Arch. Environ. Contam. Toxicol. 24, 432–439 (1993)

ARCHIVES OF
Environmental
Contamination
and Toxicology

Responses of Hemocytes and Gill Tissues to Sublethal Cadmium Chloride Poisoning in the Crab *Paratelphusa hydrodromous* (Herbst)

B. Victor

Department of Postgraduate Studies and Research in Zoology, St. Xavier's College, Palayamkottai-627 002, India

Abstract. The hemocytic and gill tissue responses of the crab *Paratelphusa hydrodromous* (Herbst) to a wide range of sublethal concentrations of cadmium chloride (0.02–0.50 μM/L) were examined after a 30-day exposure using hemocyte counts (THC and DHC) and lamellar pathology. A continued reduction in the hemocyte counts and selective changes in the numbers of hyalinocytes and eosinophilic granulocytes was evident in the toxified crabs. Under sublethal stress, the hyalinocytes developed eccentric nuclei, granular cytoplasm and membrane blebs. Atypical shape, lobate nucleus, dense cytoplasmic deposits and granuloplasmic vacuoles were frequently observed in the granulocytes. Greater proliferation of prohemocytes and abnormal hemocyte morphology indicated cadmium-induced neoplastic transformation of hemocytopoietic organs. No major structural changes in the gills were noted at 0.02 μM $CdCl_2$. Nodular gill disease (NGD), hemocytic hyperplasia and sloughing of walled off hemocytes were prominant lesions after 0.50 μM cadmium chloride exposure.

Most laboratory studies evaluating toxicity of cadmium to decapod crustaceans are concerned with the lethal concentrations (Thorp and Lake 1974; Ahsanullah 1976; Ahsanullah and Arnott 1978), strategies of accumulation (Wright 1977; Jennings and Rainbow 1979; White and Rainbow 1982, 1986; Rainbow and White 1989), localization in tissues (Nimmo *et al.* 1977), and changes in gill morphology (Papathanassiou and King 1983). Hemocytes are of known importance in the internal defenses of crustacea against microorganisms (Robin 1970; Sinderman 1971; Fontaine and Lightner 1975). The applicability of hemocyte counts for the elucidation of pathological conditions has also been suggested (Mix and Sparks 1980; Martin and Graves 1985). However the effect of metal pollutants on the hemogram during pathologic conditions has not been critically studied. It was the aim of this investigation to study the role of hemocytes during different pathological states of *Paratelphusa hydrodromous* (Herbst) exposed to sublethal doses of cadmium chloride.

Methods

Test Animal

Paratelphusa hydrodromous (Herbst) (Decapoda: Paratelphusidae) were collected during the early part of the breeding season (December–January) (Anilkumar and Adiyodi 1980), from irrigation canals of the river Thambraparani, Tamil nadu, India, Prior to experimentation, they were acclimatized for at least two weeks to 29 ± 1°C water temperature, 6.51 ± 0.21 ml/L, dissolved oxygen, 7.6 ± 0.1 pH, and 165 ± 10 mg/L total hardness (as $CaCO_3$).

Acute Toxicity Bioassay

A preliminary 96-h acute toxicity bioassay with $CdCl_2.2H_2O$ (reagent grade) was conducted in 2-L shallow glass troughs with 10 intermoult (hard shelled) crabs of uniform size (carapace width 2.80 ± 0.5 cm, weight 7.75 ± 0.50 g). All tests were static and the bioassay was repeated three times. Observations for mortality was made twice daily at regular intervals. The bioassay procedures used were those recommended by the Committee on Methods of Toxicity Tests with Aquatic Organisms (1975). The regression method of Finney (1971) was used to calculate the LC_{50} values.

Experimental Design

To study the hemogram responses, healthy adult female crabs weighing 12–14 g size were selected from the crabbery and were treated with graded doses of sublethal cadmium chloride solution (0, 0.02, 0.05, 0.10, 0.20, 0.40, and 0.50 μM $CdCl_2$/L) for 30 days. The selection of the sublethal concentrations was based on the results of acute bioassay studies. Forty animals (four groups of ten) were tested at each dose level. The crabs in exposures were fed *ad libitum* of minced fish *Lepidocephalichthys thermalis* supplemented with goat liver. The test media were replaced thrice a week.

Table 1. Lethal concentrations of cadmium chloride to *P. hydrodromous*

Exposure time (h)	Slope	Intercept	LC_0 (μM/L)	LC_{50} (μM/L)	LC_{100} (μM/L)
24	1.363	1.943	11.803	85.770	188.850
48	1.577	2.384	5.902	37.574	94.426
72	1.664	3.002	1.475	12.836	4.721
96	1.226	4.434	0.197	1.623	2.361

LC_0 and LC_{100} are actual values and LC_{50} is estimated

Hemolymph Smears

Hemolymph samples from the heart or pericardial sinus were taken every 3 days. Thin hemocyte films were prepared by carefully spreading a drop of unfixed hemolymph. They were then air-dried, fixed in 10% formalin-methanol (1:9) and flooded in Giemsa stain (Cornick and Stewart 1978). The identification of hemocyte types were based on cell size, shape, and staining properties (Wood and Visentin 1967).

Hemocyte Counts

The counting of free hemocytes (THC) was done by using a hemocytometer with improved double Neubauer ruling and a diluting fluid suitable for crustaceans (Stewart *et al.* 1967). Whenever cell rupture, agglutination and plasma clot formation appeared either in the dilution pipette or in the counting chamber, the count was not made.

Differential hemocyte counts (DHC) were made on freshly stained smears following the method of Mix and Sparks (1980). The different types of hemocytes were counted at random under the microscope (×400) and the number of each cell type expressed in percentage, to which the following formula was applied.

$$\text{DHC (\%)} = \frac{\text{number of cells of a type}}{\text{total number of cells counted}} \times 100$$

Histological Technique

Gill tissues from all control and treated animals of four sublethal doses (0.02, 0.05, 0.25, 0.50 μM $CdCl_2$/L) were dissected out after 30 days in crustacean ringer solution (Van Harreveld 1936) and fixed for at least 24 h in Bouin's fluid or Helly's fluid. Paraffin blocks were made with sections of 6–8 μm. They were stained with Harri's haematoxylin and Bowie's eosin or Masson's trichrome.

Results

The data of the cadmium bioassays performed on *P. hydrodromous* are presented in Table 1. The 24-h to 96-h lethal concentration of cadmium chloride to the crabs ranged from 85.770 to 1.623 μM/L.

Normal Hemocyte Morphology and Hemogram

In *P. hydrodromous*, prohemocytes (pr) numbered 8–9% of the total hemocyte population. They were the smallest hemocytes with a diameter of 5–6 μm and centrally placed large nuclei (Figure 1). Hyalinocytes (hy) varied from 26 to 28% of the total population. They were observed in a variety of shapes (8–12 μm in diameter). The nucleus occupied more of the cytoplasm as the cell matured. Basophilic granules were occasionally noted in the cytoplasm. Intermediate granulocytes (ig) of 10–12 μm diameter accounted for most of the hemocyte population (42–45%) (Figure 2). The eosinophilic granulocytes (eg) represented 20–25% of the circulating hemocytes. They were the largest of the hemocytes observed (14–16 μm). The nuclei were eccentric and the cytoplasm contained coarse granules (Figure 3).

Pathology of Hemocytes

Several hemocyte pathologies were observed at the light microscope level, when *P. hydrodromous* were sublethally treated with cadmium chloride for 30 days. In hyalinocytes, the nuclei were pushed towards the periphery and the cytoplasm became more granular (Figure 4). The eosinophilic granulocytes invariably developed granuloplasmic vacuoles, membrane blebs, and dense cytoplasmic deposits (Figure 5). Some of the granulocytes were aberrant in shape with lobate nuclei and highly vacuolized cytoplasm (Figure 6). Achromophilia of cytoplasm was observed in a few cells (Figure 7).

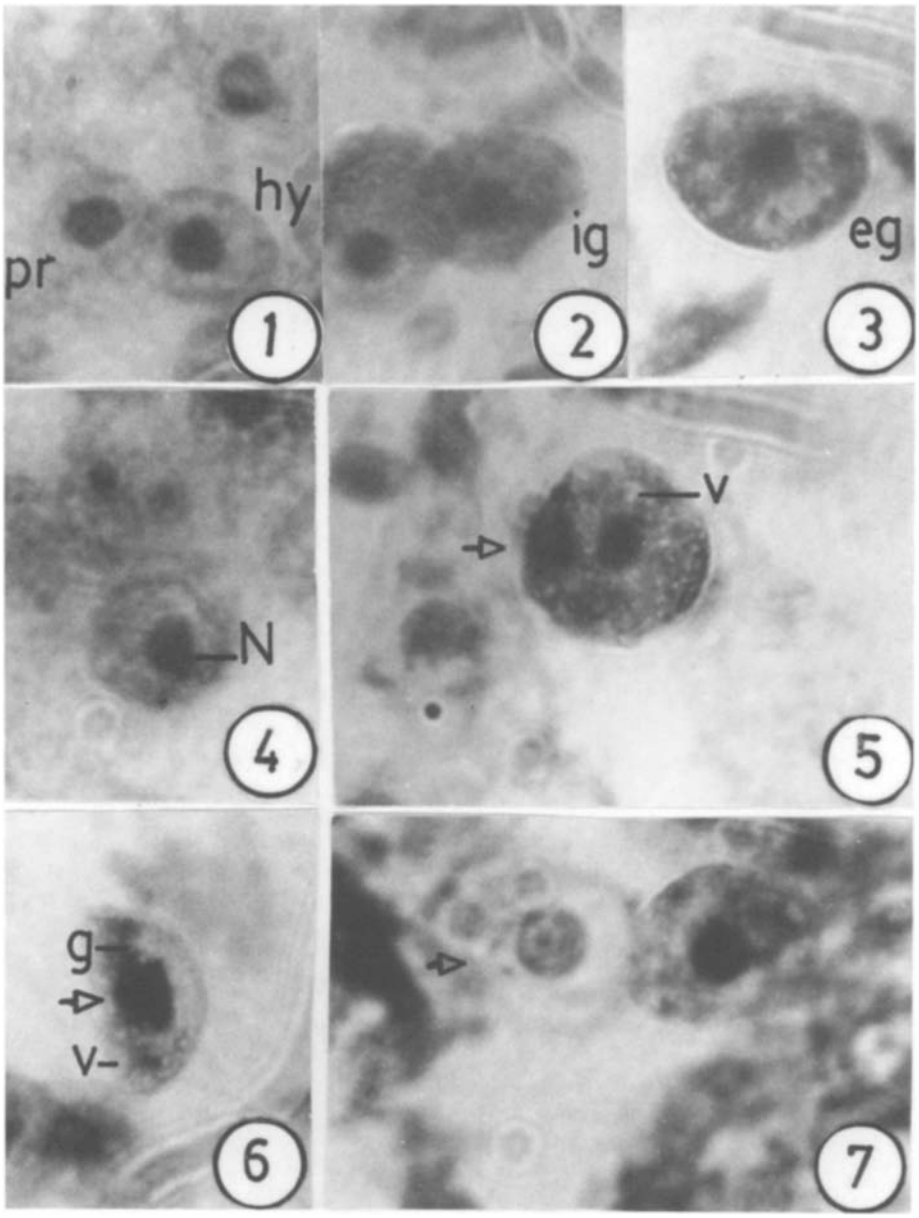
Hemogram Response

Exposure of *P. hydrodromous* to increasing sublethal levels of cadmium chloride induced significant variation in total and differential hemocyte counts (Tables 2–3). The total number of circulating hemocytes was drastically reduced to over the control at concentrations above 0.20 μM/L. Higher cadmium stress selectively reduced the percentage occurrence of hyalinocytes and eosinophilic granulocytes to 21% and 32%, respectively and enhanced the counts of prohemocytes and intermediate granulocytes to 69% and 18%, respectively. In general, prohemocytes and eosinophilic granulocytes exhibited greater coefficient of variation of 15–16% after cadmium chloride exposure.

Branchial Pathology and Hemocyte Behavior

Gills of crabs in the control exhibited no pathological alterations (Figure 8). The branchial lamellae of *P. hydrodromous* histologically resembled to that of *Palaemon serratus* (Papathanassiou and King 1983). The respiratory epithelium composed of a layer of flattened squamous cells. Internal to the epithelium was the hemolymph channel transversed at regular intervels by transverse and central cells (Figure 9). The outer lamellar surface was covered by a thin chitinous layer.

Gills of *P. hydrodromous* exposed to 0.02 μM/L of cadmium chloride for 30 days showed swelling and disruption of central cells (Figure 10). No major structural changes that could be



Figs. 1–3. Normal hemocyte morphology of *P. hydrodromous* (× 1500)
1 Prohemocyte (pr) and hyalinocyte (hy). **2** Intermediate granulocyte (ig) and eosinophilic granulocyte (eg). **3** Another focus of eosinophilic granulocyte (eg)
Figs. 4–7. Hemocytic pathology of *P. hydrodromous* induced by sublethal toxicity of cadmium chloride for 30 days. **4** Hyalinocyte showing eccentric nucleus (N) and granular cytoplasm. **5** Eosinophilic granulocyte exhibiting granuloplasmic vacuoles (v) and dense cytoplasmic deposits (arrows). **6** An aberrant eosinophilic granulocyte showing lobate nucleus (arrow) large granular masses (g) and many vacuoles (v). **7** A degranulated granulocyte (arrow) and another hypertrophied granulocyte

attributed to the dose were detected. At exposures of 0.05 μM/L, gills of test crabs exhibited necrotic foci and aggregation of blackened hemocytes around injured tissues (Figure 11). Many of the hyalinocytes showed large wavy frills pseudopodia and some of them stretched out into fibroblast-like cells. Cyto- and histotoxicity of gills were evident at 0.25 μM/L of cadmium chloride. The lamellae were irregularly disposed and the hemolymph channels were ruptured at many points (Figure 12). Proliferation of hemocytes and adherence of them to branchial lacunae were increasingly apparent. The necrotic epithelial cells were strongly vacuolized with hypertrophied nuclei (Figure 13). Penetration of granulocytes was observed in the epithelial cells displaying vacuolar degeneration. Obstruction of the hemolymph vessels by intravascular cellular clots and hemostasis were further severe pathological changes.

Nodular formations (=nodular gill disease, NGD) were extensively observed at 0.50 μM $CdCl_2$/L (Figure 14). Disruption of hemocytes was evident in many of the necrotic lesions. In the centers of more advanced lesions, hemocytic hyperplasia, and sloughing of walled-off hemocytes were noticed (Figure 15).

Discussion

Cadmium chloride, above 1.623 μM/L in the medium, proved to be acutely lethal to the crab *P. hydrodromous*. The results of the present study were comparable to the values reported for other decapod crustaceans (Eisler 1971; Collier *et al.* 1973; Thorp and Lake 1974).

Highly significant ($P < 0.001$) and dose-related decrease in total hemocyte counts were observed in the crab *P. hydrodromous*. This hemocytopenic response may be resulted from reduction in the counts of hyalinocytes and eosinophilic granulocytes ($P < 0.001$ and < 0.05). More proliferation of prohemocytes and intermediate granulocytes clearly demonstrated the neoplastic transformation of hemocytopoietic or-

Table 2. Total counts of the circulating hemocytes of *P. hydrodromous* exposed to sublethal concentrations of cadmium chloride for 30 days

Time (days)	Control	Total hemocyte counts (cells/mm^3) 0.02 μM Cd/L	0.05 μM Cd/L	0.10 μM Cd/L	0.20 μM Cd/L	0.40 μM Cd/L	0.50 μM Cd/L
3	1135 ± 24.4	1100 ± 36.4	1087 ± 55.4	1170 ± 40.9	1150 ± 43.0	1026 ± 45.6	1030 ± 39.8
6	1140 ± 35.5	1075 ± 20.0	1050 ± 32.3	1050 ± 45.6	1025 ± 39.6	910 ± 32.7	880 ± 35.4
9	1125 ± 25.0	1050 ± 32.2	980 ± 43.2	1025 ± 30.1	1055 ± 42.1	838 ± 31.4	815 ± 42.6
12	1142 ± 32.5	1025 ± 16.5	1017 ± 45.6	1035 ± 32.0	925 ± 31.4	830 ± 20.2	800 ± 22.9
15	1105 ± 20.4	1087 ± 40.7	1008 ± 25.0	1075 ± 40.3	875 ± 24.9	862 ± 25.0	810 ± 18.7
18	1162 ± 23.9	1037 ± 42.4	978 ± 37.5	985 ± 23.9	838 ± 45.6	840 ± 32.7	775 ± 20.4
21	1152 ± 22.4	975 ± 33.7	1005 ± 31.9	880 ± 25.0	813 ± 23.0	730 ± 20.4	760 ± 23.9
24	1150 ± 20.4	950 ± 31.4	912 ± 25.6	862 ± 31.0	870 ± 20.4	700 ± 23.9	680 ± 12.4
27	1140 ± 25.4	937 ± 23.9	975 ± 20.4	875 ± 35.0	762 ± 35.3	762 ± 31.5	650 ± 32.7
30	1135 ± 39.6	962 ± 25.1	937 ± 32.0	866 ± 25.0	730 ± 18.5	625 ± 37.2	610 ± 30.9
Exposure time (X) vs total hemocyte counts (−Y)							
Regression Coefficient	b 0.0996 a 1138.6	−1.3662 1042.34	−1.0716 1012.58	−2.5046 1023.83	−3.2506 957.94	−2.6577 856.15	−2.9222 829.21
Correlation Coefficient	r 0.2482	−0.8906	−0.8207	−0.9144	−0.9420	−0.9127	−0.9451
Significance	P(NS)	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001

$P < 0.001$, highly significant; $P < 0.01$, more significant; NS, not significant

Table 3. Differential hemocyte counts of *P. hydrodromous* exposed to sublethal concentrations of cadmium chloride for 30 days

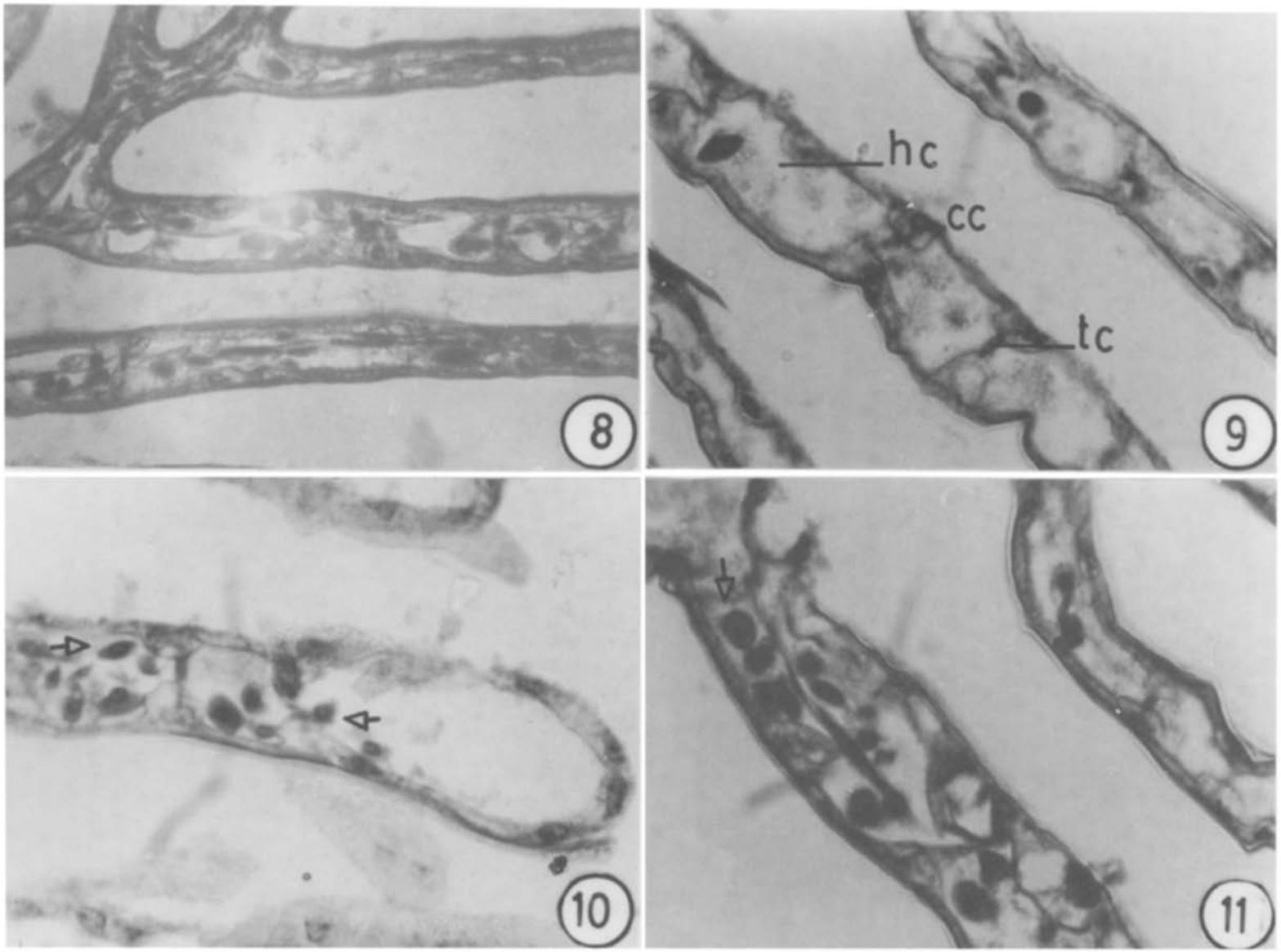
Cadmium exposure concentrations (μM/L)	Differential hemocyte count (%) Prohemocyte	Hyalinocyte	Intermediate granulocyte	Eosinophilic granulocyte
Control	8.69 ± 0.25	27.51 ± 0.48	42.00 ± 0.58	21.75 ± 0.29
0.02	11.50 ± 0.65 (+32.3)	24.25 ± 1.08 (11.9)	42.75 ± 0.63 (+1.8)	21.50 ± 0.35 (−1.1)
0.05	12.00 ± 0.71 (+38.1)	24.50 ± 0.65 (−10.9)	42.50 ± 0.71 (+1.2)	21.00 ± 1.05 (−3.4)
0.10	13.00 ± 0.91 (+49.6)	23.50 ± 0.60 (−14.6)	42.50 ± 0.65 (+3.2)	20.00 ± 0.77 (−8.0)
0.20	13.00 ± 0.40 (+49.6)	23.25 ± 0.75 (15.5)	44.50 ± 0.29 (+5.9)	19.25 ± 1.83 (−11.5)
0.40	14.25 ± 0.25 (+64.0)	22.25 ± 1.03 (−19.1)	46.00 ± 0.41 (+9.5)	14.75 ± 0.91 (−32.2)
0.50	14.00 ± 0.41 (+69.1)	21.75 ± 0.98 (−20.9)	49.50 ± 1.08 (+17.9)	14.75 ± 0.98 (−32.2)
% CV (exptl)	15.30	7.92	5.92	15.94
% CV (control)	2.27	4.62	2.03	4.09
P (control vs exptl)	<0.001	<0.001	=0.05	<0.05

Values in parentheses are per cent increase (+) and decrease (−) from the corresponding controls
P Value calculated by students t test

gans. The reduction in the number of circulating hemocytes was a common pathogenic response in lobsters and crabs experimentally infused with endotoxins (Levin 1967; Cornick and Stewart 1968; Stewart *et al.* 1969; Newman and Feng 1982). The formation of hemocyte clumps in the blood sinuses and gills may be effected the decline in the number of circulating hemocytes (Smith *et al.* 1984). In the crayfish, administration of endotoxin induced degranulation and lysis of hemocytes (Smith and Soderhall 1983).

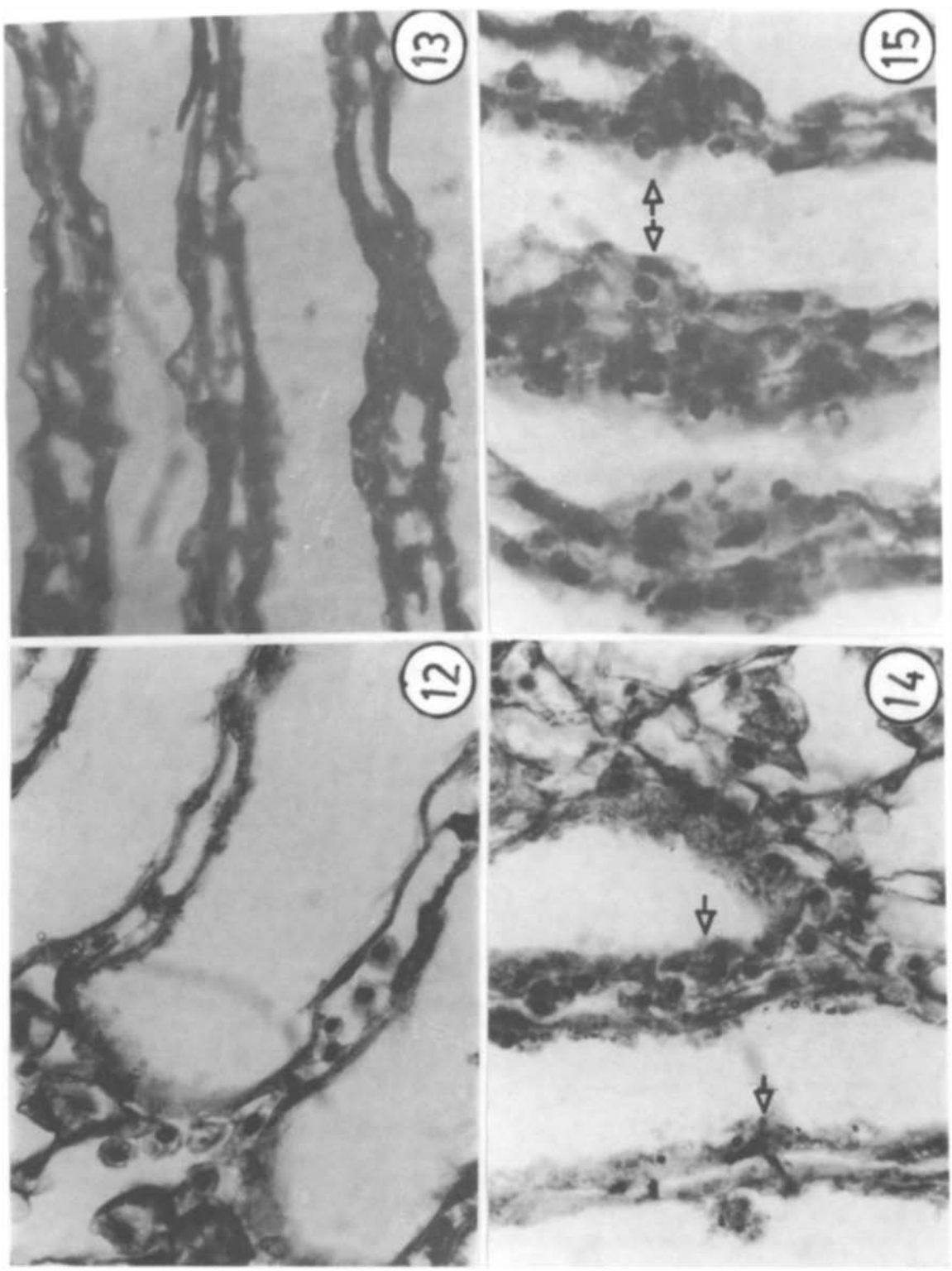
Morphologically, eosinophilic granulocytes of toxified crabs exhibited atypical shape, lobate nucleus, dense cytoplasmic deposits and more granuloplasmic vacuoles. The hyalinocytes developed eccentric nucleus, granular cytoplasm and membrane blebs. In general, alteration in the density of granules in arthropod hemocytes was observed during coagulation (Dumont *et al.* 1966), basement membrane formation (Beaulation 1968), and wound repair (Lai-Fook 1970). Lai-Fook (1970) noted that the degree of density and homogenecity of the granules was related to the physiological state of the organism. Investigations of Deruby (1918) and Myers and Dewolfa-Glade (1964) on vertebrate cells suggested that responses of degranulation, vacuolization of the cytoplasm and nuclear pycnosis were indicative of cell injury, autolysis, aging or death.

The lamellar pathology of *P. hydrodromous* varied in extent, depending on the sublethal doses of cadmium chloride from the disruption of central cells with agglutination of hemocytes to severe toxic responses of nodular gill disease (NGD) with hemocytic hyperplasia. Investigations of rock crabs *Cancer*



Figs. 8–9. Histology of normal gills of *P. hydrodromous*. **8** Gill lamellae with normal tissue arrangement (× 200). **9** Hemolymph channel (hc) of lamellae with transverse (tc) and central cells (cc) (× 250)

Figs. 10–11. Gill pathology of *P. hydrodromous* exposed to $CdCl_2$. **10** Disruption of central cells in the lamellae treated with 0.02 μM $CdCl_2$/L for 30 days (× 250). **11** Agglutination of pleomorphic hyalinocytes and blackened granulocytes (arrow) in the necrotic centers of lamellae treated with 0.05 μM $CdCl_2$/L for 30 days (× 250)



Figs. 12–15. Gill pathology of *P. hydrodromous* exposed to $CdCl_2$. **12** Diffuse distension and rupture of hemolymph channel after exposure to 0.25 μM $CdCl_2$/L (× 200). **13** Note the highly ruffled epithelial surface due to intense vacuolation (× 200). **14** Nodular gill disease (NGD) and hemocytic exudation (arrow) after exposure to 0.50 μM $CdCl_2$/L (× 250). **15** Focus of hemocytic hyperplasia (arrow) and sloughing off walled of hemocytes (× 250)

irroratus collected from a sewage disposal site, which polluted with heavy metals showed nodular formations, necrosis, and hemocyte aggregation in the gills (Greig *et al.* 1982). Couch (1978) reported that heavy metal exposure may be one of the predisposing factors for black gill disease in penaeid shrimps. Similarly, Estrella (1984) observed a high incidence of black gill and shell disease in *Homarus americanus* collected from the coast of Massachusetts contaminated with industrial wastes.

In *P. hydrodromous*, desquamation of the upper hyperplastic tissue plaque along with walled off hemocytes was recognized as an important protective response in cadmium extrusion or neutralization. According to Corner and Rigler (1958), many decapod crustaceans were capable of using detoxification and excretory processes to afford some degree of protection against heavy metal pollution. The sloughing off dead cellular material from the gills and body surface were suggested as a response to get rid off cadmium (Eisler 1974). Nimmo *et al.* (1977) stated that cadmium was collected by hemocytes of the shrimp, accumulated in the gills and eliminated by sloughing off gill epithelium. However, whatever may be the adaptive response to metals, the present study suggests the need for investigating in more detail, the role of hemocytes in detoxification and excretion.

Changes in the hemogram and gill pathology of *P. hydrodromous* could quantify the sublethal effects of cadmium chloride. This finding may represent further evidence that cadmium induces nodular gill disease (NGD) in crustaceans.

References

Ahsanullah M (1976) Acute toxicity of cadmium and zinc to seven invertebrate species from Western Port Victoria. Aust J Mar Freshwater Res 27:187–196

Ahsanullah M, Arnott GH (1978) Acute toxicity of copper, cadmium and zinc to larvae of the crab *Paragrapsus quadridentatus* (H. Milne Edwards) and implications for water quality criteria. Aust J Mar Freshwater Res 29:1–8

Anilkumar C, Adiyodi KG (1980) Ovarian growth, induced by eyestalk ablation during the prebreeding season is not normal in the crab *Paratelphusa hydrodromous* (Herbst). Int J Invertebr Reprod 2:95–105

Beaulaton J (1968) Etude ultrastructural et cytochimie des glandes prothoraciques de vers a soie aux quatrieme et cinquieme ages larvaires I. La tunica propria et ses relations avec les fibres conjonctives et les hemocytes. J Ultrastruct Res 23:474–498

Collier RS, Miller JE, Dawson MA, Thurberg FB (1978) Physiological responses of the mud crab *Eurypanopeus depressus* to cadmium. Bull Environ Contam Toxicol 10:378–382

Committee on Methods for Toxicity tests with Aquatic Organisms (1975) Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. National Water Quality Laboratory, Duluth, Minnesota, USEPA-660/3-75-009

Corner EDS, Rigler FH (1958) The modes of action of toxic agents III. Mercuric chloride and n-amylmercuric chloride on crustaceans. J Mar Biol Assoc UK 37:85–96

Cornick JW, Stewart JE (1968) Interaction of the pathogen Gaffkya homari with natural defense mechanisms of *Homarus americanus*. J Fish Res Board Canada 25:695–709

Cornick JW, Stewart JS (1978) Lobster (*Homarus americanus*) hemocytes: classification, differential counts and associated agglutinin activity. J Invertebr Pathol 31:194–203

Couch JA (1978) Diseases, Parasites and toxic responses of commercial penaeid shrimps of the Gulf of Mexico and south Atlantic coasts of North America. Fish Bull 76:1–44

Deruby KG (1918) A study on autolysis of animal tissues. J Biol Chem 35:179–219

Dumont JN, Anderson E, Winner G (1966) Some cytological characteristics of the hemocytes of *Limulus* during clotting. J Morphol 119:181–208

Eisler R (1971) Cadmium poisoning in *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and other marine organisms. J Fish Res Board Canada 28:1225–1234

Eisler R (1974) Radiocadmium exchange with seawater by *Fundulus heteroclitus* (L) (Pisces: Cyprinodontidae). J Fish Biol 6:601–612

Esterlla BT (1984) Black gill and shell disease in American lobster (*Homarus americanus*) as indicators of pollution in Massachusetts Bay and Buzzards Bay, Massachusetts. Mass Div Mar Fish Pub No 14049-19-125-5-85-CR, 17 pp

Finney DJ (1971) Probit analysis. Cambridge University Press, London

Fontaine CT, Lightner DV (1975) Cellular response to injury in penaeid shrimp. Mar Fish Rev 37(5–6):1–10

Greig RA, Sawyer TK, Lewis EJ, Galasso ME (1982) A study of metal concentrations in relation to gill color and pathology in the rock crab. Arch Environ Contam Toxicol 11:539–545

Jennings JR, Rainbow PS (1969) Studies on the uptake of cadmium by the crab *Carcinus maenas* in the laboratory. I. Accumulation from seawater and a food source. Mar Biol 50:131–139

Lai-Fook J (1970) Haemocytes in the repair of wounds in an insect (*Rhodnius prolixus*). J Morphol 130:297–313

Levin J (1967) Blood coagulation and endotoxin in invertebrates. Fed Proc 26:1707–1712

Martin GG, Graves BL (1985) Fine structure and Classification of shrimp hemocytes. J Morphol 185:339–348

Mix MC, Sparks AK (1980) Hemocyte classification and differential counts in the dungeness crab *Cancer magister*. J Invertebr Pathol 35:134–143

Myers DK, DeWolfe-Glade D (1964) Some effects of cationic condensing agents on rat thymocyte. Exp Cell Res 33:344–349

Newman MC, Feng SY (1982) Susceptibility and resistance of the rock crab *Cancer irroratus* to natural and experimental bacterial infection. J Invertebr Pathol 40:75–88

Nimmo DR, Lightner DV, Bahner LH (1977) Effects of cadmium on the shrimp *Penaeus duorarum*, *Palaemonetes pugio*, *Palaemonetes vulgaris*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of marine biota to pollutants. Academic Press, New York, p 131

Papathanassiou E, King PE (1883) Ultrastructural studies on the gills of *Palaemon serratus* (Pennant) in relation to cadmium accumulation. Aquat Toxicol 3:273–284

Rabin H (1970) Hemocytes, hemolymph and defense reactions in crustaceans. J Reticuloendoth Soc 7:195–207

Rainbow PS, White SL (1989) Comparative strategies of heavy accumulation by crustaceans: Zinc, copper and cadmium in a decapod, an amphipod and a barnacle. Hydrobiologia 174:245–262

Stewart JE, Cornick JW and Dingle JR (1967) An electronic method for counting lobster (*Homarus americanus* Milne Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Can J Zool 45:291–304

Stewart JE, Arie B, Zwicker BM, Dingle JR (1969) Gaffkemia a bacterial disease of the lobster *Homarus americanus:* effects of the pathogen *Gaffkya homari* on the physiology of the host. Can J Microbiol 15:925–932

Sindermann CJ (1971) Internal defenses of Crustacea: A review. Fish Bull US 69:455–489

Smith VJ, Soderhall K (1983) B-1,3 glucan activation of crustacean hemocyte *in vitro* and *in vivo*. Biol Bull 164:299–314

Smith VJ, Soderhall K, Hamilton M (1984) B, 1,3-Glucon induced cellular defence reactions in the shore crab *Carcinus maenas*. Comp Biochem Physiol 77A:635–639

Thorp VJ, Lake PS (1974) Toxicity bioassays of cadmium on selected freshwater invertebrates and the interaction of cadmium and zinc

on the freshwater shrimp *Paratya tasmaniensis* Riek. Aust J Mar Freshwater Res 25:97–104

Van Harreveld A (1936) A physiological solution for freshwater crustaceans. Proc Soc Exper Biol Med 34:428–432

White SL, Rainbow PS (1982) Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. Mar Ecol Prog Ser 8:95–101

Wood PJ, Visentin LP (1967) Histological and histochemical observations of the hemolymph cells in the crayfish *Orconectes virilis*. J Morphol 123:559–568

Wright DA (1977) The effect of salinity on cadmium uptake by the tissues of the shorecrab *Carcinus maenas*. J Exp Biol 67:137–146

Manuscript received November 15, 1990 and in revised form November 28, 1992.