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LYSOSOMAL MEMBRANE DAMAGE AS AN *IN VITRO* MARKER OF CONTAMINANT IMPACT UNDER FIELD AND EXPERIMENTAL CONDITIONS .

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ABSTRACT

These investigations examined lysosomal damage in a variety of cell types *in vitro* from a diverse range of marine animal species under both experimental and field conditions and compares the results with histopathological determinants of cell damage and tissue, particulate, sediment and water chemistry. The results demonstrated that membrane damage, as indicated by the reduced ability of the lysosomal compartment to retain the cationic probe neutral red, is a highly sensitive indicator of contaminant impact that is in good agreement with histopathological and immunocytochemical determinants of cell damage as well as to contaminant burdens

INTRODUCTION

Lysosomes are known to be linked to pathological changes in both plants and animals and these changes have been shown to be associated with a variety of degenerative disease conditions as well as to diseases induced by environmental pollutants. A remarkable feature of lysosomes is their ability to accumulate a diverse range of toxic metals and fat soluble organic chemicals including heterocyclic compounds and PCBs. The presence of these toxins is believed to contribute to the process of cell injury.

Damage to lysosomes has been demonstrated histochemically following contaminant impact as well as a result of non-toxic stressors, such as anoxia and starvation, in a variety of marine and terrestrial species. The principal

determinant of damage to lysosomes is membrane fragility, with associated changes including enlargement and increases in numerical density. However, one of the limiting factors of a histochemical approach to a study of lysosomal processes is that whilst it may be possible to demonstrate the enzymes the cell infrastructure is essentially morbid and cannot be manipulated further. By contrast *in vitro* studies offer the facility to further challenge the cells, and thereby their lysosomes, with a view to investigating the processes of lysosomal injury and cellular function as well as the more subtle effects of contaminant synergism's.

These investigations involved the use of *in vitro* cationic probe retention as a biomarker of lysosomal membrane damage in fish hepatocytes (*Limanda limanda*) as well as molluscan blood and digestive cells (*Mytilus edulis*, *Mytilus galloprovincialis* and *Littorina littoria*) under field (North Sea and Lagoon of Venice) and laboratory conditions. Lysosomal integrity is used as a predictive biomarker for impairment of the health of the individual animal and the results were correlated with available tissue, sediment and water contaminant burdens as well as with histopathological determinants of cell damage and tissue.

METHODS

The cells and tissues used for these studies are derived from a variety of sources and investigations including:

1. Dab liver cells and tissues from the ICES/IOC Bremerhaven Workshop Transect and from a collaborative study between the PML and the MAFF Fish Diseases Laboratory, Weymouth, investigating the pathological consequences of exposure to contaminated sediments
2. Laboratory exposures of the winkle (*Littorina littoria*) to the model hydrocarbon fluoranthene.
3. Laboratory exposure of mussel digestive cells *in vivo* to the model hydrocarbon fluoranthene.
4. Studies on mussel (*M.galloprovincialis*) blood cell lysosomes from field sites in the Lagoon of Venice.

The assay works on the principle that while undamaged lysosomes in healthy cells take up and retain the dye neutral red, by contrast, damaged lysosomes

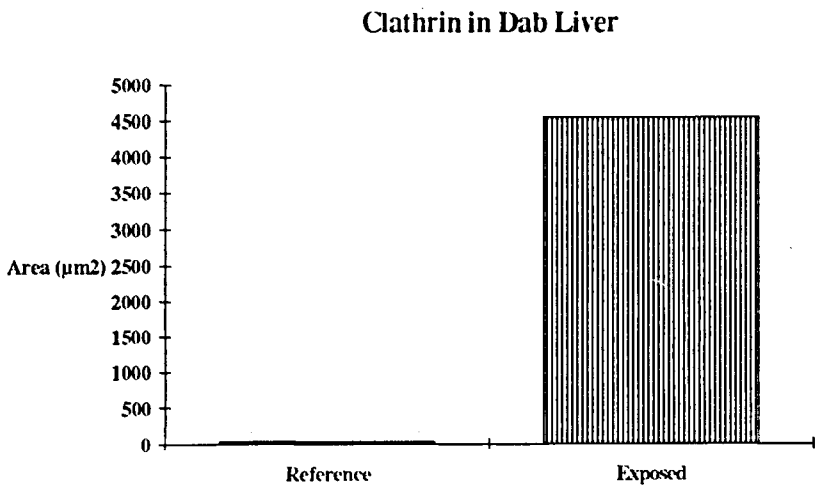

Fig. 6.

When the test was applied to digestive cells isolated from the gastropod mollusc *Littorina littoria* following experimental *in vivo* exposure to fluoranthene for 5 days at 440ppb the results indicated that retention time was significantly depressed ($P<0.01$ Fig. 7). However, following 2 days relaxation of the contaminant dosing the lysosomal membranes demonstrated a capacity for recovery and the retention time returned to that of the control cells.

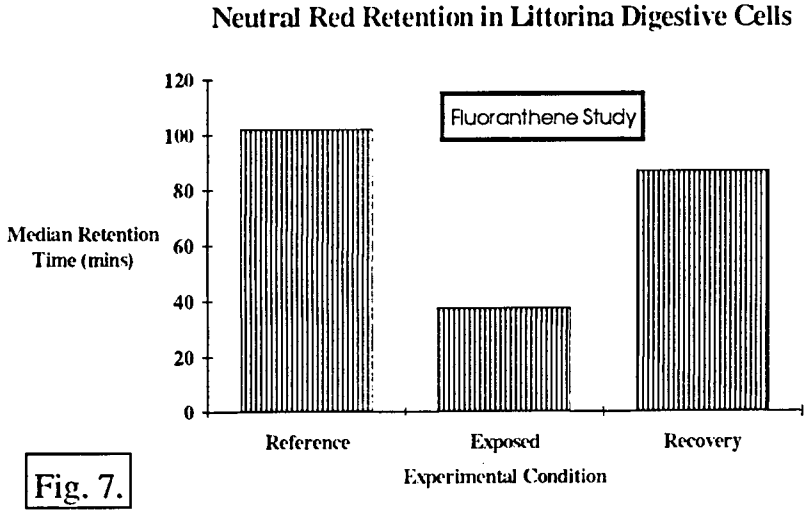

Fig. 7.

Figure 8 shows the effects following *in vivo* exposure for 7 days to the model hydrocarbon fluoranthene on the retention capacity of isolated mussel (*Mytilus edulis*) digestive cells. Once again retention capacity was significantly reduced ($P<0.01$). The total activity of the lysosomal marker enzyme N-acetly-ß-D-hexosaminidase (NAH) was also determined in the isolated digestive cells and found to be significantly elevated in the exposed mussels ($P<0.05$, Fig. 9).

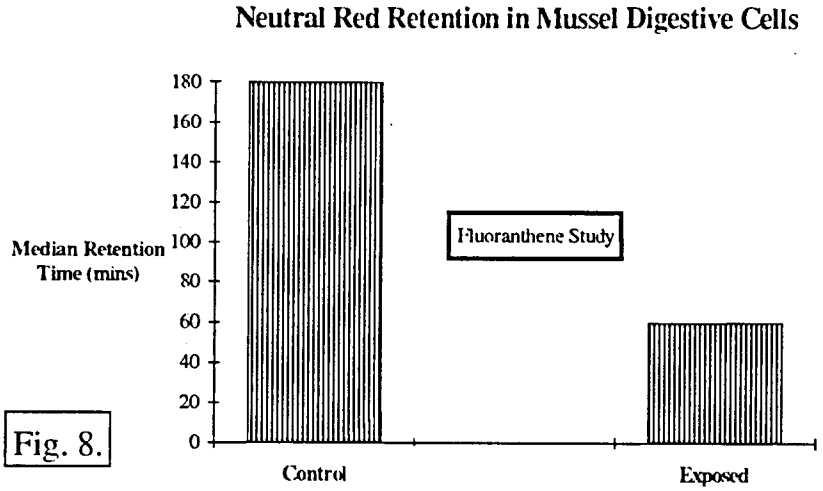

Fig. 8.

All of the results presented so far involve the use of cells isolated from their host tissues using enzymic digestion. This tends to be a time consuming process and one that does not readily lend itself to field work and so the method was modified for use on blood cells. Initial studies have concentrated on mussels.

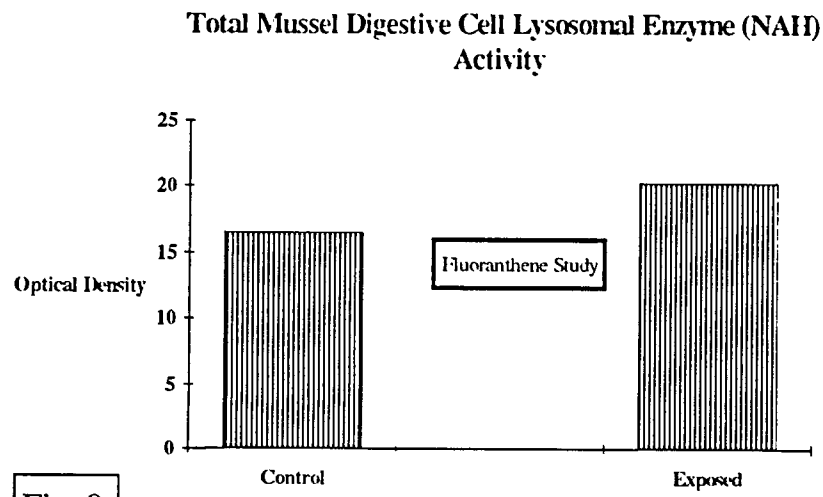

Fig. 9.

Figure. 10 shows that retention of the probe in blood cell lysosomes from the mussel *Mytilus galloprovinciali s* was significantly reduced at 2 contaminated sites in the Lagoon of Venice (Table 1) as compared to a reference

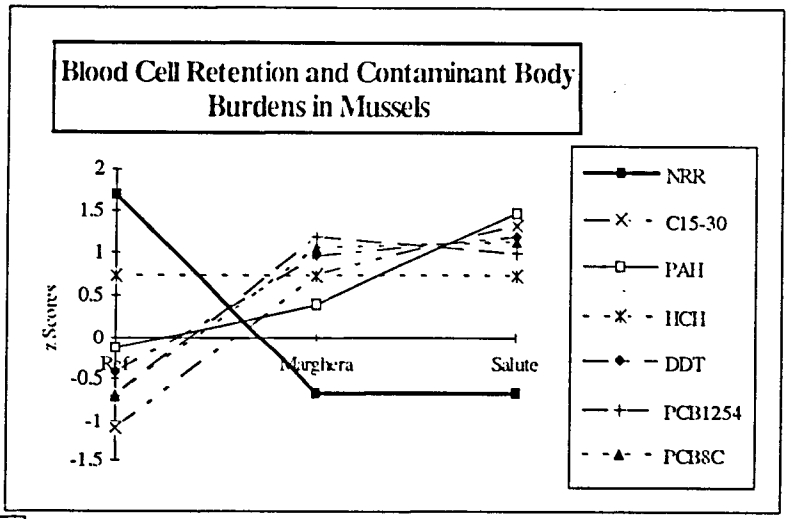

Fig. 10

also take up the dye but quickly lose it back to the cytosol. The time taken to lose the probe into the cytosol being a measure of the degree of damage.

RESULTS

Figure 1 shows the results of the analysis when applied to isolated dab hepatocytes taken from stations on the Bremerhaven Workshop transect. Dye retention was longest at station 7, which was the least contaminated site and shortest at stations 3 and 5, those being the most contaminated (Lohse 1990; Cofino *et al.* 1992).

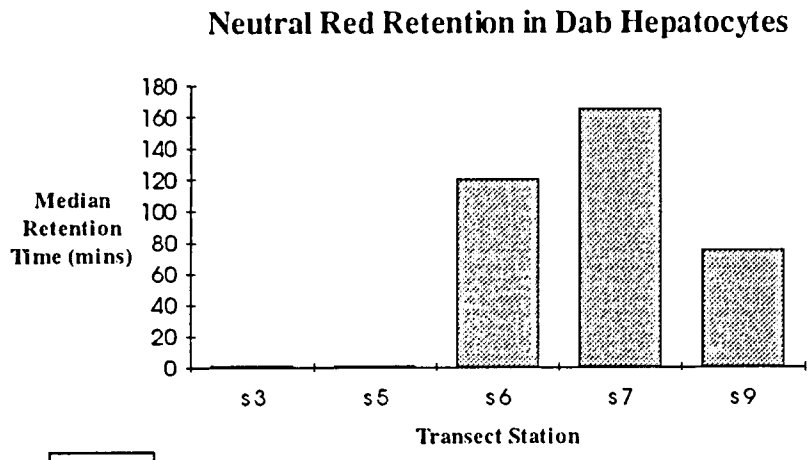

Fig. 1.

Stations 6 and 9 were significantly longer than 3 and 5 (Lowe *et al.* 1992) That damage had occurred to the liver cells was corroborated by histopathological evidence of atypical lipid accumulation in large cytoplasmic vacuoles.(Fig. 2)

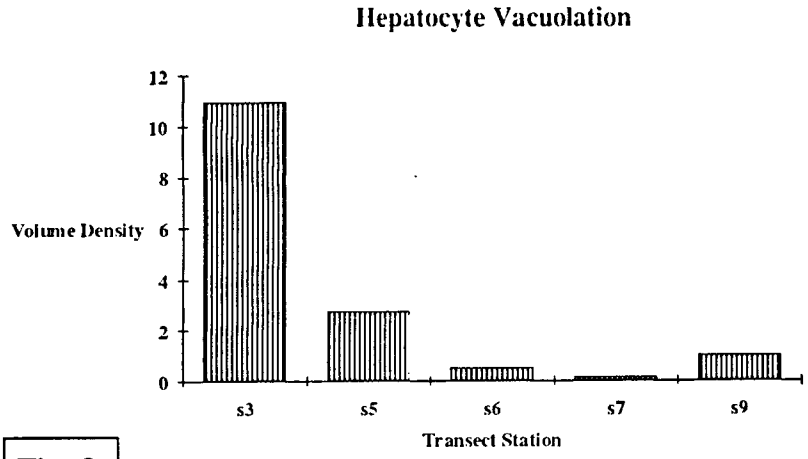

Fig. 2.

In an experimental study where dab were exposed to contaminated sediments from Liverpool Bay there was a significant decrease ($P<0.01$) in the retention time in isolated hepatocytes (Fig. 3) and a corresponding significant increase ($P<0.01$) in exposed dab for low density lipoproteins which are required as a source of cholesterol for membrane repair (Fig. 4) In addition,

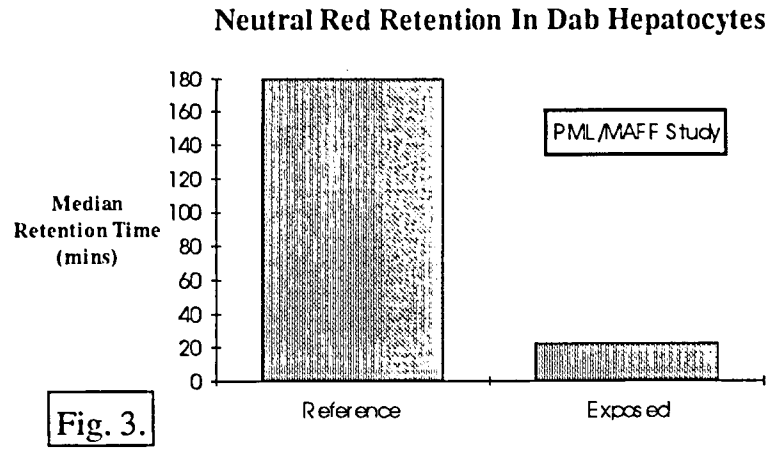

Fig. 3.

epidermal growth factor receptors were significantly increased (Fig. 5, P<0.03) which indicates a stimulus for the cells to divide for tissue regeneration.

There was also evidence of a significant increase in the levels of the structural protein clathrin (P<0.01 Figure 6) indicating enhanced endocytic processes probably, in this instance related to cell repair.

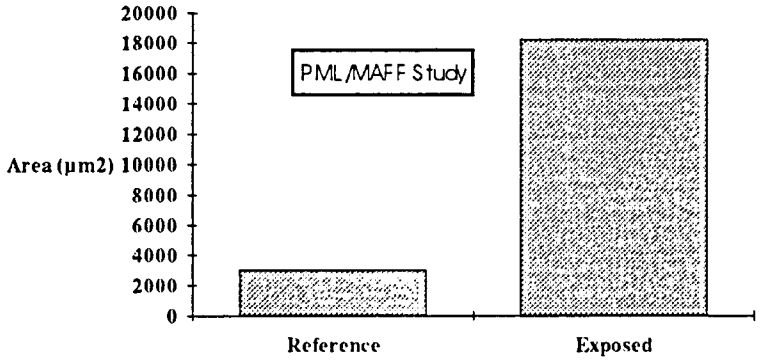

Fig. 4.

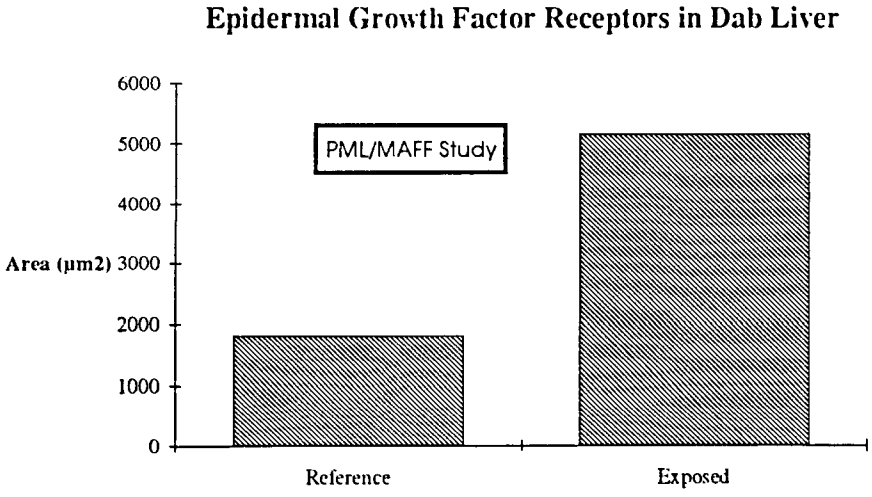

Fig.5.

site in the Adriatic Sea.

The results are plotted as z-scores ($\frac{x-\bar{x}}{\theta}$) in order to accommodate all of the data which have different ranges on a single plot; this does not effect the shape of the plot which remains the same as that of the raw data.

Table 1 Total body burdens of contaminants in mussels

Site	ΣC15-C30	PAH	ΣHCH	ΣDDT	PCB	PCB Σ8C
Reference	6.80	312	3.3	38.5	309	180
Marghera	28.87	358	3.3	110.7	1864	952
Salute	36.0	453	3.3	122.8	1702	987

C15-C30 total n-paraffin's, range nC_{15} - nC_{30}
PCB quantified as Aroclor1254
PCB Σ8C total of 8 congeners

DISCUSSION

In conclusion these studies indicate that lysosomal membrane damage, as demonstrated by a reduction in the capacity to retain the cationic probe neutral red, is in good agreement with elevated tissue contaminant burdens. The use of neutral red retention as a biomarker of lysosomal damage and cell injury is supported by histopathological evidence of cellular dysfunction and enhanced tissues maintenance and repair in the contaminated animals.

Acknowledgements
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