

Cystic kidneys in copper exposed mussels

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ABSTRACT: *Mytilus edulis* L. were exposed to 0.2 mg l^{-1} copper for 24 h in aquaria before being transferred to cages submerged in the sea. The first renal cysts appeared 4 mo after exposure. On average 20% of the exposed mussels (166 of 830 survivors) developed cystic kidney lesions within 1 yr. No cysts were found in control mussels. The cysts were thin walled, fluid filled sacs arising from the kidney epithelium and filling part of the mantle cavity. The cyst wall was composed of 2 cell layers: the inner layer consisting of kidney epithelium which gradually changed to columnar epithelium, and the outer layer of single columnar epithelium which changed to squamous epithelium. Both cell layers were proliferative. The lesions were accompanied by an inflammatory response. The cysts were considered to be dilatations of the kidney tubules due to polypoid tubular obstruction.

INTRODUCTION

Kidneys are the principal site of accumulation of heavy metals in the mussel *Mytilus edulis*. Metals are immobilized and detoxified in kidney cells by cellular compartmentation into granules which are eventually excreted in the urine (George et al. 1982). The mussel kidney is composed of a tubular structure on both sides of the visceral mass at the base of the gills, one central tubule giving rise to numerous blind ending branches. The kidney tubules are made up of an infolded single layer of columnar epithelium (kidney cells) and are connected to the pericardial cavity through the pericardial funnel and renopericardial canal. Ultrafiltration takes place in the pericardial cavity through the walls of the auricle (Pirie & George 1979, Yevich 1980).

Cystic kidney lesions have not previously been described in molluscan pathology. In vertebrate pathology, however, renal cysts have been reported in several species. The lesions are either polycystic or single cysts, and are thought to be congenital or acquired. In man, simple cysts are common in elderly patients. These are solitary or multiple acquired abnormalities of undetermined, possibly diverse causes (Walsh et al. 1986). In experimental animals a wide variety of chemical compounds are known to induce renal cysts (Safouch et al. 1970, Dobyan et al. 1981). Drug-induced cysts are considered to originate from tubular obstruction (Goodman et al. 1970) or a structural defect of the tubular basement membranes (Carone et al. 1974).

In long-term tests, copper exposed mussels *Mytilus edulis* were observed to develop cysts which arose between gills and mantle, and often invaded most of the mantle cavity. This paper reports a high occurrence of cystic kidneys in copper exposed mussels and describes their histopathology.

MATERIAL AND METHODS

Samples were collected by a diver in the vicinity of the Tvärminne Zoological Station in the Gulf of Finland (Baltic Sea, $29^{\circ}51' \text{ N}$, $60^{\circ}12' \text{ E}$). *Mytilus edulis* (2.0 to 3.5 cm maximum length) were enclosed in cages and exposed to 0.2 mg l^{-1} copper ($\text{CuSO}_4 \cdot 3 \text{ H}_2\text{O}$ analytical grade, static exposure) in aerated aquaria for 24 h.

Renal cysts were induced in 2 experiments. In the first experiment (August, exposure temperature 20°C), the number of mussels with cystic lesions was counted 4 and 12 mo after exposure, and samples of cysts fixed for histological evaluation. In the second experiment (April, exposure temperature 11°C), the mussels were fixed whole 4 mo after exposure and those with kidney cysts studied histologically. In each experiment 500 mussels were exposed (total 1000); the 500 control mussels were kept in clean brackish water in aquaria during the time of exposure.

Cages with the exposed or control mussels were fastened to an unused pier and anchored to the sea bottom. For histology the mussels were fixed in Helly's fixative and embedded in paraffin. $8 \mu\text{m}$ thin sections were stained with Harris haematoxylin-eosin and

Alcian-blue-PAS. Sections from 10 mussels were stained with Twort's Gram-method to detect any bacterial infection associated with cyst formation.

RESULTS

In the first and second experiment, cystic kidneys were found in 22 and 18 % of the survivors, respectively (166 of 830 survivors). There were no cysts in control mussels. The cysts arose between mantle and gill at the posterior end of the mussel. They were thin-walled, round, brownish and collapsed easily; their size varied from barely visible to 1 cm (Fig. 1). Cysts were mostly single although some multiple cysts were found. The gills of affected mussels were deformed. The cyst wall was composed of 2 cell layers (Fig. 2A). The inner layer consisted of kidney epithelium, which at the stalk looked normal, but gradually changed to columnar epithelium. The outer layer

consisted of columnar epithelium with mucous secretory cells, changing on top of the cyst to very flat squamous epithelium. The cysts were proliferative, both cell layers forming distensions and polypoid structures (Fig. 2B, C). The lumen between the 2 cell layers was filled with granular haemocytes. The cyst was filled with acellular material which gave a weak periodic acid-Schiff reaction. The cysts seemed to form as dilatations of the kidney tubules and were still partially connected to the rest of the kidney.

There was an acute inflammatory response associated with the cysts (Fig. 2A). No signs of bacterial infection could be detected on the slides.

DISCUSSION

Long-term exposure experiments with caged *Mytilus edulis* have been carried out for a number of years at the

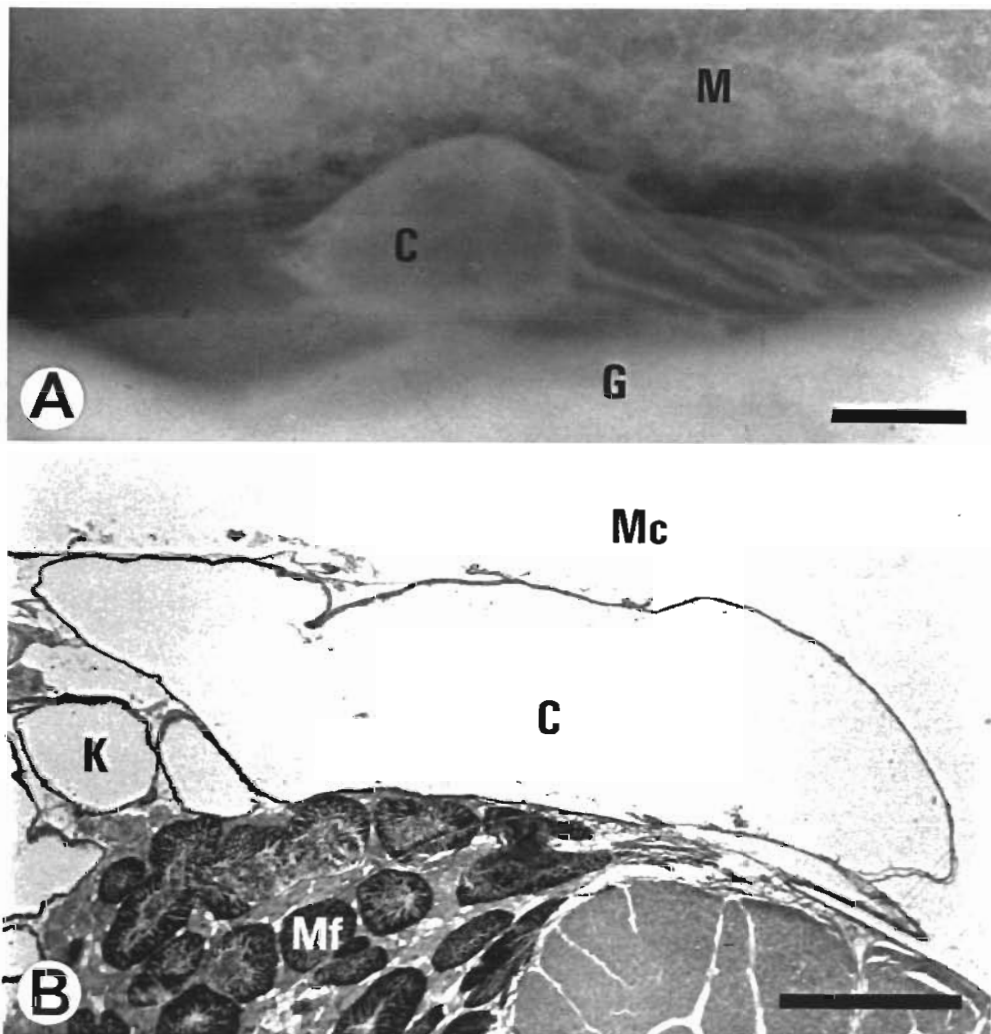


Fig. 1. *Mytilus edulis*. (A) Stereophotomicrograph of a renal cyst in an unstained total preparation; C: cyst; M: mantle; G: gills; scale bar 0.5 cm. (B) Paraffin section of a mussel; C: cyst; Mf: male follicles; K: kidney tubules; Mc: mantle cavity; scale bar 1 mm

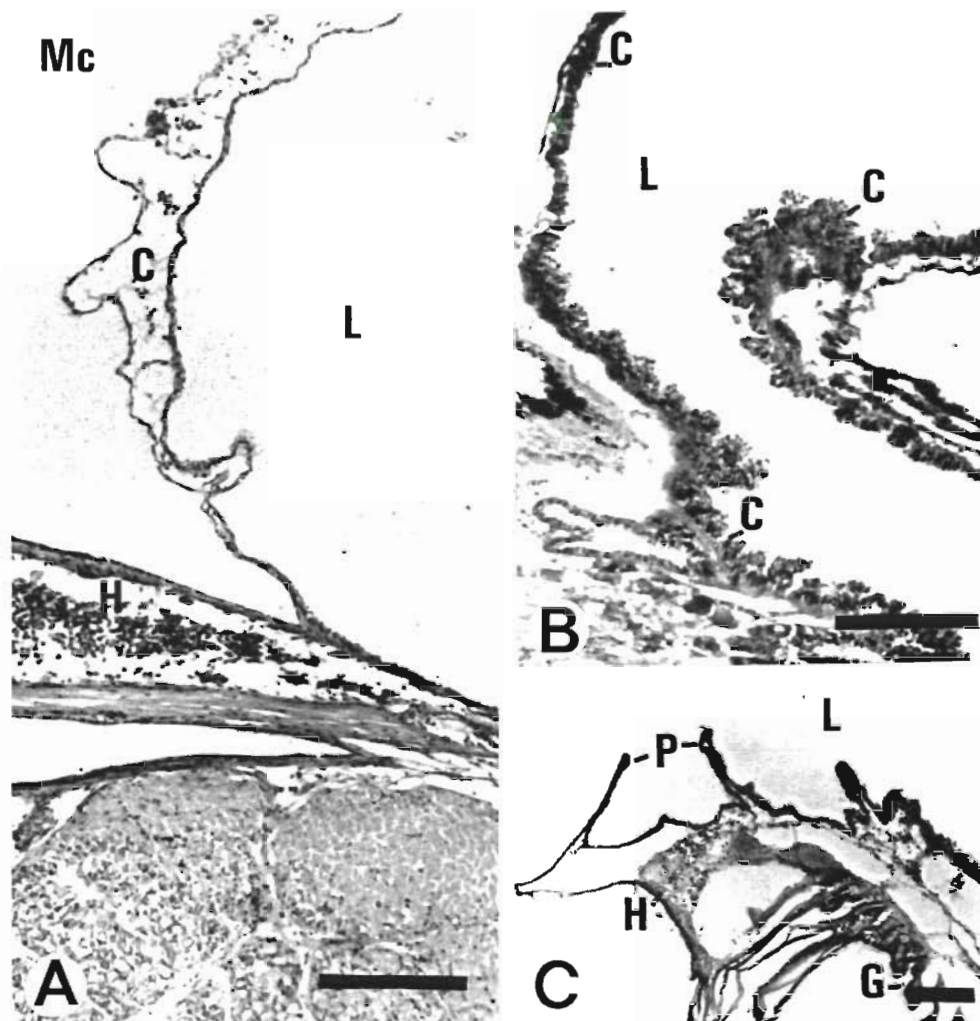


Fig. 2. *Mytilus edulis*. (A) Paraffin section of a mussel; C: cyst wall; Mc: mantle cavity; L: lumen of cyst; H: haemocytes, demonstrating an inflammatory response; scale bar 200 μm . (B) Infolded epithelium inside cyst; C: cyst wall; L: lumen of cyst; scale bar 200 μm . (C) Polypoid epithelium inside cyst; P: Polyps; L: lumen of the cyst; G: gill arch; H: haemocytes; scale bar 200 μm

Tvärminne Zoological Station in the Gulf of Finland (salinity 7‰). The LC_{50} value for copper in brackish water is 0.32 to 0.40 mg l^{-1} (Sunila 1981, Sunila & Lindström 1985). Exposure to 0.2 mg l^{-1} of copper has been found to cause shell and gill deformities in long-term tests. In one study, shell deformities appeared in 46% of copper-exposed caged mussels, but were also common (26%) in control individuals (Sunila & Lindström 1985). Of the copper-exposed mussels (0.2 mg l^{-1}), 44% developed gill lesions. Deformed gills were low and had a folded edge. According to histological studies these abnormalities were due to fusions of the gill epithelia. These were classified as fusions of the frontal parts of the filaments, fusions of the abfrontal parts of the filaments or interlamellar fusions of the abfrontal parts of the filaments of opposite lamellae (Sunila 1986).

In the present study there was no evidence of bacterial infection or of chronic inflammation. There was, however, an acute inflammatory response between the

cyst walls and in the tissues surrounding it. No sporozoan parasites were found. Cystic kidneys in the exposed mussels occurred concurrently with gill lesions. This correlation is not found in the field. Cysts are rare in the study area, only 1 mussel with a cyst having been found, but gill lesions are common in polluted areas (Sunila 1987).

Dobyan et al. (1981) induced cyst formation in rat kidney by cis platinum administration. The cysts were lined by simple squamous epithelium. Safouch et al. (1970) induced experimental cystic disease in rat kidney with diphenylamine. The cysts formed as dilations of the kidney tubules and were lined with tubular epithelium. According to Gardner et al. (1976), cysts are the consequence of partial tubular obstruction and elevated intratubular pressure. Goodman et al. (1970) induced multiple cysts in rat kidney with nordihydroguaiaretic acid. The contents of small cysts were strongly PAS-positive but only slightly so in

larger cysts. Cyst walls were composed of tubular epithelium and were thought to originate from tubular blockage.

Carone et al. (1974) induced cyst formation in rat kidney with diphenyl thiazole. No evidence of tubular obstruction was found, and the condition was considered to originate from alterations in the structure of the tubular basement membrane, which in the presence of a normal transtubular pressure gradient results in progressive tubular dilatation. Their results confirmed previous findings that cysts are not closed sacs but occur as tubular distensions with functioning nephrons, cell proliferation accompanying tubular enlargement.

There have been several reports of cystic enlargement of the kidneys of cyprinids. The aetiology is either the presence of a sporozoan parasite (*Mitraspora cypriini*) or unknown. Environmental pollution as a cause of polycystic kidneys in goldfish *Carassius auratus* has been suggested by Munkittrich et al. (1985). They found a prevalence of 6.3% (5 of 80) goldfish with cystic kidneys from a heavily polluted industrial bay.

Renal cysts in mussels have many features in common with vertebrate renal cysts despite major functional and anatomical differences in the organ. In the mussel kidney, the ultrafiltrate passes from the pericardial cavity into the renopericardial canal which opens by a convoluted ciliated funnel within the kidney lumen just above the excretory duct. According to Pirie & George (1979), the fluid from the renopericardial canal may pass directly to the exterior without passing through the entire length of the kidney lumen. However, in this study, partial obstruction due to polyps caused dilatation of the distal tubules. This might originate from pressure caused by an inflow of ultrafiltrate. As a result, the tubular epithelium starts to proliferate, forming a large cyst which expands into the mantle cavity.

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