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HISTOLOGY OF LIVER TISSUE FROM DOVER SOLE

The liver functions in the digestion of food, the formation of excretory products, the degradation of complex cellular materials (e.g., hemoglobin), the detoxification of unnatural compounds, the synthesis of plasma proteins, and the maintenance of energy and vitamin stores. Because of this functional complexity, and the dependence of liver function on its structure, any changes in liver anatomy become important considerations in the study of diseased or abnormal organisms.

Changes in liver size and condition have been noted in demersal fish collected from areas of municipal wastewater discharge in both southern California and Washington. Enlarged livers with pathologic lesions have been found in the English sole (*Parophrys vetulus*) and starry flounder (*Platichthys stellatus*) populations of the contaminated Duwamish River Estuary in Seattle (Pierce 1976). In southern California studies, Dover sole (*Microstomus pacificus*) collected from the Palos Verdes shelf have been found to have enlarged livers, as have individuals of this species exposed to Palos Verdes sediment in the laboratory (Sherwood and Mearns 1976).

The objectives of this preliminary study were to determine (1) if there were differences between the histologic characteristics of the livers of Dover sole collected from the Palos Verdes shelf and those of specimens from a control area and (2) if these differences were similar to those found in populations of English sole and starry flounder from contaminated and control areas in Seattle waters.

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METHODS

The Dover sole to be examined were selected from otter trawl collections taken in May 1976; the station locations and the size and condition of the specimens are given in Figure 1. Liver tissue from each specimen was removed and preserved immediately in 10 percent phosphate-buffered formalin. Subsamples from four different areas of each liver were processed. Paraffin-embedded tissue was sectioned at 4 microns and stained with Hematoxylin-eosin, periodic acid-Schiff reagent, Masson's trichrome stain, and May-Grunwald-Giemsa solution. Frozen tissue was sectioned at 7 microns and stained with Oil Red 0 and Sudan Black B.

RESULTS

The normal fish liver consists of branching sheets of tissue (muralia), each one or two cells thick, radiating out from central veins; these veins empty into the hepatic vein, which carries blood away from the liver. The sheets of tissue are separated by sinusoids, which receive blood from the hepatic artery and from the alimentary canal, pancreas, and spleen via the portal vein.

The livers of the Dana Point Dover sole exhibited the normal structure (Figure 2). There were lipid-containing vacuoles (or cavities) in the cytoplasm of most liver cells. Melanin-macrophage centers—collections of cells containing yellow-brown to black pigment—were found associated with the liver blood vessels; these were small, few in number, and vacuolated. Little fibrotic tissue was found between the liver cells.

In Palos Verdes specimens without fin erosion, there was an increase in the size and irregularity of fatty vacuoles in the liver cells. The structure of the tissue (muralia and sinusoids) showed evidence of disarray but was still recognizable (Figure 3). Melanin-macrophage centers were larger and more numerous, and there appeared to be an in-crease in the amount of interstitial fibrotic tissue present.

In two of the three Palos Verdes specimens with severe fin erosion, the changes were more extreme. Livers were characterized by severe vacuolation of cells, complete disarray of tissue structure with disappearance of sinusoid spaces, larger and more numerous melanin-macrophage centers, increased amounts of interstitial fibrotic tissue, and areas of degenerating liver cells (Figure 4). In one of these two specimens, there were spores of a myxosporidan, a protozoan parasite, in the liver tissue.

The condition of the liver of the third specimen with severe fin erosion (which was collected at Station 2, Figure 1) resembled that of the Palos Verdes specimens without fin erosion.

DISCUSSION

The pathologic lesions in the livers of Dover sole with severe fin erosion from the Palos Verdes shelf were similar to those seen in starry flounder from the

Duwamish River Estuary. These lesions included severe fatty vacuolation of the liver cells, structural disarray, degeneration of liver cells, and blood congestion. The enlarged nonvacuolated liver cells common in affected starry flounder were absent in affected Dover sole. Spores of a myxosporidan were found in starry flounder and English sole from all areas sampled in Puget Sound and did not appear to be associated with the liver lesions seen in specimens from the Duwamish River Estuary (Pierce 1976). Although affected individuals of species from both regions showed an increase in the size and number of melanin-macrophage centers, only in Dover sole did the increase appear to be associated with the occurrence of fin erosion. There are several possible explanations for this association. A common mechanism could be responsible for the development of both responses, or both could occur together independently if they were dependent on length or degree of exposure to the environment on the Palos Verdes shelf. It has been suggested that melanin-containing cells respond to exposure to toxic cyclic com-pounds because of the great affinity of melanin for these types of compounds (Roberts 1975).

Liver abnormalities in fishes have been reported to be caused by a number of factors, including infectious micro-organisms, parasites, improper diet, and exposure to toxic compounds such as chlorinated hydrocarbons. There was no indication that the Dover sole, English sole, and starry flounder from the waste discharge areas were affected by a systemic infection or were differentially parasitized in a manner that would result in the lesions observed. Improper diet was not examined and cannot be eliminated as a possible cause. However, in both areas, the bottom sediments and the fish themselves are contaminated with chlorinated hydro-carbons—PCB's in the Duwamish River Estuary and PCB's and DDT's off Palos Verdes. Additional field and laboratory studies will be required to investigate potential cause and effect relationships between the structural changes observed in liver tissue and environmental contamination and to determine the effects of such changes on liver function.

REFERENCES

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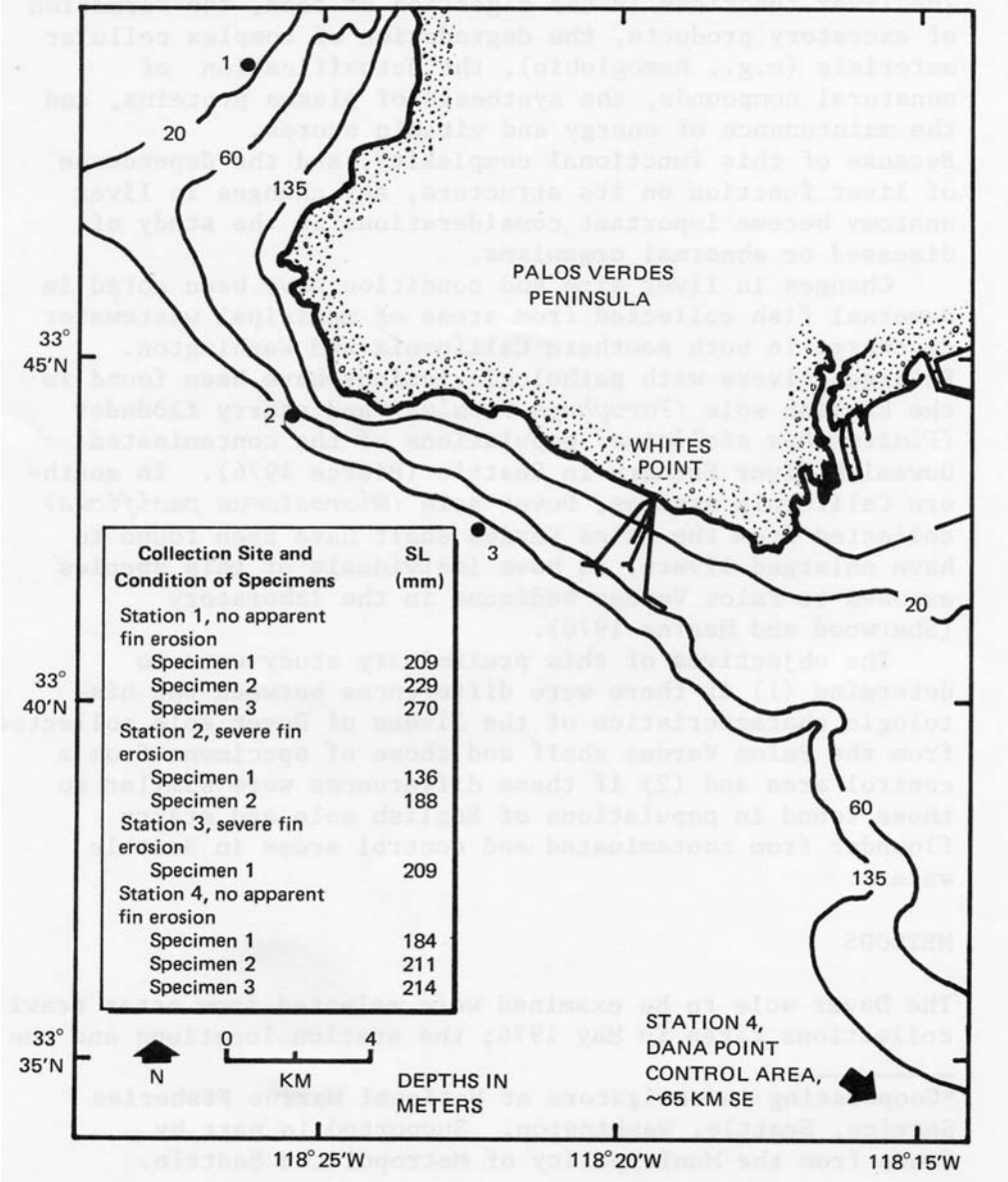

Collection Site and Condition of Specimens	SL (mm)
Station 1, no apparent fin erosion	
Specimen 1	209
Specimen 2	229
Specimen 3	270
Station 2, severe fin erosion	
Specimen 1	136
Specimen 2	188
Station 3, severe fin erosion	
Specimen 1	209
Station 4, no apparent fin erosion	
Specimen 1	184
Specimen 2	211
Specimen 3	214

Figure 1. Collection sites and condition of Dover sole used in liver histology study.

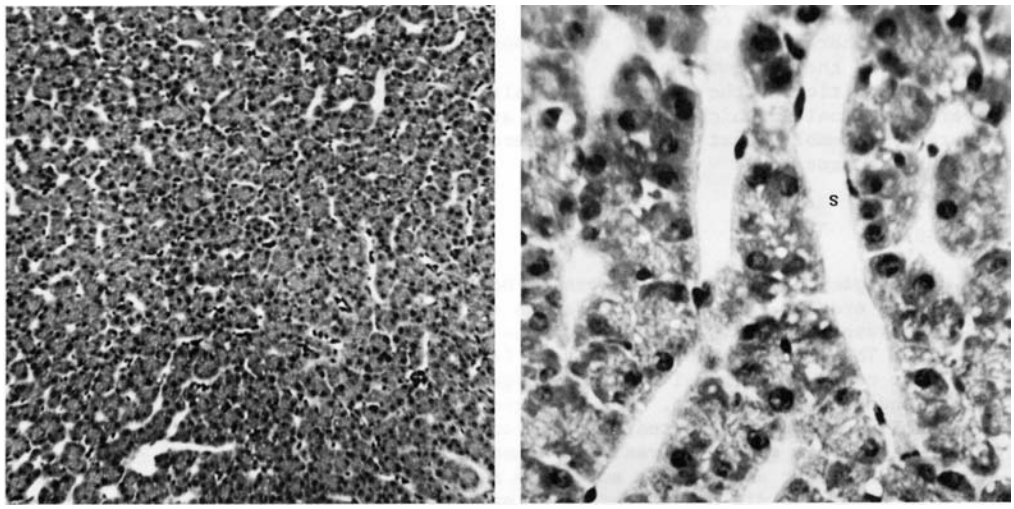

Figure 2. Liver from Dana Point Dover sole, stained with hematoxylin-eosin. Left: Cord structure, magnified 150 times. Right: Muralia and associated sinusoid spaces (S), magnified 900 times; note normal fatty vacuolation of liver cells.

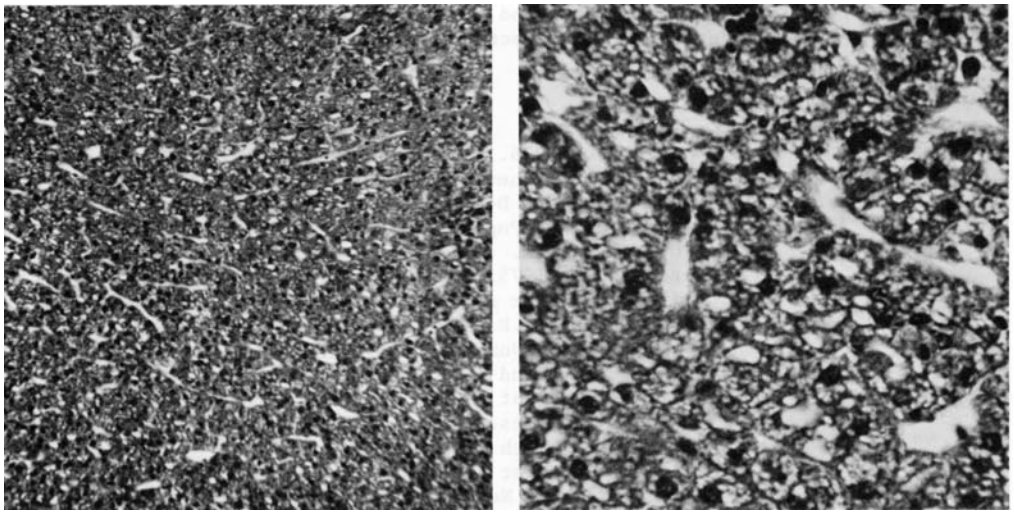

Figure 3. Liver from Palos Verdes Dover sole without fin erosion, stained with hematoxy-lin-eosin. Left: Cord structure magnified 180 times, showing slight distortion. Right: Muralia, magnified 630 times; note enlargement and irregularity of fat vacuoles.

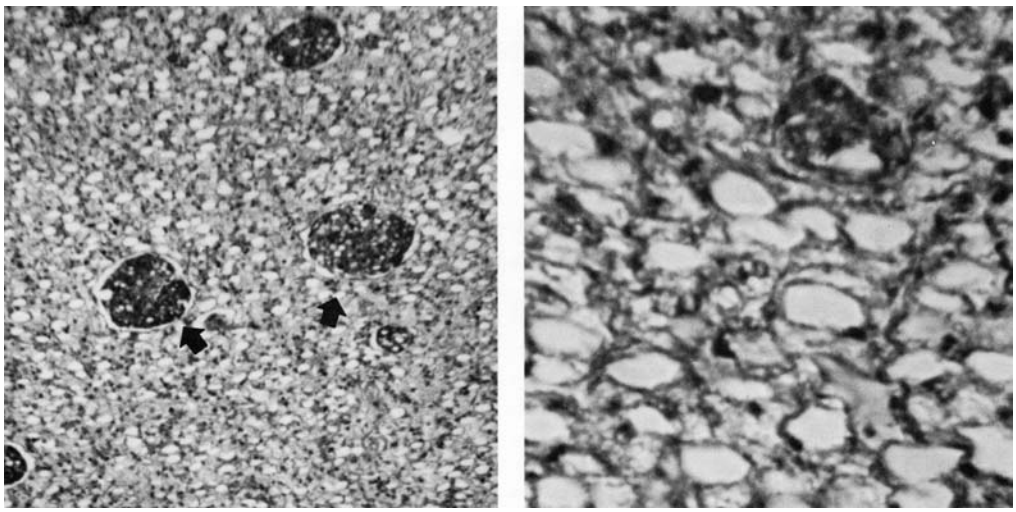

Figure 4. Liver from Palos Verdes Dover sole with severe fin erosion, stained with periodic acid-Schiff reagent. Left: Cord structure, magnified 210 times; sinusoid spaces are absent, and melanin-macrophage centers (arrows) are numerous and enlarged. Right: Muralia, magnified 940 times, showing extreme fatty vacuolation in liver cells.