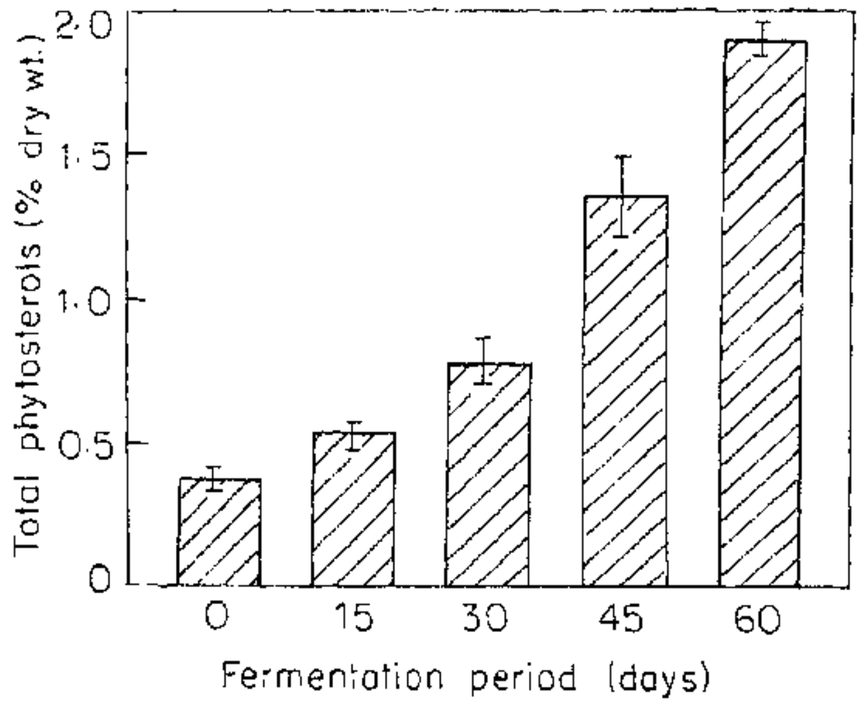

Figure 2. Enrichment of phytosterols during fermentation.

matter and resulted in enrichment of phytosterols (Figure 2).

These observations show that bamboo shoots (succulent ones) are a good source of phytosterols. The phytosterols so obtained can be used for the manufacture of steroidal drugs after microbial conversion into 1,4-androstadiene-3,17-dione using *Arthrobacter oxidans* as was done earlier[9,10]. Thus the fermented succulent bamboo shoots can replace diosgenin from *Dioscoria* and solasodine from *Solanum* as the starting material in the production of steroidal drugs from plant steroids.

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Cadmium-induced cancer in the gills of the crab *Scylla serrata* (Forskal)

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The commercially important edible estuarine portunid crab *Scylla serrata* was exposed to three sublethal concentrations of cadmium (1/10, 1/7 and 1/4 of the LC_{50} value of 8 ppm) for 30 days. After exposure, the histological changes effected by cadmium were observed. The changes noticed were: thickening of gill lamellae, increase and accumulation of haemocytes, and infiltration of cells into the haemocoelic space of the gill. Among the infiltrated cells there were present proliferated cancerous cells. The observations are discussed.

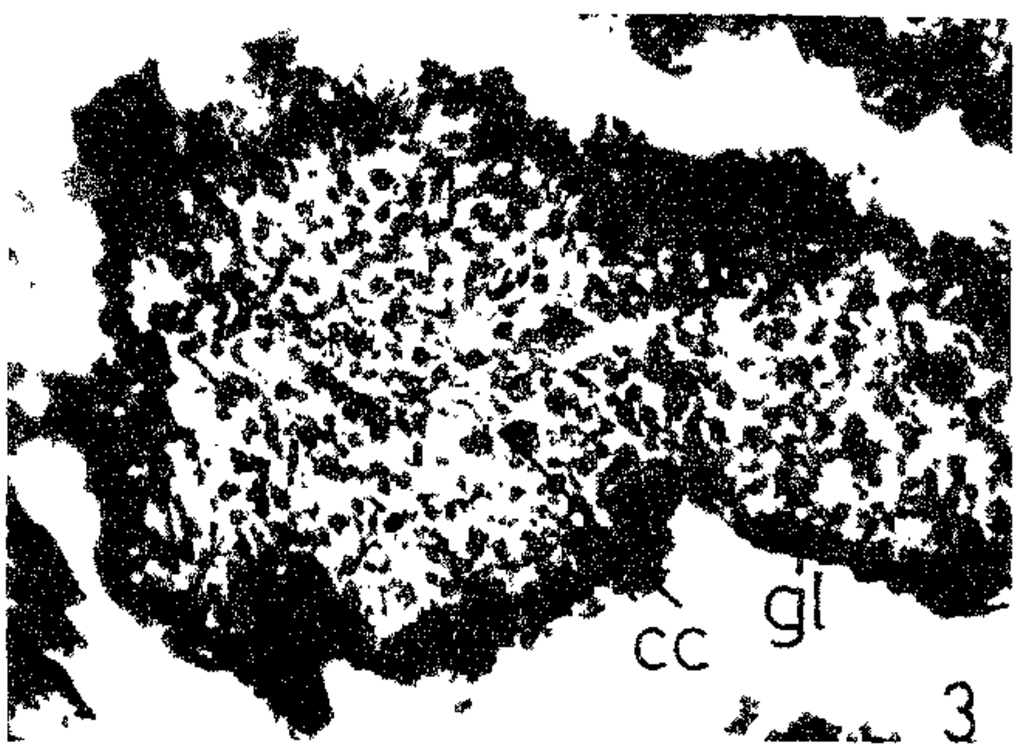
METAL ions are serious pollutants in the marine environment as they are concentrated by the marine organisms[1-3]. The accumulation of cadmium in different tissues of marine crustaceans has been studied, and gills have been shown to be the organs of accumulation, both under natural and experimental conditions[4-6]. Gills are the primary sites of respiration and of transport system involved in osmoregulation and it has been confirmed that accumulation of metal ions within them may have an adverse effect on these functions[7-9]. Ultra-structural changes related to bronchial cells in crustaceans have been reported by various authors[10,11]. Some studies have emphasized that histological changes occur within the gills, when aquatic animals are subjected to different concentrations of metal ions[12,13]. It is of interest to note that no spontaneous or idiopathic neoplasms have been reported for crustaceans even though thousands of crabs, shrimps and other crustaceans have been examined histologically in a variety of toxicological and pathological studies[14]. Here we report our observation of cancerous cells induced by cadmium toxicity in the edible crab *Scylla serrata*, which has good market potential. In all probability, this is the first report of metal-induced carcinoma in a crustacean, particularly edible crabs.

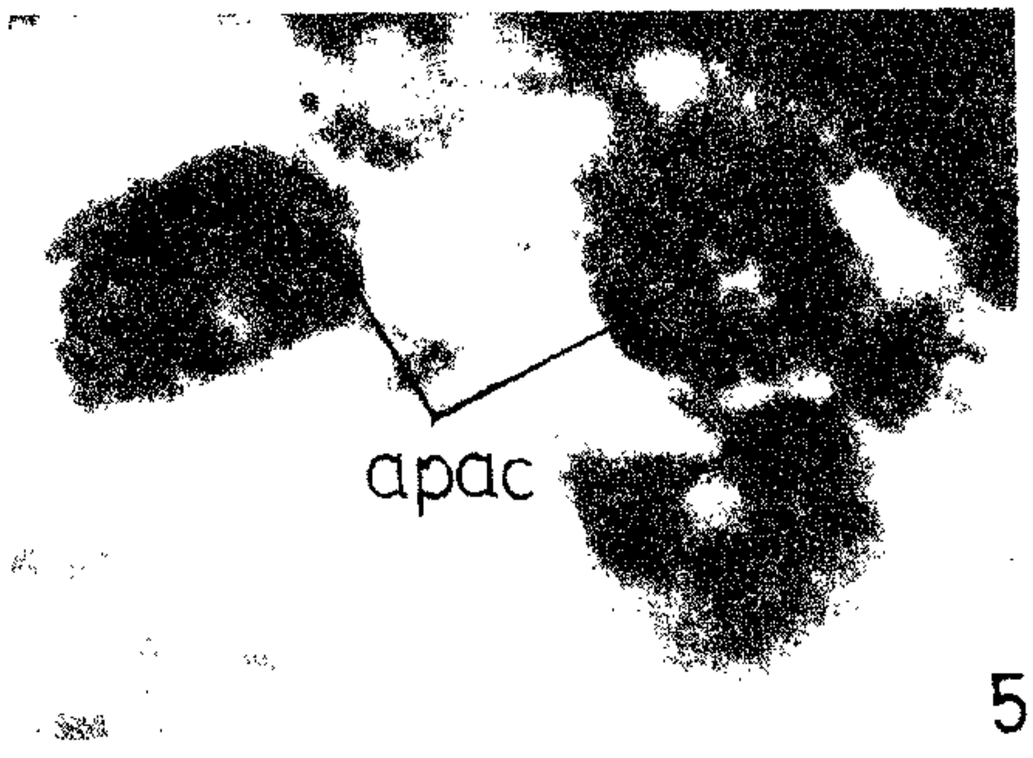
Healthy specimens of *S. serrata* were collected from the Pitchavaram mangrove (Lat. 11°29'N, Long. 79°47'E) and kept alive in estuarine water of salinity $27 \pm 1‰$ for a week. After acclimation, based on the LC_{50} value (8 ppm of cadmium) 30 crabs (ten in each concentration) were exposed to three sublethal concentrations (0.8, 1.1 and 2.0 ppm) representing 1/10, 1/7 and 1/4 of the LC_{50} value for 30 days. A control group (10 crabs) was also maintained. During the experimental period, the crabs were fed with chopped clam meat. After the exposure period, the gills of the control and experimental animals were dissected out and fixed in

Zenker, embedded in celloidin-paraffin, sectioned at 7 μm, and stained in Masson's trichrome, for histopathology[15] and also tested for alkaline phosphatase. For localizing alkaline phosphatase, the gills were fixed in ice-cold acetone, following the method of Gomori[16].

The gills of the crab *S. serrata* are composed of a double row of closely spaced lamellae extending anteriorly and posteriorly from the gill shaft. The lamellae are lined by a thin layer of epithelial cells enclosing the central haemocoelic sinus. Pillar cells are specialized epithelial cells, which extend into the lamellar sinus at intervals and about with similar cells extending from the opposite surface. Elongate clumps of these cells are arranged in curved rows presumably facilitating even perfusion of the lamella with haemolymph and preventing detention due to blood pressure. In addition, epithelial cells are supported and separated by pillar cells. Within the haemocoelic space of the gill, haemocytes and haemolymph are present (Figure 1).

Figure 1. Typical gill structure with haemocoelic space of a gill showing haemocytes (hcy). Pillar cell (p) and epithelial cell (e) can also be seen. Zenker, Heidenhain's iron haemotoxylin (ca × 240).





Figures 2–5. **2**, Gills of crab exposed to 0.8 ppm of cadmium. Gill lamella thickened (gl) with proliferated and infiltrated cells into the haemocoelic space of the gill. Zenker, Heidenhain's iron haemotoxylin (ca × 240). **3**, Gills of crab exposed to 1.1 ppm of cadmium. Pronounced thickening of gill lamella and the haemocoelic space of the gill being filled with infiltrated cells of different kinds including cancerous cells (cc). Zenker, Heidenhain's iron haemotoxylin (ca × 240). **4**, Gills of crab exposed to 2 ppm of cadmium. Extremely thickened gill lamella with a haemocoelic space being filled with proliferated and infiltrated cells. Cancerous cells can be seen. Zenker, Heidenhain's iron haemotoxylin (ca × 240). **5**, Gills of crab exposed to 2 ppm of cadmium. Alkaline phosphatase activity of the cancer cell (apac) (ca × 2000).

Distinct pathological changes could be noticed in the gills of all the crabs exposed to different concentrations of cadmium (Figures 2–4). At 0.8 ppm concentration, disruption of lamellae with proliferation and infiltration of cells into the haemocoelic space of the gill was noticed (Figure 2). At 1.1 ppm of cadmium pronounced thickening of gill lamellae and necrosis of cells were observed. The haemocoelic space was fully infiltrated by several kinds of proliferated cells. The gills were thickened greatly as a result of the proliferation (Figure 3). At the highest concentration (2 ppm) extreme thickening of gills was found with the haemocoelic space being almost obliterated by the proliferated and infiltrated cells. The cytomorphological observations in six of the ten crabs revealed that there were many proliferated cells having large nuclei among the infiltrated cells which occupy the haemocoelic space of the gill (Figure 4). These cells are characteristic in being cancerous as confirmed by enhanced alkaline phosphatase activity (Figure 5). When the exposure was continued with another set of organisms (10) the afflicted animals became weak. While two animals died on the 53 day of exposure, another five animals died between 62 and 69 days of exposure. The cancerous nature of the cells was tested again and it was confirmed by enhanced alkaline phosphatase activity. But in none of the animals, the cells had grown into big tumours.

Pollution monitoring is very essential to protect the aquatic life on which man is partly dependent. Because of the recent awareness that some aquatic animals are susceptible to carcinogenic substances prevalent in the environment, considerable effort has been expended in the last two decades all over the world to study selected aquatic invertebrates and fishes as indicators and models of carcinogenesis. This paper reports a case of carcinogenesis in the gills of the most important portunid crab, *S. serrata*, exposed to cadmium ions.

Pathological changes induced by cadmium include accumulation of haemocytes (whose number has increased manifold due to pollution), and swelling of the gills. Similar findings due to pollution by oil and cadmium have been reported earlier[6,17,18]. In the highest concentration of 2 ppm, the proliferation and infiltration of cells reached their peak. In six of the ten crabs of this concentration, among the cells, there were some exceptionally large ones with conspicuously large nuclei. These cells are different from the others. These were diagnosed to be cancerous based on their cytomorphology. To confirm this, they were tested for their alkaline phosphatase activity[19]. This was found to be high, and distinct intracellular granules positive to the test could be located in the cytoplasm (Figure 5). Thus, it is evident that there is cadmium-induced carcinogenesis.

It is necessary to continue the investigations on the responses of invertebrate and fish to carcinogenic agents under controlled laboratory conditions. The role of promoters of development of neoplasms should be studied, since most pollutants discharged into the environment contain mixtures of substances, some of which may be initiators, and some, promotors of carcinogenesis.

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Evaluation of ferritin and calcitonin as possible markers in leukaemia and lymphoma

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In an attempt to search for biological markers for leukaemias and lymphomas, assessment of serum ferritin and calcitonin levels in these patients, in comparison to anaemic patients and normal subjects, was made. Our initial study on a small group of patients indicates that serum ferritin may be considered a potential marker for both leukaemias and lymphomas and merits further scrutiny.

STUDIES on tumour markers have helped biologists in the understanding of differentiation and dedifferentiation,