection of liver samples from English sole, 10 additional liver samples were taken from five other sole species. Selection of the 10 samples was based on evidence of any gross visible liver abnormalities such as presence of white cysts or unusual colour patterns (mottled appearance). These samples were intended only for preliminary histological analysis and not used in determining the prevalence of idiopathic lesions. The species represented were: flathead sole (Hippoglossoides elassodon); hybrid sole (Inopsetta ischyra); rex sole (Glyptocephalus zachirus); sand sole (Psettichthys melanostictus) and starry flounder (Platichthys stellatus).

In addition to the liver samples collected in September 1986 for histological analysis, during every survey 10 - 15 sole from each size class from all species, were routinely necropsied onboard for any gross visible liver abnormalities. During each of the five field surveys between May 1985

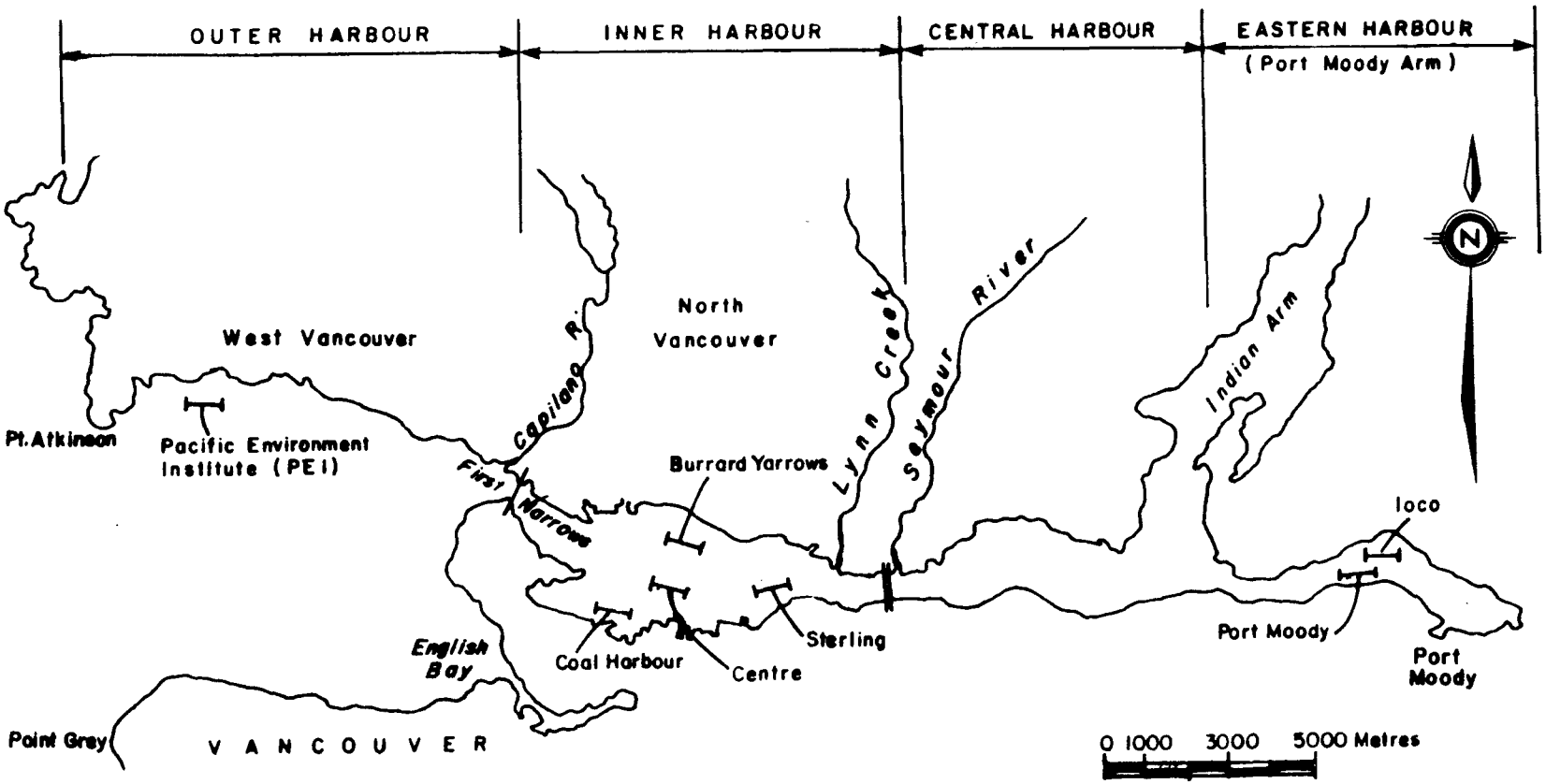


FIGURE 1 OTTER TRAWL LOCATIONS - VANCOUVER HARBOUR

and September 1986, all flatfish were examined for obvious external abnormalities such as parasitic infections, epidermal lesions, unusual skin colouration.

3.2 Histological Technique and Histopathologic Diagnosis

For histological sectioning of representative areas of the liver and proper penetration of histological solutions, the liver samples were divided into three to five equal portions (approximately 1 cm^2) while still in buffered formalin. Liver tissues were then washed in water, dehydrated in ethanol and embedded in glycol methacrylate, JB4. Skip serial sections were cut at two and five micrometers using a Sorval JB4 porter-blum microtome and glass knives. Slides were stained with Mayer's haematoxylin and xylidine ponceau, which is comparable to the standard haematoxylin/eosin stain (H & E) (Humason, 1979).

Liver abnormalities were classified according to nomenclature used by Myers et al., (1987), and documented on light micrographs from a Zeiss ultraphot microscope on Tech Pan black and white film. Sample areas corresponding to liver samples were not revealed to the histologist prior to the histopathological analysis to eliminate bias.

4 RESULTS

4.1 Histopathology

Histopathological observations of each liver sample are given in Appendix I. Plate 1 (#'s 1 to 4) shows the histological structure of normal liver tissue. Photographs illustrating the features of the various tissue lesions are given in Plates 2 through 6 (#'s 5 to 25). Plate 7 (#'s 26 to 28) illustrates gross visible features of external skin lesions found on English and rex sole.

4.1.1 Liver. Five types of idiopathic lesions were observed in English sole collected from Vancouver Harbour, ranging from nonspecific necrotic lesions to neoplasms. Lesions were also found due to parasitic (protozoan and helminths) infection. Cellular vacuolation and tinctorial characteristics of the hepatocellular cytoplasm varied among specimens.

4.1.1.1 Normal liver structure. (Plate 1) Normal English sole liver is composed of hepatocytes with a muralial tubulosinusoidal (1 to 2 cell layers) architecture, often irregularly arrayed about the central veins (Plate 1.1). Bile ducts, pancreatic acini (Plate 1.1) and melano-macrophage centers (Plate 1.4) are found scattered irregularly throughout the parenchyma. The hepatocytes are often vacuolated due to the presence of glycogen and lipid (Plates 1.2 and 1.3) and generally stain eosinophilic or occasionally basophilic depending on the spawning and nutritional status of the fish (Myers et al., 1987).

4.1.1.2 Nonspecific necrotic lesions. (Plate 2) These lesions are non-nodular and have a focal to diffuse distribution throughout the liver. The muralial architecture is normal but exhibits degenerative-necrotic conditions such as hepatocellular coagulation necrosis, liquefactive necrosis, hydropic degeneration, pyknosis and hyalinization (Plates 2.5 and 2.7).

4.1.1.3 Specific degenerative condition - megalocytic hepatosis. These lesions were only found in medium size class English sole from Vancouver Harbour. The lesions are non-nodular, diffusely distributed in a non-zonal pattern and have a normal muralial architecture. Common characteristics are marked enlargement of both nuclear and cellular diameters (Plates 2.6 and 2.7) and hydropic degeneration of affected cells. Surrounding hepatocytes near these lesions are often regenerative.

4.1.1.4 Intracytoplasmic storage disorder - hepatocellular steatosis. Lesions of this type are also non-nodular, diffusely distributed in a non-zonal pattern and have normal muralial architecture but are characterized by fatty changes within the hepatocytes that are visible as round smooth-edged vacuoles peripherally displacing the nuclei (Plate 2.8). This condition is suggestive of metabolic disorders (Myers et al., 1987) and was found in only one sand sole specimen. Sand sole occur in relatively small numbers in Vancouver Harbour and not in sufficient quantity to confidently establish the prevalence of this type of disorder.

4.1.1.5 Foci of cellular alteration (Plate 3). Lesion types in this category are arranged in discrete micronodular foci with borders blending indistinctly into the surrounding muralia with minimal or no compression of adjacent parenchyma. Foci rarely contained other hepatic elements (i.e. bile duct, pancreatic acini or melanomacrophages). The muralial architecture within the foci was generally normal. Foci of cellular alterations were the most dominant lesions in samples from Vancouver Harbour.

Clear Cell Foci. Clear cell foci contained vacuolated hepatocytes due to either lipid accumulation or glycogen storage (Plate 3.9). These types of lesions are rarely found close to other types of idiopathic lesions (Myers et al., 1987).

Eosinophilic Foci. These foci show a slight to dramatic hepatocellular hypertrophy, increased cytoplasmic eosinophilia with a granular texture (Plate 3.10), and varying degrees of pleomorphism of the nuclei (Plate 3.11). Eosinophilic foci are occasionally found proximate to basophilic foci (Myers et al., 1987).

Basophilic Foci. Basophilic foci characteristically possess hyperbasophilic cytoplasm in normal-sized hepatocytes with pleomorphic nuclei (Plate 3.12). This lesion has been shown to be a precursor in the pathogenesis of liver neoplasms in rodents, however this relationship remains to be confirmed (Myers et al., 1987).

4.1.1.6 Neoplasms - liver cell adenoma (Plate 4). The only type of neoplasm observed among Vancouver Harbour English sole was the liver cell adenoma. The adenomas were detected grossly as cream, firm nodules with smooth borders and occurred either singular or multiple within a single liver (Plate 4.13). Histomorphologically, adenomas exhibit compression of surrounding tissue, well-defined separation of proliferative tissue from normal tissue, increased cellular density, normal muralial architecture and the absence of other hepatic elements (bile ducts, pancreatic acini and melano-macrophage) (Plates 4.14-4.16). Cytologically, there was an increase in the nuclear:cytoplasm ratio and the tinctorial quality was either a mixed population of clear cells and basophilic cells (Plate 4.14) or basophilic type alone (Plates 4.15 and 4.16).

4.1.1.7 Zooparasitic infection (Plate 5). Parasites were present in both normal liver sample and those with idiopathic lesions. The most common was the plasmodial form of the sporozoan genus Myxidium (Butschli), family Myxidiidae (Thelohan), found within the bile ducts and ductules (Plate 5.17). Myxidium spp. infection was observed in all fish examined, with severe infections occurring in flathead and hybrid soles (Appendix I). Severe infections were characterized by proliferation of bile ductules containing plasmodia and spores with parasites scattered throughout the parenchyma. This resulted in severe fibrosis along with hepatocellular necrosis (Plate 5.18).

One English sole liver sample was infected with an ooctye-like body (Plate 5.19) believed to be a protozoan which has been associated rarely with liver tissue and occasionally with kidney tissue of feral fish (Myers, pers. comm.). To date, little is known regarding the identity of this apparent infectious agent.

Acanthocephalans and nematodes were present either externally or internally (Plate 5.20). Associated with these helminth infections was an inflammatory response, resulting in the formation of granulomas (Plate 5.21).

4.1.2 **Skin Lesions and Fin Erosion (Plates 6 and 7).** Field observations of over 10,000 flatfish caught in Vancouver Harbour indicated that few individuals (less than 5%) were affected by skin lesions or epidermal papillomas. Of the 16 species represented in the catch, skin lesions were confined to the English sole, characteristically as grey to black cauliflower-like proliferations of the epidermis (Plates 7.26 and 7.27) and the rex sole as filamentous, tan coloured growths on the skin (Plate 7.28)

Epidermal papillomas from five English sole were examined microscopically. These growths were typically papillary in form, composed of irregular, branching stalks of connective tissue covered externally by multiple layers of polygonal cells of variable size. The latter cells were characterized by a granular, eosinophilic cytoplasm and a large nucleus with a prominent nucleolus (Plates 6.22 and 6.23). These cells were originally described by Brooks et al, (1969) as "x-cells".

Microscopically, lesions found on the rex sole were characterized by epidermal hyperplasia, dermal fibrosis, aggregation of melanophores around sclerotic blood vessels and an increase in eosinophilic granular cells (EGC), in the epidermis (Plate 6.24). These features are described by Roberts et al., (1971). Ulceration of the epidermis with necrosis of the epithelial cells was also noted (Plate 6.25). Due to the staining properties of haematoxylin and xylidine, it was not possible to identify the presence of bacteria or fungi in any of the histologic preparations.

4.2 Prevalence of Idiopathic Liver Lesions

For the five locations where 10 or more English sole liver samples were taken (PEI, Centre Channel, Burrard Yarrows, Coal Harbour and Ioco), the prevalence of idiopathic lesions was strongly dependant on location of capture (chi square = 12.1, d.f. = 4, prob. = 1.58). The Ioco station demonstrated the highest prevalence with 58.8% of the samples affected by one

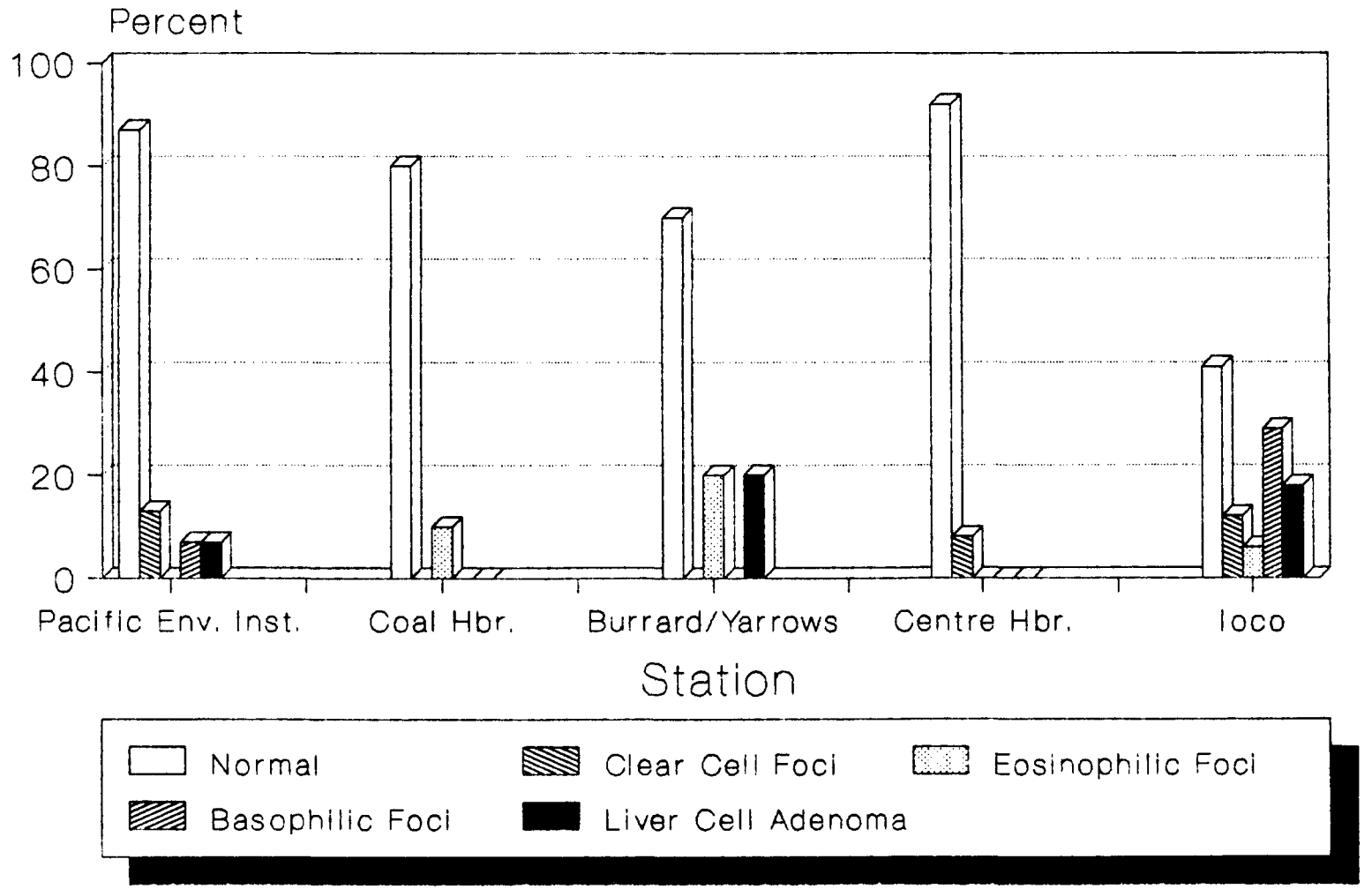
or more types of idiopathic liver lesions. PEI and Centre Channel had the lowest frequencies, 13.3% and 8.3% respectively (Table 1). The Burrard Yarrows station along the north shore of the inner harbour, and Coal Harbour, on the south shore, were 30.0% and 20.0%, respectively (Table 1). Port Moody and Sterling would have given false prevalences of 100% due to the sample size being equal to one. Percentages in Table 1 are based on both medium-sized (mean=27 cm) and large-sized (mean=33 cm) sole and include all types of idiopathic liver lesions. Figure 2 compares the percentages of English sole with normal liver tissue, various foci of cellular alteration (clear cell (CCF), eosinophilic (EF), and basophilic foci (BF)) and neoplasms (liver cell adenomas (LCA)), by sampling station.

TABLE 1 **PREVALENCE OF IDIOPATHIC LIVER LESIONS IN MEDIUM TO LARGE ENGLISH SOLE (>25 cm, fork length) FROM VANCOUVER HARBOUR - SEPTEMBER 1986**

SAMPLE AREA	SAMPLE SIZE*		PREVALENCE OF IDIOPATHIC LIVER LESIONS	
	Total Catch	Number of Subsamples	Number of Fish Affected	%
Centre Channel	17	12	1	8.3
Pacific Environment Institute	32	15	2	13.3
Coal Harbour	16	10	2	20.0
Burrard Yarrows	20	10	3	30.0
Ioco	19	17	10	58.8
Port Moody	1	1	1	-
Sterling	1	1	1	-
Total	106	66	20	30.3%

* For specific information on relative abundance and distribution of English sole and other benthic species in Vancouver Harbour refer to Goyette and Thomas, 1987.

FIGURE 2 PERCENT NORMAL VS. PRENEOPLASTIC AND NEOPLASTIC LIVER LESIONS IN ENGLISH SOLE FROM VANCOUVER HARBOUR - 1986



5 DISCUSSION

5.1 Epidermal Abnormalities

From external appearances, the majority of the fish caught in Vancouver Harbour appeared to be relatively healthy. A few individuals were affected by skin lesions, some were heavily parasitized along the fin rays (nematode) and occasionally a high proportion of the English sole catch showed a distinctive, diffuse dark red discolouration to the skin and fins. The latter condition had not been observed in numerous catches from other coastal areas using the same gear and fishing techniques (Goyette, pers. obs.). No attempt was made to ascertain the cause of this discolouration. Histological examination of the skin lesions on five English sole collected in January 1986 indicated that the lesions were x-cell pseudotumors. The pseudotumors are thought to be caused by a protozoan parasite infection (probably amoebic) and not the direct result of chemical exposure; nor are they considered to be neoplasms (Dawe, 1979 and 1981; Myers, 1981 and in review; Mix 1986).

Stich et al., (1977) had previously reported a high percentage (58%) of epidermal papillomas in English sole from the Vancouver area near the mouth of the Fraser River. The present study found only a few individuals with external papillomas within the harbour area. Similarly, a few rex sole were found with epidermal lesions, however this species was largely confined to the outermost station (PEI). Their limited numbers and distribution prevented establishing any correlation between prevalence and sediment associated chemical contaminants.

5.2 Liver Abnormalities

The sporozoan, Myxidium spp. was commonly found in the bile ducts of the English sole liver samples which were examined histologically, and some were heavily infested (Plate 5.18). No relationship between parasitic infection and geographic area was apparent.

Myers et al., (1987) provided a detailed synthesis of the pathologic anatomy of idiopathic liver lesions in English sole from Puget Sound, their co-occurrence patterns and association to hepatotoxins and hepatocarcinogens. These hepatic conditions are significantly more prevalent

in fish from polluted areas close to urban centres than fish taken from non-urban areas. Many of the liver lesions described by Myers et al., (1987) are found in English sole from the Vancouver Harbour area.

Idiopathic liver lesions are basically catagorized as degenerative-necrotic conditions, intracytoplasmic storage disorders, various foci of cellular alteration and neoplasms. The latter category of strictly hepatocellular origin can be either liver cell adenomas or hepatocellular carcinomas. Adenomas are partially distinguished from carcinomas by a well-defined border between proliferative tissue and the normal tissue. In contrast, carcinomas invade the surrounding tissue. Both exhibit signs of cellular proliferation through compression of surrounding tissue.

Although the histogenetic pathway of liver adenomas to carcinomas in rodent studies is still being debated, the occasional observation of carcinomatous foci within adenomas of English sole suggest that carcinomas may arise directly from adenomas (Myers et al., 1987). However, only liver cell adenomas were found in Vancouver Harbour with no evidence of heptocellular carcinomas detected. Prevalence of liver cell adenomas and hepatocellular carcinomas in English sole from a highly polluted area of Puget Sound are reported as 18.5% and 15.2%, respectively (Myers et al., 1987). The prevalence of liver cell adenomas in Vancouver Harbour was 10.4% for the total study area.

Extrahepatic metastasis of adenomas was not observed in English sole livers from Vancouver Harbour, but the observation of multiple adenomas in a single liver sample suggest an independent multifocal origin or intrahepatic dissemination of the tumor tissue.

All of the types of foci of cellular alteration found in English sole resemble those described in rodent hepatocarcinogenic studies (Squire and Levitt, 1975; Frith and Ward, 1980). In mammalian hepatocarcinogenesis models, these lesions are generally considered to be early, focal, proliferative lesions that can be sites for hepatocellular neoplasm development (Myers et al., 1987). Through co-occurence analysis, Myers et al., (1987) hypothesized that clear cell and eosinophilic foci are putatively preneoplastic lesions; basophilic foci are presumptively preneoplastic lesions which may immediately precede the development of the neoplasms, liver cell adenomas and hepatocellular carcinomas.

Prevalences in English sole from Eagle Harbour, one of the more contaminated areas of Puget Sound, were 30.5%, 26.5% and 8% for eosinophilic, basophilic and clear cell foci, respectively. In contrast, prevalences in Vancouver Harbour were 7.5%, 10.4% and 7.5%, for the three types of foci. Liver samples from the Ioco area showed a high prevalence of foci of cellular alteration (39%) as compared to 8.3% and 13% found at the Centre Channel and PEI stations.

Nonspecific necrosis is a response to either toxins or pathogens. If a cellular inflammatory response is present then a biological agent is the cause of the necrosis. If there is no inflammatory response and liver cell adenomas are present, then cellular necrosis may represent necrogenic action by toxic chemicals or carcinogens (McCain et al., 1982). Two liver samples from English sole showed evidence of necrosis that might be attributed to causes other than severe parasitic infection.

Similarly, megalocytic hepatosis was only found in two specimens of English sole, medium size class. Frequencies were much higher in Puget Sound sole (53.6%) as reported by Myers et al. (1987). It has been inferred that megalocytic hepatosis is an initial, subchronic to chronic hepatocellular lesion manifested by the cytotoxic effects of exposure to hepatocarcinogens (Myers et al., 1987). This lesion is frequently associated with eosinophilic and basophilic foci and often with liver cell adenomas. Along with other degenerative-necrotic conditions, this condition is widely known to occur in mammals and fish exposed to xenobiotic hepatotoxins and hepatocarcinogens.

Hepatocellular steatosis which was present only in one sample of sand sole, is a degenerative lesion commonly associated with dietary deficiencies or toxic chemical administration. Its role in the progression of lesions towards neoplasm formation in sole is not presently known (McCain et al., 1982).

Idiopathic liver lesions were found in English sole at all sampling locations in Vancouver Harbour. As mentioned earlier, these lesions were morphologically similar to those described by Myers et al. (1987). This description was based on studies of wild fish from Puget Sound and relationships to lesions induced in fish and rodents exposed to hepatocarcinogens-hepatotoxins in controlled laboratory experiments.

Most of the non-urban reference sites in Puget Sound have shown little or no evidence of preneoplastic or neoplastic lesions in fish liver tissue (Tetra Tech, 1987). In the present study, none of the sampling locations could be considered true reference sites, sufficiently removed from the influence of urban activity. As indicated earlier, 30.3% of the English sole sampled were affected by idiopathic liver lesions (Table 1). Statistically, the prevalence of idiopathic liver lesions was strongly dependant upon sampling location and increased in a west to east direction. Lesion prevalence was 13.3% in the outer harbour (Pacific Environment Institute), averaged 19% in the inner harbour (Coal Harbour, Burrard Yarrows and Center) and 58.8% in the eastern portion of the harbour (Ioco). Dependance upon sampling location however, appears to be largely from the dominance of the high lesion prevalence at the Ioco site. Excluding the Ioco site, subsequent analysis showed no significant differences between stations within the inner harbour nor the station in the outer harbour (chi square = 2.0, d.f. = 3, prob. = 0.56). This might be partly due to the physical restrictions in the harbour and strong homing instincts present in English sole.

Tagging studies conducted in Puget Sound by Day (1976) showed that fish, after being displaced, rapidly returned to the area of capture. Those that migrated out of the area during the fall and winter spawning period also returned to their home territory. Assuming the English sole in Vancouver Harbour exhibit similar homing behaviour, it is probable that fish in Port Moody Arm remain relatively separate from populations in the other sampling areas. The restricted passage at Second Narrows and distance from other areas would also limit frequent intermingling. For the other sampling stations, the opportunity for fish to intermingle would likely be greater. Sampling sites in the inner harbour were relatively close together with no physical restrictions between them. However, relatively higher prevalences of liver lesions in fish from the shore stations (Coal Harbour and Burrard Yarrows) compared to the Centre Channel station suggest the possibility of separation between flatfish populations in the inner harbour. This might result from the strong lateral current patterns present in the inner harbour. Little time is required to induce hepatic lesions in fish. Couch and Harshbarger (1985, in Tetra Tech, 1987) reported that in 59 cases or 56.2% of

the studies, laboratory exposure to carcinogenic chemicals induced hepatic neoplasms within six months; in 98 cases or 93.3% within one year of exposure.

Studies of wild fish populations have shown a higher prevalence of hepatic lesions in fish from polluted environments. In a number of areas in Puget Sound including the Duwamish Waterway and Eagle Harbour positive correlations between sediment-associated polycyclic aromatic hydrocarbons (PAHs), one of the major chemical contaminants present, and prevalences of several categories of idiopathic liver lesions in English sole (Malins et al., 1984 and 1988) have been found.

Fish in Vancouver Harbour can be exposed to a wide range of chemical contaminants including compounds with hepatotoxic and hepatocarcinogenic properties. Field observations and chemical analysis of the bottom sediments conducted concurrently with the fish histopathology studies in Vancouver Harbour suggest that the chemical exposure to fish in Port Moody Arm is much greater. Elevated levels of petroleum hydrocarbons, PAHs and certain trace metals have been found in the bottom sediments throughout Port Moody Arm (D. Goyette and M. Thomas, reports in prep.). Petroleum hydrocarbon concentrations ranged between 338 ug/g and 1897 ug/g (13 stations). PAH concentrations at 6 stations in Port Moody Arm ranged between 3.1 ug/g and 6.6 ug/g. Present in the samples were some of the more carcinogenic high molecular weight PAHs such as benzo(a)pyrene and benzo(b,k)fluoranthene. Elevated concentrations of chromium and lead were also found in Port Moody Arm up to 267 ug/g and 182 ug/g, respectively.

Although higher concentrations, of petroleum hydrocarbons (up to 6587 ug/g) and PAHs, have been found in the inner harbour, these were mainly confined to the immediate foreshore areas. The chances of flatfish in the inner harbour coming in contact with elevated levels of contaminants, or the risk of sustained exposure to contaminated sediments, would likely be much less. This might explain the presence of idiopathic lesions in other areas of the harbour but at a lower prevalences.

The fine texture, blackened appearance and strong odour of hydrogen sulphide observed during field collections, suggests that the flushing capacity of Port Moody Arm is relatively poor. Waldichuk (1965) calculated about a 30% exchange on each tidal cycle. Poor water circulation coupled

with a history of urban and industrial activity in the area are probably responsible for the higher prevalence of liver lesions in English sole relative to other areas of the harbour. However, it is important to note that moderate prevalences were also found in other regions of the harbour.

Although many of the English sole liver samples showed no visual signs of abnormalities, histological examination revealed a significant number of idiopathic liver lesions. This stresses the need for detailed histological examination to determine the impact of chemical exposure to fish populations or other marine species. Further studies on the histopathological condition of bottomfish in Vancouver Harbour, their migratory and distribution patterns in relation to contaminant levels and positive measures to reduce sources of chemical input, are warranted.

PLATES

PLATE 1 - NORMAL LIVER TISSUE

Plate 1.1 - Section of normal liver tissue from the English sole showing hepatocytes (h), pancreatic acini (pa), bile duct (bd), and melano-macrophage (m). H&E. Bar = 100 um.

Plate 1.2 - Section of normal liver tissue with vacuolated hepatocytes (h) due to glycogen/lipid storage. pa = pancreatic acini. H&E. Bar = 100 um.

Plate 1.3 - Normal liver tissue with mottled areas of vacuolated hepatocytes (h). pa = pancreatic acini. H&E. Bar = 500 um.

Plate 1.4 - Normal liver tissue with numerous macrophage aggregates or melano-macrophage centres (m). h = hepatocytes, pa = pancreatic acini. Bar = 100 um.

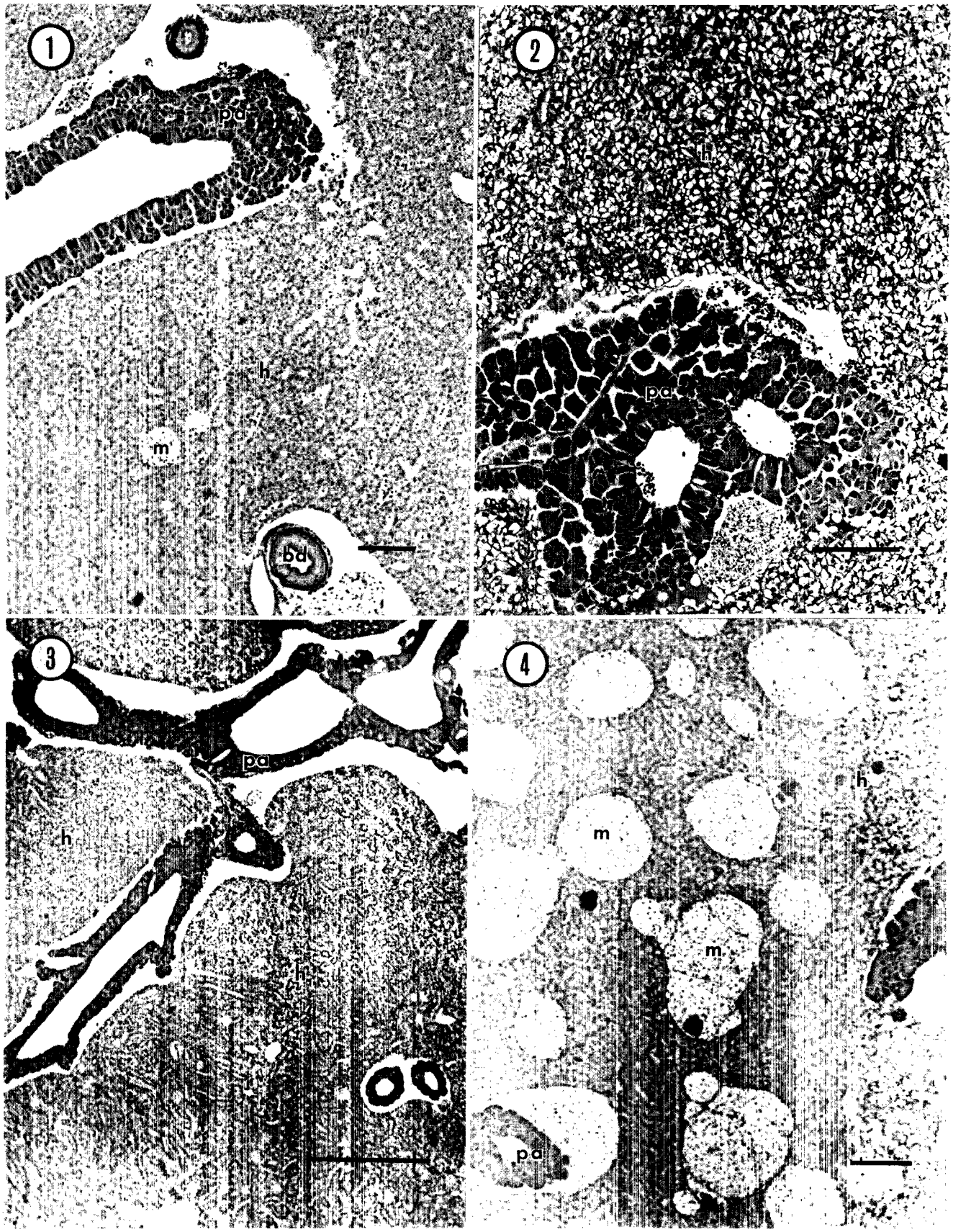


PLATE 1

PLATE 2 - NONSPECIFIC NECROTIC LESIONS, SPECIFIC DEGENERATIVE CONDITIONS AND INTRACYTOPLASMIC DISORDERS

Plate 2.5 - Nonspecific necrosis of the hepatocytes. hb = hyaline bodies, p = pyknotic nuclei, arrows = hydropic cells. H&E. Bar = 10 um.

Plate 2.6 - Megalocytic hepatosis. Arrows point to enlarged nuclei. H&E. Bar = 50 um.

Plate 2.7 - Megalocytic hepatosis and necrosis. Arrows point to enlarged nuclei. Note inflammation among necrotic cells (nec). H&E. Bar = 50 um.

Plate 2.8 - Heptacellular steatosis (hs). Note the round vacuoles and peripherally displaced nuclei due to probable lipid accumulation. Normal tissue is at far right. H&E. Bar = 100 um.

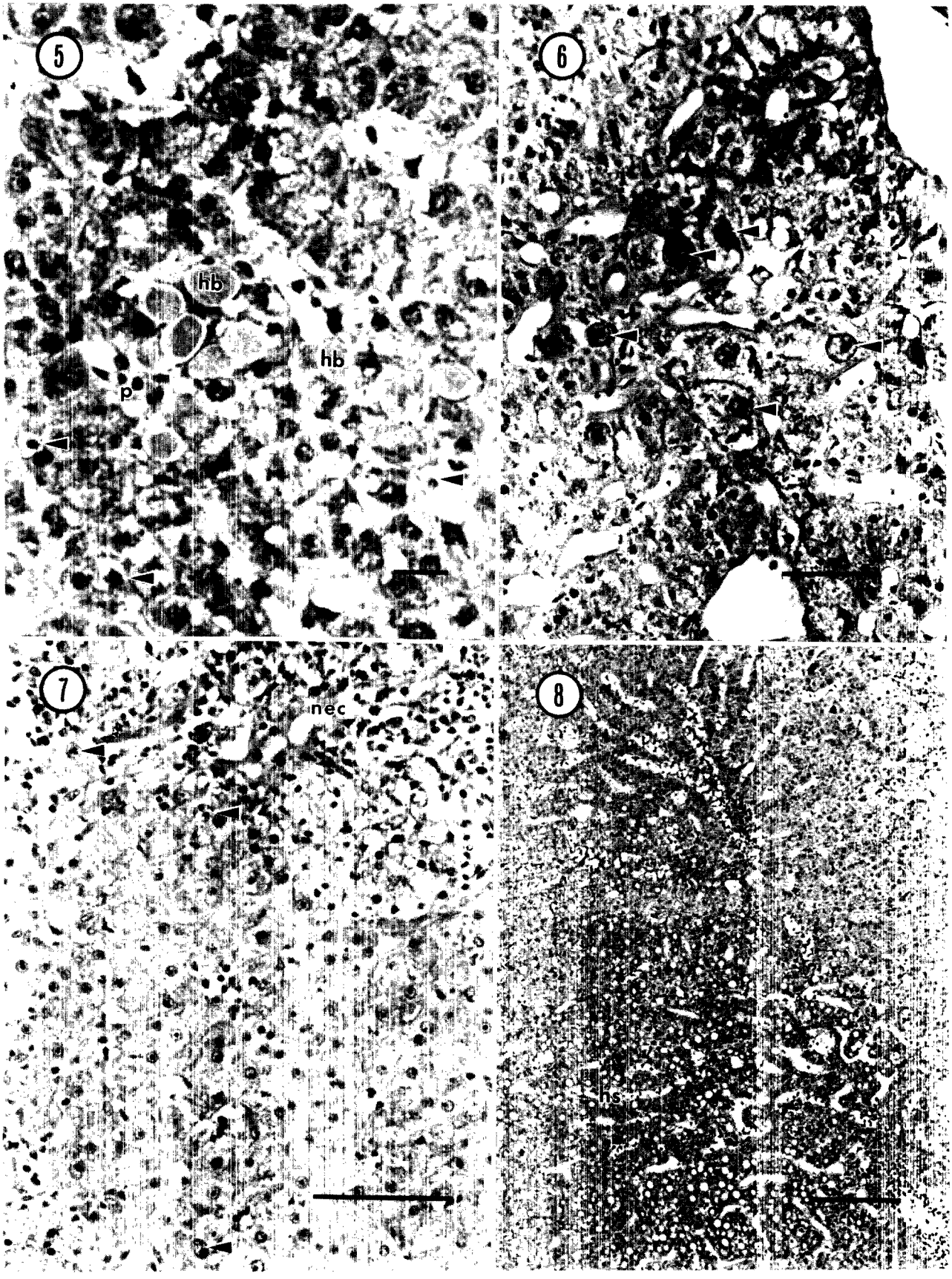


PLATE 2

PLATE 3 - FOCI OF CELLULAR ALTERATION

Plate 3.9 - Clear cell foci (CCF). Note the blending of focus muralia with the surrounding parenchyma with no compression. Arrowheads delineate focus from the normal tissue. H&E. Bar = 100 um.

Plate 3.10 - Eosinophilic foci (EF). Section shows increased cytoplasmic eosinophilia, and blending in of the focus with the surrounding parenchyma. Arrowheads delineate focus from the normal tissue. H&E. Bar = 100 um.

Plate 3.11 - Eosinophilic foci (EF) with slight nuclear pleomorphism and hepatocellular hypertropohy. Arrowheads delineate focus from the normal tissue. H&E. Bar = 50 um.

Plate 3.12 - Basophilic foci (BF) with hyperbasophilic cytoplasm in normal sized hepatocytes that blend into the surrounding tissue. Arrows delineate focus from normal tissue. H&E. Bar = 100 um.

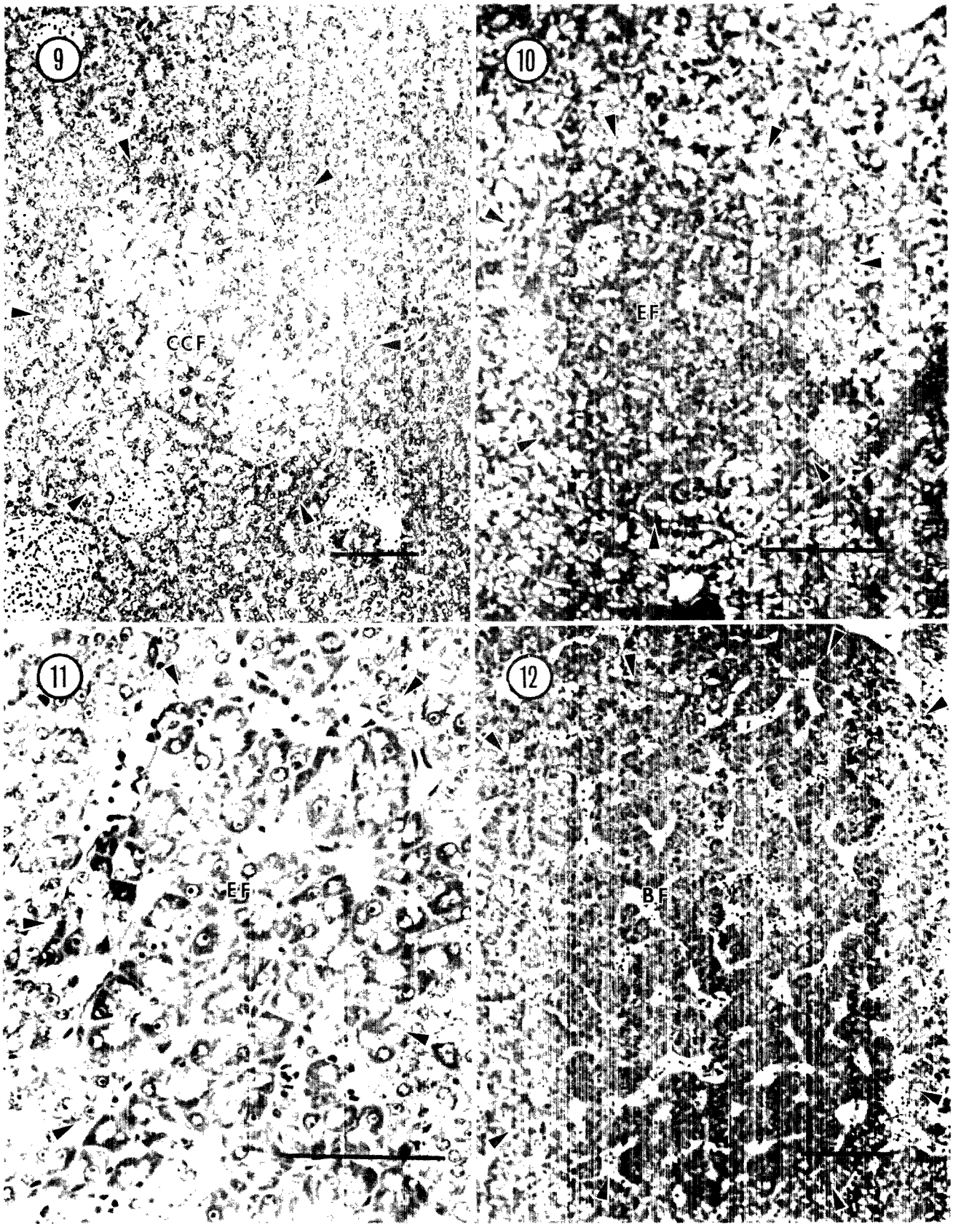


PLATE 3

PLATE 4 - NEOPLASMS

Plate 4.13 - Gross appearance of liver cell adenoma in English sole liver. The neoplasms (n) were cream in colour and raised from the surface. This liver was also affected with basophilic and clear cell foci.

Plate 4.14 - Liver cell adenoma (LCA), clear cell/basophilic type. Note the absence of normal hepatic elements within the adenoma. H&E. Bar = 500 um.

Plate 4.15 - Liver cell adenoma (LCA), basophilic type. Note the well defined separation of proliferative tissue from the normal tissue (arrows), compression of the surrounding tissue and lack of normal hepatic elements. H&E. Bar = 500 um.

Plate 4.16 - Liver cell adenoma (LCA), basophilic type. Note the compression and separation (arrows) of the surrounding parenchyma and the normal muralial architecture of the hepatocytes within the adenoma. H&E. Bar = 500 um.

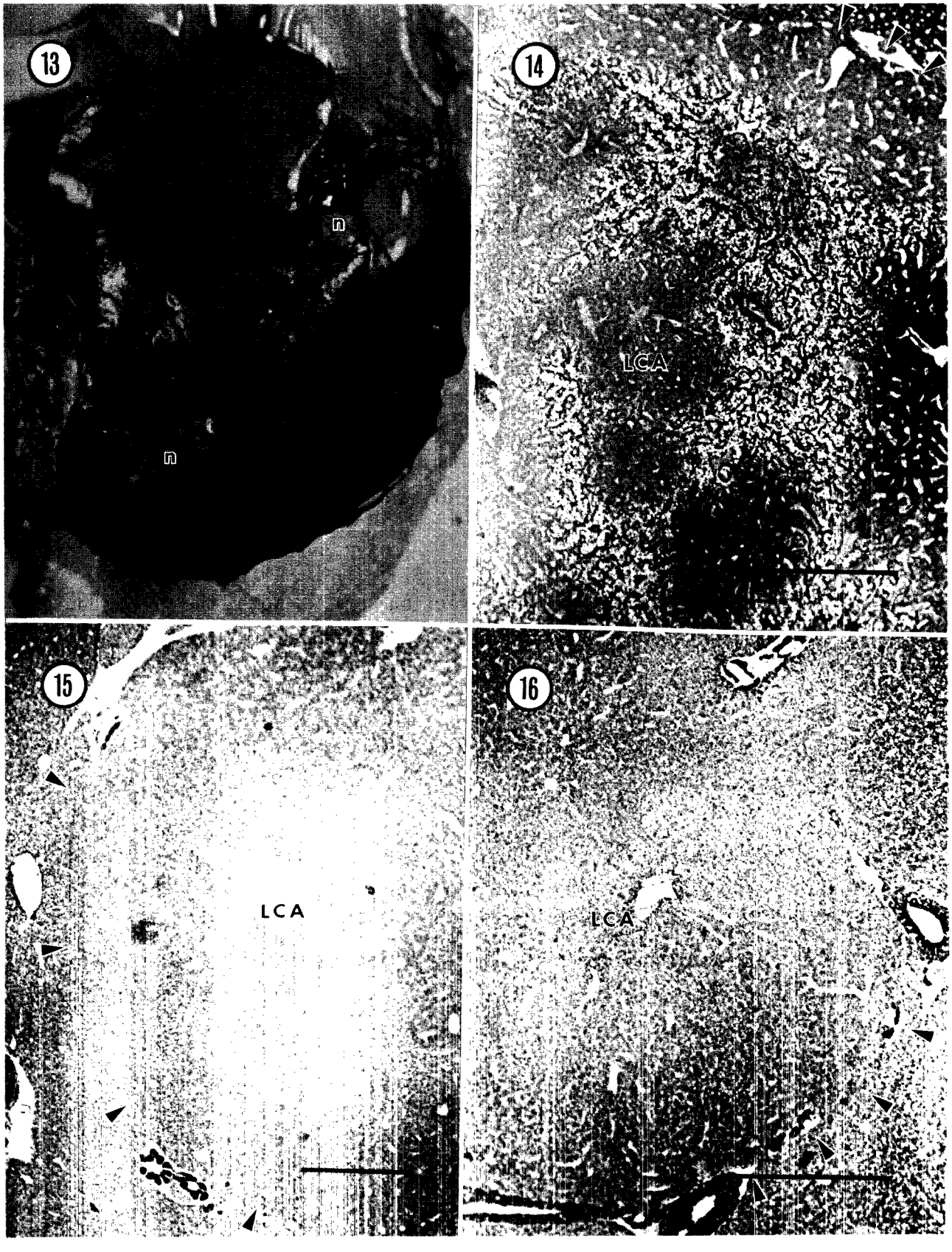


PLATE 4

PLATE 5 - ZOOPARASITIC INFECTION

Plate 5.17 - Infection of the bile ducts (bd) by the sporozoan Myxidium sp. (m). Note the absence of an inflammatory response. H&E Bar = 500 um.

Plate 5.18 - Severe Myxidium sp. infection (m). Note the bile ductule proliferation, parenchymal fibrosis and hepatocellular necrosis. H&E. Bar = 100 um.

Plate 5.19 - Oocyte-like body (ob) infection surrounded by normal liver hepatocytes. H&E. Bar = 100 um.

Plate 5.20 - Helminth (h), probably a nematode, located in the parenchymal interstitium. H&E. Bar = 100 um.

Plate 5.21 - Inflammatory response manifested by the formation of a granuloma (g) due to infection by an undetermined type of helminth. Arrows delineate granuloma from surrounding tissue. H&E. Bar = 500 um.

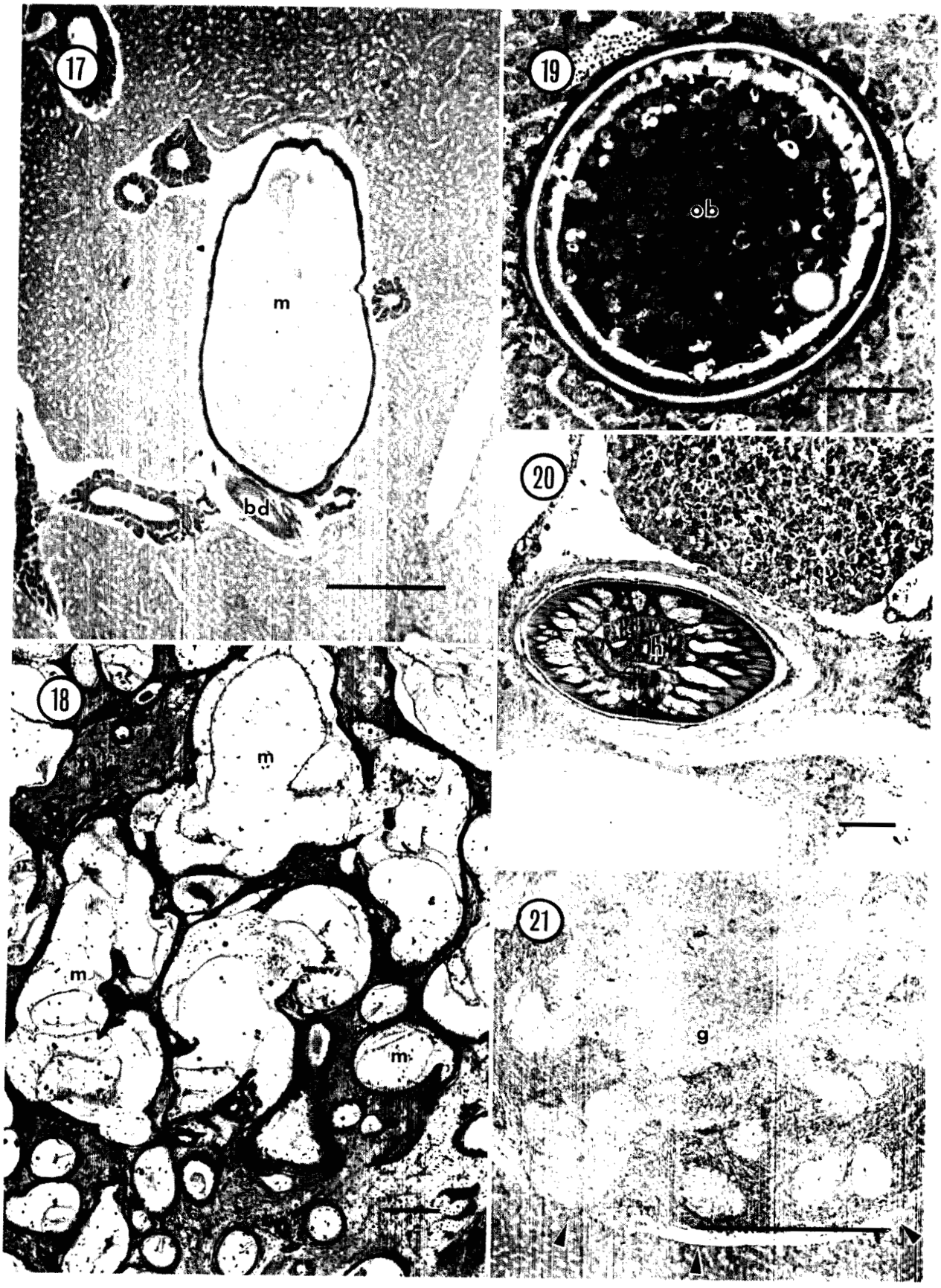


PLATE 5

PLATE 6 - SKIN LESIONS AND FIN EROSION

Plate 6.22 - Epidermal papilloma (EP). Note the increase in the eosinophilic epidermal cells that give rise to the marked papillomatous proliferation of the epidermis. H&E. Bar = 500 um.

Plate 6.23 - The higher magnification of an epidermal papilloma showing branching stalks of connective tissue (c) and "X" cells (x). H&E. Bar = 100 um.

Plate 6.24 - Fin erosion with epidermal hyperplasia containing eosinophilic granular cells (ECG). Arrows indicate the aggregation of melanophores. H&E. Bar = 500 um.

Plate 6.25 - Fin erosion with necrosis (nec) of the epithelial cells. H&E. Bar = 500 um.

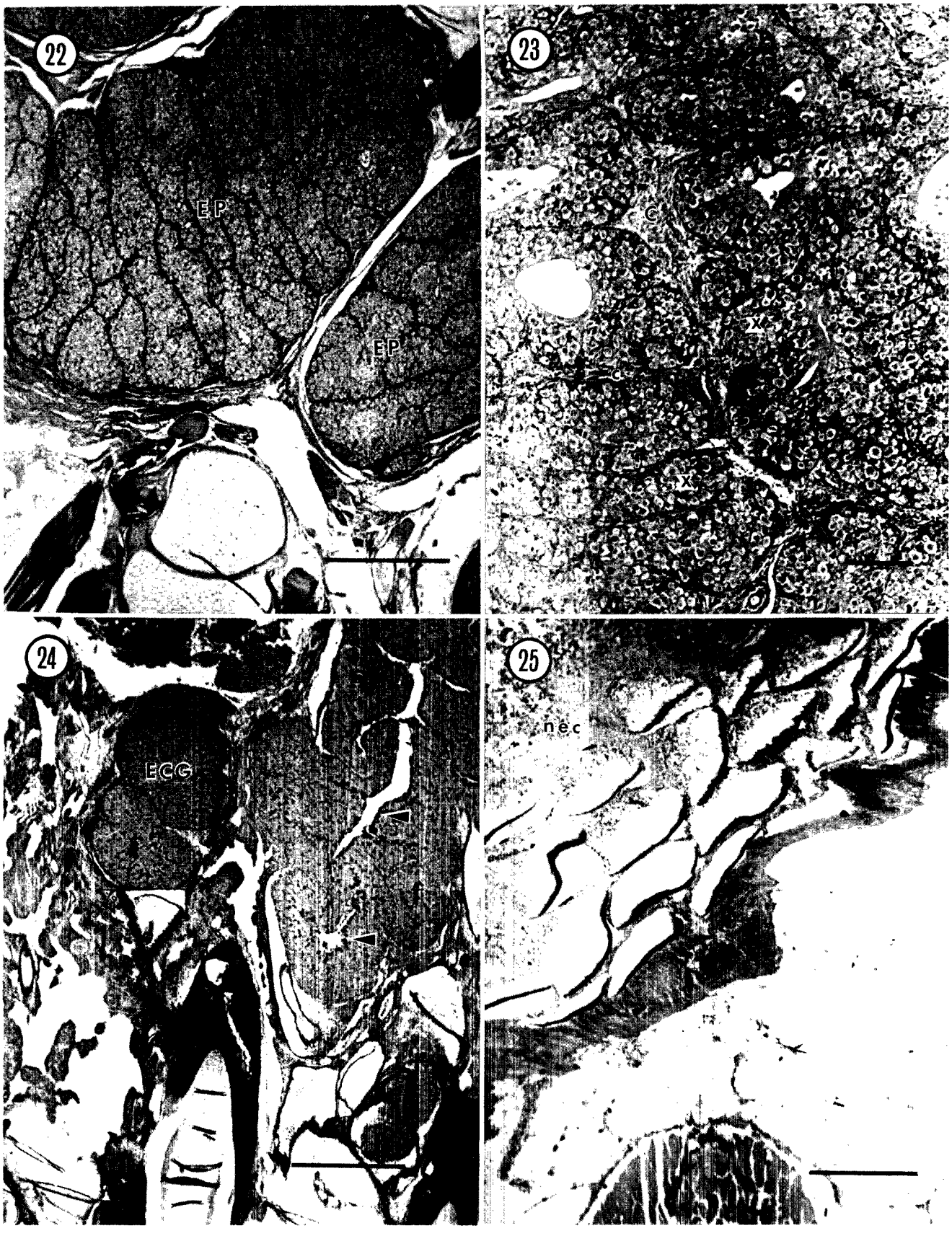


PLATE 6

PLATE 7 - GROSS MORPHOLOGY - EPIDERMAL PAPILLOMAS

Plate 7.26 - Epidermal papilloma - English sole (Parophrys vetulus). Bar = 2.5 cm.

Plate 7.27 - Epidermal papilloma - English sole (Parophrys vetulus). Bar = 2.5 cm.

Plate 7.28 Epidermal papilloma - rex sole (Glyptocephalus zachirus).

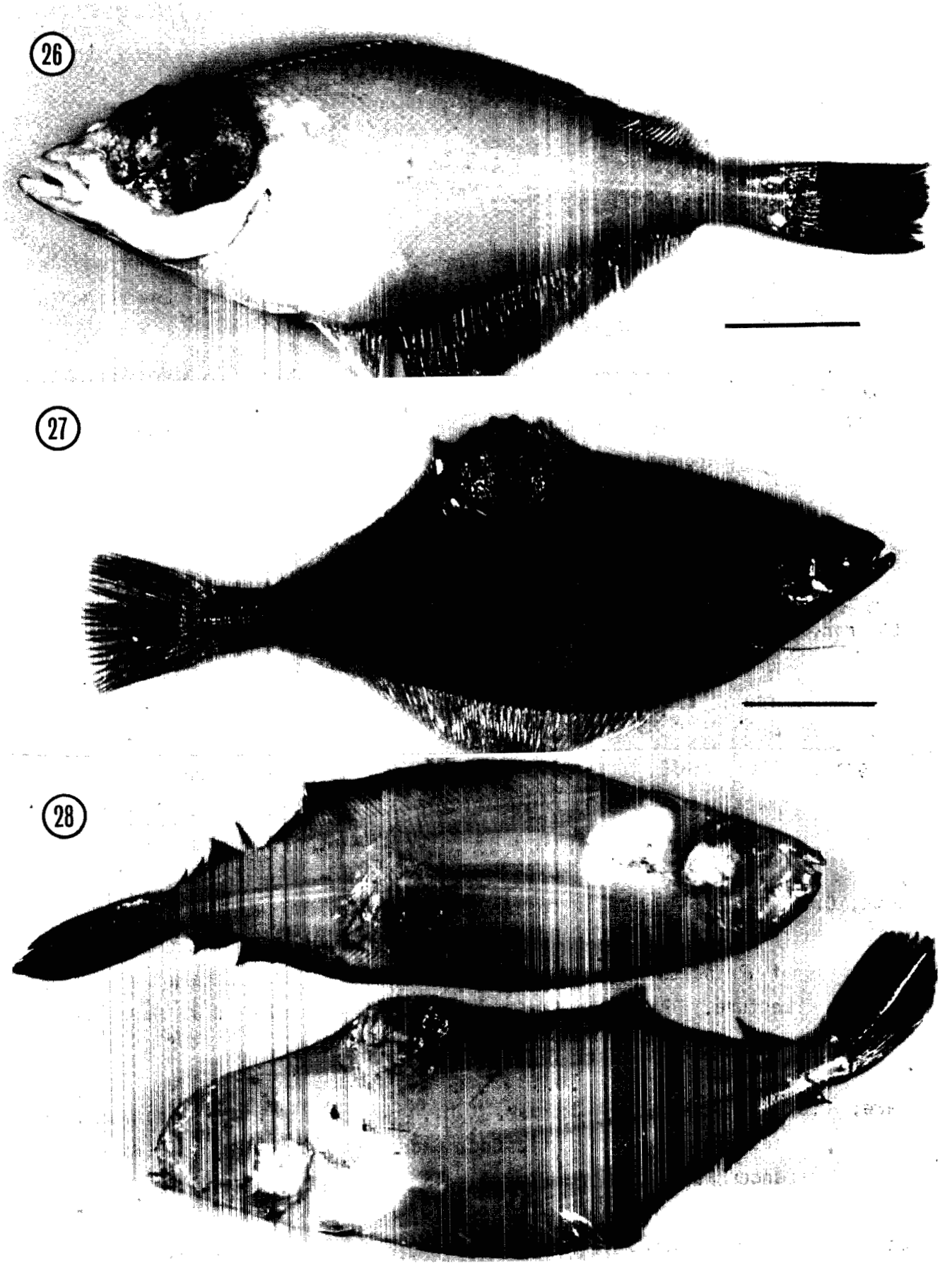


PLATE 7

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APPENDICES

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Pacific Environment Institute

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
Flathead	M	white cyst	NEC	M	+
sole	M	Normal	NEC	SMI	-
English	M	Normal	Normal	M	-
sole	M	Normal	CCF	G	-/+
	L	Normal	Normal	-	-
	L	white cyst	OB	M	-
	L	Normal	Normal	M	-
	L	parasite	Normal	G	-/+
	L	Normal	Normal	M,G	-
	L	Normal	Normal	M	-
	L	Normal	Normal	M	-
	L	Normal	Normal	H	+
	L	Normal	Normal	-	-
	L	mottled	Normal	M	-
	L	Normal	CCF, BF, LCA	-	-
	L	Normal	Normal	M	+
	L	Normal	Normal	M,G	+
Hybrid s.	M	dual colour	NEC	SMI	-

M - medium
L - large

NEC - necrosis
CCF - clear cell foci
OB - oocyte-like body
BF - basophilic foci
LCA - liver cell adenoma

M - Myxidium spp.
SMI - Myxidium spp., severe infection
G - granuloma
H - helminth (nematode or acanthocephalon)

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Coal Harbour

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
Rex sole	M	parasite	Normal	G	+
Starry f.	L	white cyst	Normal	G,H	-
English s.	M	mottled	MH	M	-
	L	Normal	Normal	M,G	-
	L	Normal	Normal	M,G	-
	L	Normal	Normal	G	+
	L	Normal	Normal	M,G	+
	L	Normal	Normal	-	-
	L	Normal	EF	G	-
	L	Normal	Normal	M	-
	L	Normal	Normal	G	+
	L	Normal	Normal	G	+/-

M - medium
L - large

MH - megalocytic hepatosis
EF - eosinophilic foci

G - granuloma
H - helminth (nematode and acanthocephalon)
M - *Myxidium* spp.

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Burrard Yarrows

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
English sole	M	dual colour	LCA, EF	M	+
	M	mottled	EF, RHV	M	+
	M	Normal	Normal	M	-
	M	Normal	Normal	M	+/-
	M	Normal	RHV	M	+
	M	white cyst	Normal	M	-
	L	Normal	Normal	M	-
	L	Normal	LCA	M	-
	L	Normal	Normal	M	+
	L	Normal	Normal	M	-

M - medium
L - large

LCA - liver cell adenoma
EF - eosinophilic foci
RHV - hepatocellular vacuolation

M - *Myxidium* spp.

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Ioco

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
Flathead s	S	mottled	NEC	SMI	+/-
Starry f.	M	mottle/cyst	Normal	H	-
English	S	white cyst	Normal	G	-
sole	L	white cyst	Normal	G	-
	L	Normal	Normal	M,G	+
	L	Normal	Normal	M,G	-
	L	Normal	EF	G	+
	L	Normal	BF, NEC	G	+
	L	blood spot	BF	M	-
	L	Normal	Normal	M	-
	L	Normal	BF	M	-
	L	Normal	BF, EC	-	+
	L	Normal	Normal	-	+
	L	Normal	Normal	M	+
	L	white cyst	LCA	M	-
	L	mottled	Normal	M	+
	M	white cyst	LCA, BF, CCF	H	-
	M	white cyst	LCA	-	-
	M	white cyst	MH, NEC, IH	M	+
	M	blood spot	CCF	-	+
Sand sole	L	white cyst	HS	M,H	-

S - small
M - medium
L - large

NEC - necrosis
EF - eosinophilic foci
BF - basophilic foci
LCA - liver cell adenoma
CCF - clear cell foci
MH - megalocytic hepatosis
IH - inflammation hepatosis
HS - heptacellular steatosis

SMI - severe Myxidium spp. infection
H - helminth (nematode or acanthocephalon)
G- granuloma
M - Myxidium spp.

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Port Moody

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
Flathead sole	M	mottled	NEC	SMI	+
English sole	M	white cyst	LCA	H	-

M - medium

NEC - necrosis
LCA - liver cell adenoma

SMI - severe Myxidium spp. infection
H - helminth (nematode or acanthocephaton)

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Centre Channel

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
English	M	Normal	Normal	-	+
sole	M	Normal	Normal	-	-
	M	Normal	CCF	-	+
	M	Normal	Normal	M	-
	M	Normal	Normal	-	-
	M	Normal	Normal	M	-
	M	dual colour	Normal	M,H	+
	M	Normal	Normal	M	-
	M	Normal	Normal	M	-
	M	Normal	Normal	M	-
	M	white cyst	Normal	G	+
	M	white cyst	Normal	G	-

M - medium

CCF - clear cell foci

M - *Myxidium* spp.
H - helminth (nematode or acanthocephalon)
G - granuloma

APPENDIX I HISTOPATHOLOGICAL OBSERVATIONS OF FLATFISH LIVER SAMPLES FROM VANCOUVER HARBOUR - SEPTEMBER, 1986

Station: Sterling

FISH SPECIES	SIZE CLASS	GROSS MORPHOLOGY	HISTOPATHOLOGY	PARASITE	VACUOLE
Sand sole	L	dual colour	Normal	H,M	-
Flathead sole	S	mottled	NEC	SMI	+/-
English sole	M	white cyst	LCA, BF	-	-

S - small
M - medium
L - large

NEC - necrosis
LCA - liver cell adenoma
BF - basophilic foci

H - helminth (nematode or acanthocephalon)
M - *Myxidium* sp.
SMI - severe *Myxidium* spp. infection