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Environmental Contamination and Toxicology

Submicroscopic Hepatic and Renal Pathology of a Teleost Maintained in Sewage

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Sewage is a major contaminant of freshwater; it forms an integral part of the ecological picture, particularly of countries with a socio-economic order like that of India. Surprisingly, however, practically no attention has been paid to this pollutant in India. This laboratory has undertaken the study of the biological ill-effects of sewage on *Heteropneustes fossilis* and other freshwater fish of Gorakhpur. The blood picture of *Heteropneustes fossilis* exposed to sewage (Narain and Srivastava 1979; Srivastava and Narain 1981, 1985; Narain and Nath 1982; Narain et al. unpublished) presents features which are known clinical signs of disorder in tissues, particularly liver and kidney (Narain 1981). Histological observations (unpublished work) also reveal hepatic and renal damage in this stressed fish. The present study aims to further confirm, through electron microscopy, the production of damage in the liver and kidney of *Heteropneustes fossilis* subjected to an environment polluted by sewage.

MATERIALS AND METHODS

Sewage samples were collected from the mostly open sewer which carries refuse from the major part of Gorakhpur region to Ramgarh Lake, the chief freshwater body of the region (Narain and Srivastava 1979).

Chemical analysis of the sewage samples used for experiments and the tap water used as control medium revealed as follows:

	Sewage	Control (tap water)
pH	8.00	7.300
Dissolved oxygen	....	7.200 ppt
Free carbon dioxide	19.50 ppt	90.000 ppt
Total nitrogen	0.30 ppt	0.002 ppt
Ammonia nitrogen	1.75 ppt	0.002 ppt
Phosphate	43.59 ppt	0.010 ppt
Sulphate	0.18 ppt	0.004 ppt
Bicarbonate alkalinity	0.45 ppt	0.001 ppt
Calcium	0.11 ppt	0.060 ppt

ppt = parts per thousand

Bacterial culture, fungal culture and microscopic examination of sewage samples revealed the presence of the following micro-organisms:
Bacteria:-*Pseudomonas* sp., *Klebsilla* sp. and *Bacillus subtilis*; Fungi:- *Candida* sp.; Ciliates, ++; Flagellates, +; Other protozoans, ++; *Ascaris* ova, +/-.

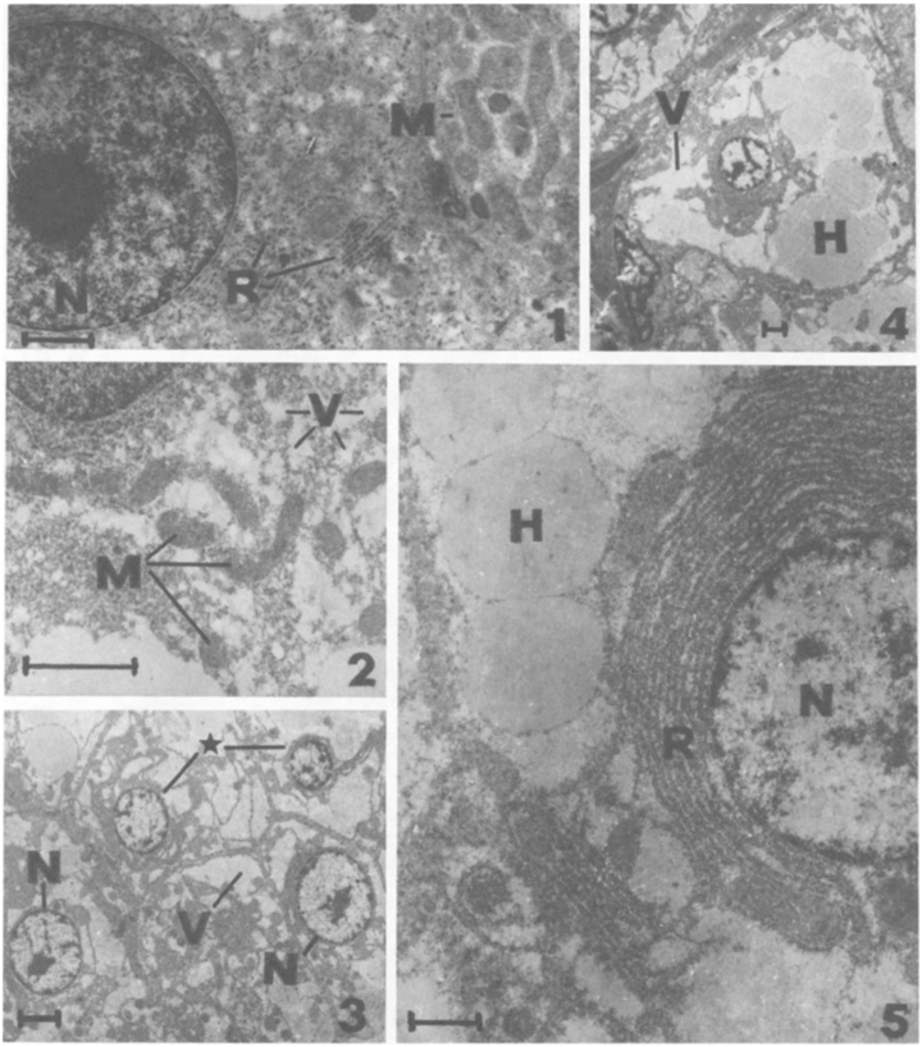
Fish were collected from unpolluted habitats, and were maintained for 2-3 weeks in tap water. The fish were fed dried shrimp powder during this period. They were then transferred to cylindrical glass aquaria (diam, 18 in; capacity, 12 l) each containing 10 fish (body weight, 12-24 g; fork length, 10-17 cm) in 10 l of medium. The experimental aquaria contained sewage diluted to 25% with tap water, while the control aquaria contained unpolluted tap water. The medium of all the aquaria was changed every 24 hr. The experiment was continued for 2 weeks. The fish were not fed during this period. The experiment was repeated 5 times, and was conducted at room temperature of 35-40°C.

Liver and kidney tissues of experimental and control fish were fixed in 3% glutaraldehyde and 1% osmic acid at the end of 2 weeks. Ultrathin sections were stained with lead citrate, and observed under a Philips EM 301 transmission electron microscope.

RESULTS AND DISCUSSION

Almost all the hepatocytes of exposed fish showed extensive cytoplasmic vacuolization. The vacuoles were localized around the mitochondria (Fig. 2) or coalesced so that the entire cytoplasm was affected (Fig. 3). The vacuolated areas contained hyaline bodies which were mostly rounded to ovoid in shape, varied in size, and were lined by a thin membrane(Figs. 4, 5). The mitochondria decreased in number. Their cristae became indistinct, and the matrix appeared dense and homogeneous (Fig. 6). The RER was markedly disorganized and the strands were usually concentrated around the nucleus (Fig. 5). Due to RER damage, the free ribosomes increased in number (Fig. 6). The macrophageal activity of the sinusoids increased. Macrophages were observed frequently; they contained vacuoles with or without granular debris, electron-dense bodies, lysosomes, autophagic vacuoles enclosing electron-dense matter, and engulfed blood cells many of which were degenerated (Figs. 7, 8). The nuclei were often greatly shrunken, and showed nucleolar degeneration (Fig. 3). Sometimes, binucleate hepatocytes were also observed (Fig. 9).

The cells lining the sinusoids of the liver, however, looked normal (Fig. 10).



<u>Liver (Control)</u>:

Figure 1. Hepatocyte showing: nucleus (N); mitochondria (M); RER (R). Scale, 1 µm

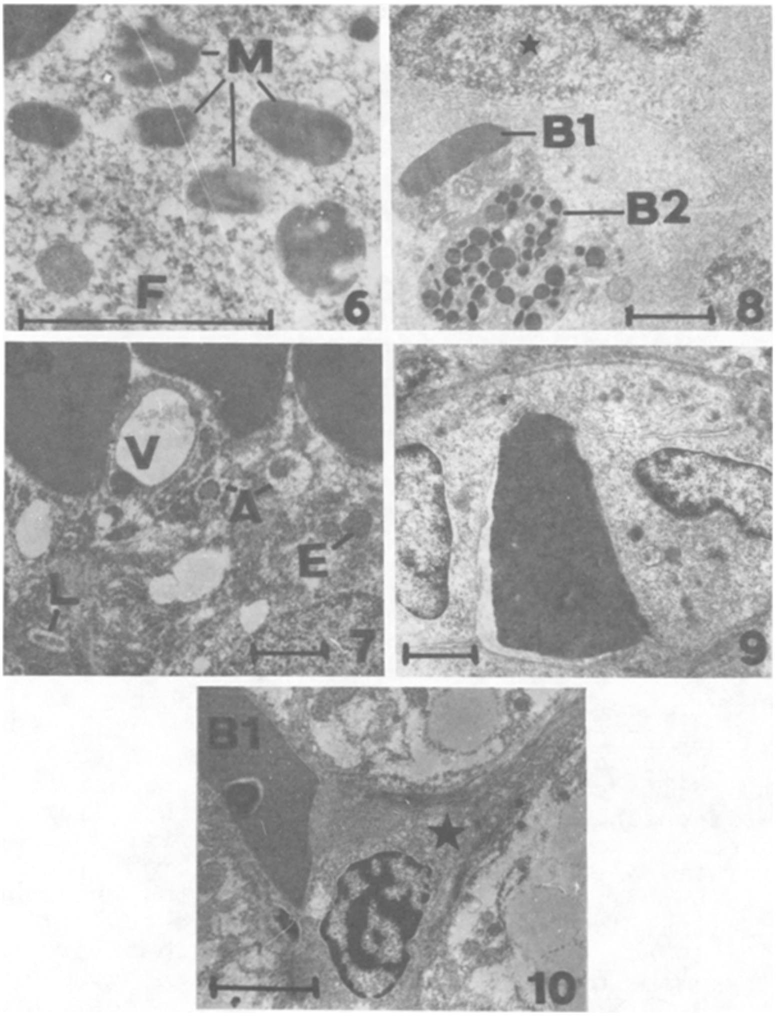
<u>Liver (Experimental)</u>:

Figure 2. Hepatocyte showing: vacuoles (V) around mitochondria (M). Scale, 1 µm

Figure 3. Hepatocytes showing: vacuolated areas (V); normal nuclei (N); shrunken nuclei (asterisk) having degenerated nucleoli. Scale, 1 µm

Figure 4. Hepatocyte showing: vacuolated area (V) containing hyaline bodies (H). Scale, 1 µm

Figure 5. Hepatocyte showing: disorganized RER (R) concentrated around nucleus (N); membrane-bound hyaline bodies (H). Scale, 1 µm



Liver (Experimental):

Figure 6. Hepatocyte showing: damaged mitochondria (M); free ribosomes (F). Scale, 1 μm

Figure 7. Macrophage showing: vacuoles (V); electron-dense bodies (E); lysosomes (L); autophagic vacuoles (A). Scale, 1 μm

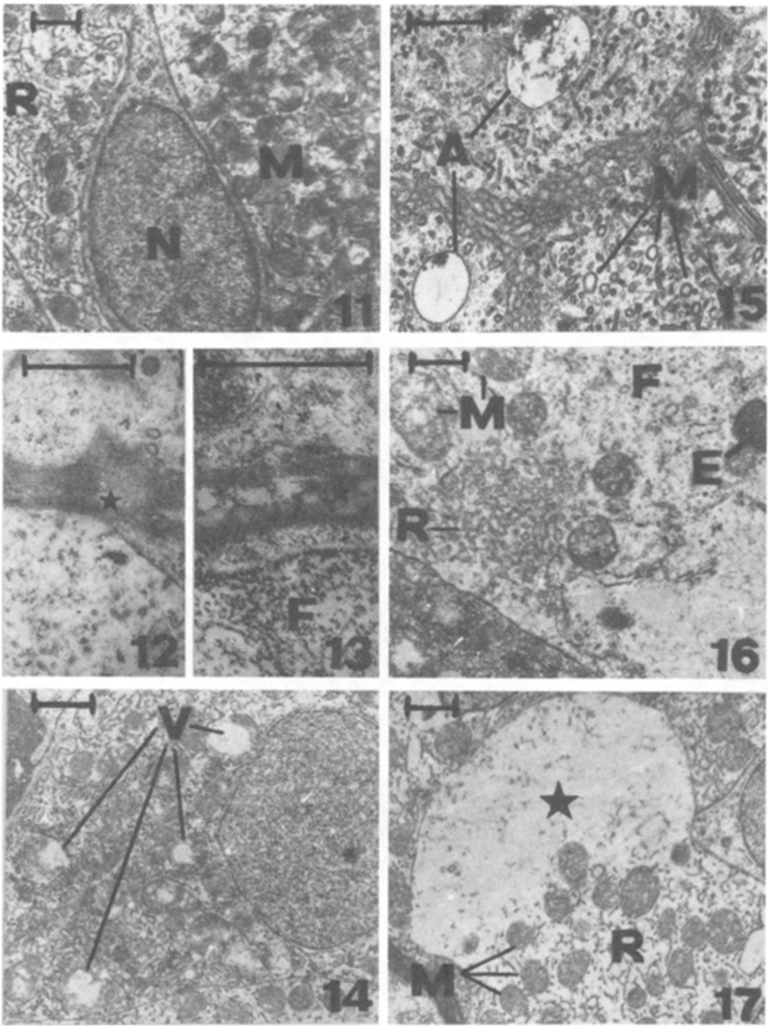
Figure 8. Macrophage showing engulfed: erythrocyte(B1); leucocyte (B2); damaged blood cells (asterisk). Scale, 1 μm

Figure 9. A binucleate hepatocyte. Scale, 1 μm

Figure 10. Sinusoid showing: lining cell (asterisk);red blood cell (B1). Scale, 1 μm

The cells lining the kidney tubules of exposed fish frequently showed cytoplasmic vacuolization, and their basement membrane presented a disrupted appearance (Figs. 13,14). The mitochondria frequently became vacuolated and swollen and presented a disrupted appearance, their cristae becoming indistinguishable (Figs. 15, 16, 18). The RER frequently got disrupted, and clusters of its fragments could be observed (Fig. 16). Consequently, free ribosomes increased in number (Figs. 13, 16, 18, 21). Many electron-dense hyaline bodies, and a few autophagic vacuoles containing granular debris were also observed (Figs. 15, 16, 18). Cellular destruction was widespread, and most cells had portions where no subcellular elements could be distinguished (Fig. 17). Many cells also showed fibrosis; some of these cells were completely fibrosed, and no organelles except a few mitochondria could be discerned, the entire cytoplasmic area being filled with fibrous matter and granular debris (Figs. 18, 19). The lumen of the tubules was invariably engorged with various kinds of cellular and non-cellular debris (Fig. 20). Some cells were seen to contain accumulations of rounded or oval bodies which had an electron-dense core and looked like virus particles (Fig. 21).

The submicroscopic hepatic and renal pathology of stressed Heteropneustes fossilis generally reflects a widespread degenerative and necrotic inflammatory condition. The condition seemingly tends to assume the nature of chronicity in case of kidney since fibrosis developes within many of the effected cells; proliferative lesions, progressing to fibrosis, are known to appear during chronic inflammation when the acute inflammatory lesions do not resolve quickly (Roberts 1978). Tubular necrosis and fibrosis are also known symptoms of hepato-renal syndrome, a pathological condition caused in fish by dietary imbalances (Cowey and Roberts 1978; Roberts 1978). A nutritional disorder could also be indicated by the degenerative changes observed in the liver of stressed Heteropneustes fossilis. The hyaline bodies, so frequently observed within the highly vacuolated hepatocytes, could be representing fatty infiltration of liver, and the macrophagic invasion of liver tissue could indicate an aggravated state of degenerative fatty change of hepatocytes. These features are known (Cowey and Roberts 1978) to exist in fish suffering from lipoid liver disease, a nutritional disease occurring mostly on account of diets containing rancid fats. Stressed Heteropneustes fossilis are very likely to be faced with unusual dietary situations, specially those on account of rancidity of food in the environment, because the environment of the fish is contaminated with sewage and hence subjected to organic enrichment and putrefaction.



Kidney tubule cells (Control):

Figure 11. Showing: nucleus (N); mitochondria (M); RER (R). Scale, 1 µm

Figure 12. Showing basement membrane (asterisk). Scale, 1 µm

Kidney tubule cells (Experimental):

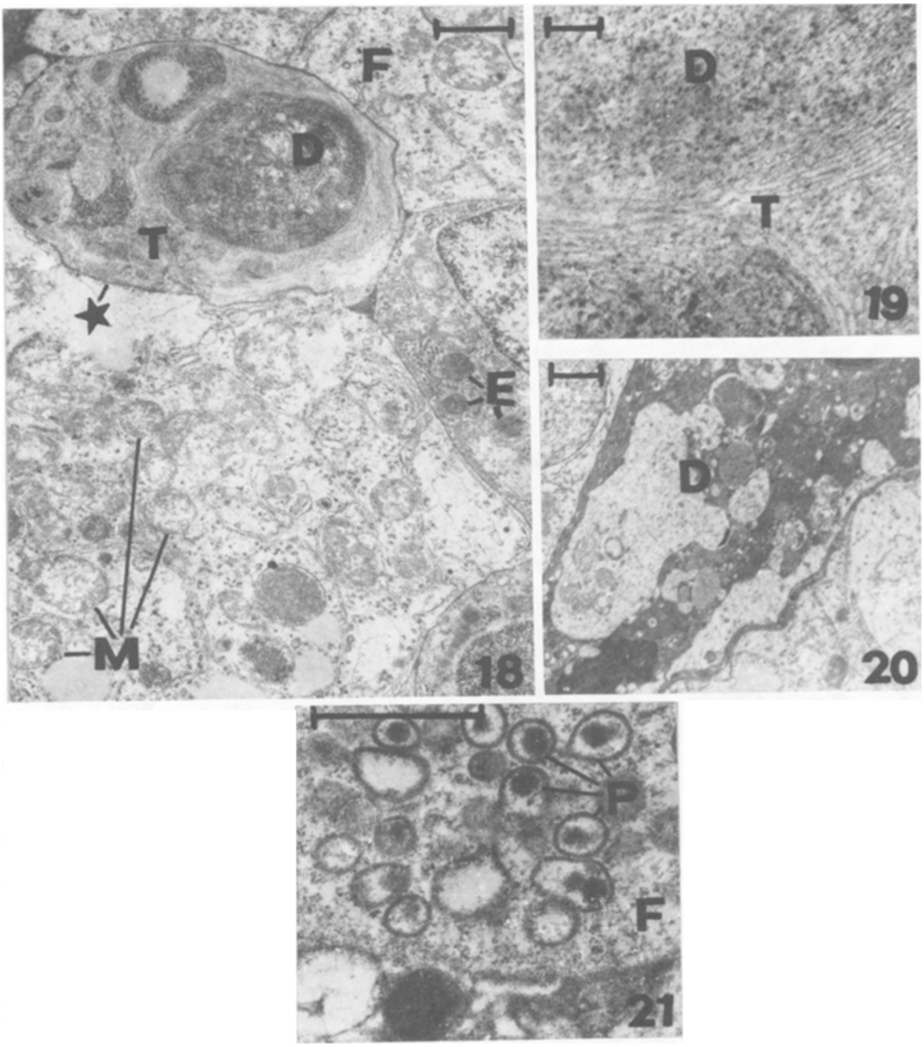
Figure 13. Showing: disrupted basement membrane (asterisk); free ribosomes (F). Scale, 1 µm

Figure 14. Showing: cytoplasmic vacuoles (V). Scale, 1 µm

Figure 15. Showing: vacuolated mitochondria (M); autophagic vacuoles (A). Scale, 1 µm

Figure 16. Showing: RER fragment cluster (R); swollen, vacuolated mitochondria (M); electron-dense body (E); free ribosomes (F). Scale, 1 µm

Figure 17. Showing: damaged area (asterisk); normal mitochondria (M); RER (R). Scale, 1 µm



<u>Kidney tubule cells (Experimental)</u>:

Figure 18. Showing: completely fibrosed cell (asterisk) filled with fibrous matter (T) and granular debris (D); adjacent cells with many disrupted looking, swollen and vacuolated mitochondria (M), free ribosomes (F), and electron-dense bodies (E). Scale, 1 µm

Figure 19. Showing: fibrous material (T); granular debris (D). Scale, 0.1 µm

Figure 20. Showing assorted debris (D) filling tubular lumen. Scale, 1 µm

Figure 21. Showing: accumulation of unidentified bodies (P)(virus?); free ribosomes (F). Scale, 1 µm

It has constantly been felt in this laboratory that sewage refuse is not as insignificant a polluting eco-factor as it has been made to appear by the negligible attention which it has attracted in comparison to other pollutants like heavy metals, pesticides and detergents. In fact, in a broad comparison with the agrochemicals tested here on fish, sewage has been found to be more toxic in many respects (Narain and Srivastava 1979). The marked subcellular damage to vital organs like liver and kidney, as observed in the present study, would further substantiate this view.

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