

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 cytological 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<sup>6,17,18</sup>. 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 cytology. To confirm this, they were tested for their alkaline phosphatase activity<sup>19</sup>. 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, promoters 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,

while clinicians find them useful for detection, diagnosis, prognostication and treatment follow-up.

In view of the importance of marker substances, many of which are already known for other cancers, we attempted to identify them in leukaemias and lymphomas<sup>1,2</sup>. The present paper reports our initial observations on the potential use of serum ferritin and calcitonin as markers for this group of diseases.

Clinically diagnosed cases of different types of leukaemia and lymphoma, confirmed by cytohistology were selected. Venous blood samples were collected from 35 normal subjects and patients with leukaemia (40 cases), lymphoma (48 cases) and non-malignant anaemia (20 cases). Only male subjects were included in this study. Anaemia cases in this study were anaemia of chronic diseases only and not of iron-deficiency group. Lymphomas were histologically classified as Hodgkin's, non-Hodgkin's and unclassified. Leukaemias were of both acute and chronic type of myeloid and lymphoid groups.

Serum ferritin and calcitonin were determined by radio immunoassay using commercial kits (Diagnostic Systems Lab, Texas). Simultaneous determinations of serum ferritin and blood haemoglobin were made and correlated with clinical observation of anaemia.

The normal range of ferritin was found to be 3.8 to 70 ng ml<sup>-1</sup> and that of calcitonin 0 to 30.5 pg ml<sup>-1</sup>. In case of anaemia, the incidence of elevated serum ferritin was 100% while in leukaemias and lymphomas high levels were noted in 45.4 and 68.7% respectively. Mean value of serum ferritin was highest in lymphoma, followed by leukaemia and anaemia (Table 1). A positive correlation was observed with respect to anaemia and serum ferritin elevation. Cases with moderate to severe anaemia among the malignant group (haemoglobin below 9 g%) were accompanied with very high levels of ferritin (300–500 ng ml<sup>-1</sup>). This observation is important in view of the fact that ferritin is an iron storage protein whose level in the serum reflects the level of storage iron in the body<sup>3</sup>. Moreover, elevated levels of serum ferritin have been reported in conditions of iron overload and some malignant diseases<sup>4,5</sup>. It is reported that anaemia of chronic diseases is associated with high ferritin<sup>4</sup> as found in the present observation. Early reports on serum ferritin in

**Table 1.** Serum ferritin in normal subjects and anaemia, leukaemia and lymphoma patients.

Group	Ferritin (ng/ml) Mean (range)	% cases with higher serum ferritin
Normal	37 (3.8–70)	
Anaemia	221.6 (100–365)	100
Leukaemia	326 (UD*–560)	45.4
Lymphoma	354.2 (UD*–1100)	68.7

\*Undetectable.

**Table 2.** Serum calcitonin in normal subjects and anaemia, leukaemia and lymphoma patients.

Group	Calcitonin (pg/ml) Mean (range)	% cases with elevated serum calcitonin
Normal	28.9 (UD*–30.5)	
Anaemia	28.3 (UD*–31.5)	Nil
Leukaemia	UD	Nil
Lymphoma	39 (UD*–44)	7.1

\*Undetectable.

leukaemia<sup>6</sup> and Hodgkin's disease<sup>7</sup> are also in agreement with our present findings. We observed that in both Hodgkin and non-Hodgkin types serum ferritin was considerably elevated in about 85% of cases. Among leukaemias we failed to note any definite pattern of serum ferritin with respect to the cytological types but the incidence of elevated ferritin was higher in the acute forms (83%).

Calcitonin is one of the peptide hormones secreted by C-cells of thyroid gland for regulating serum Ca levels and activity of osteoclasts<sup>8</sup>. Medullary thyroid cancer secretes calcitonin in excess<sup>9</sup> and ectopic production of this hormone in carcinoma of lung and breast is also known<sup>10,11</sup>. This study reveals that circulating levels of calcitonin in leukaemias and lymphomas were mostly in the normal range (Table 2) except for a few cases of lymphomas thereby negating its value as a tumour marker.

As ferritin, when present, was found to be elevated much above the normal range and its presence detected in a significant number of patients, this substance merits consideration as a useful marker and holds promise for its clinical application in future. Moreover, assessment of the ferritin levels with individual types of leukaemias or lymphomas requires further scrutiny. It would therefore seem worthwhile to pursue investigation on ferritin in a larger number of patients prior to, during and after treatment to establish the utility of ferritin as marker for human leukaemias and lymphomas. Furthermore, the present study indicates that the relationship between anaemia and iron-deficiency or overload states as reflected by serum ferritin, as well as the causes of high ferritin in malignant condition of the blood-forming system needs to be scrutinized in detail. This will help to clarify the problem of anaemia associated with malignancy.

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## Reactivation of acetylcholinesterase activity by two new oximes in diisopropylfluorophosphate-intoxicated mice

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Diisopropylfluorophosphate (0.5 LD<sub>50</sub>, i.p.) significantly inhibited acetylcholinesterase (AChE) activity in blood and in cerebral cortex and corpus striatum of brain 1 h after administration in mice. Percentage reactivation of blood AChE was greater with pralidoxime than with the new oximes DEV-9 and DEV-10 at a dose of 50 mg/kg, i.p. along with atropine at 10 mg/kg, i.m. However, cortical AChE was reactivated with DEV-9 and DEV-10 without any effect on striatal AChE. We conclude that the protection afforded by oximes was due to peripheral reactivation of AChE.

It is widely accepted that most of the organophosphorus compounds inhibit the enzyme acetylcholinesterase (AChE), thereby causing toxic effects in humans and in animals<sup>1</sup>. The phosphorylated (inhibited) enzyme can be reactivated by certain drugs (cholinesterase reactivators) such as hydroxylamine, hydroxamic acid and oximes<sup>2</sup>. Among the oximes, pralidoxime, obidoxime and trimidoxime are well-known cholinesterase reactivators<sup>3</sup>. But the controversy on the cholinesterase reactivating drug which would be universally preferred is still open. In the present study we have evaluated reactivation of AChE by the oximes DEV-9 (1-[3-(hydroxyiminomethyl)-1-pyridino]-3-[4-carbamoyl-1-pyridino]-propane dibromide) and DEV-10 (1-[3-(hydroxyiminomethyl)-1-pyridino]-3-[3-(carboxymethyl)-1-pyridino]-propane dibromide) (Figure 1), synthesized by the condensation reaction of 1-bromo-3-[3-(hydroxyiminomethyl)-1-pyridino]-propane bromide with isonicotinamide and carboxymethylnicotinamide respectively as described by Sikder *et al.* (unpublished data), in diisopropylfluorophosphate (DFP)-treated mice.

Thirty male Swiss mice (20–25 g) were randomly

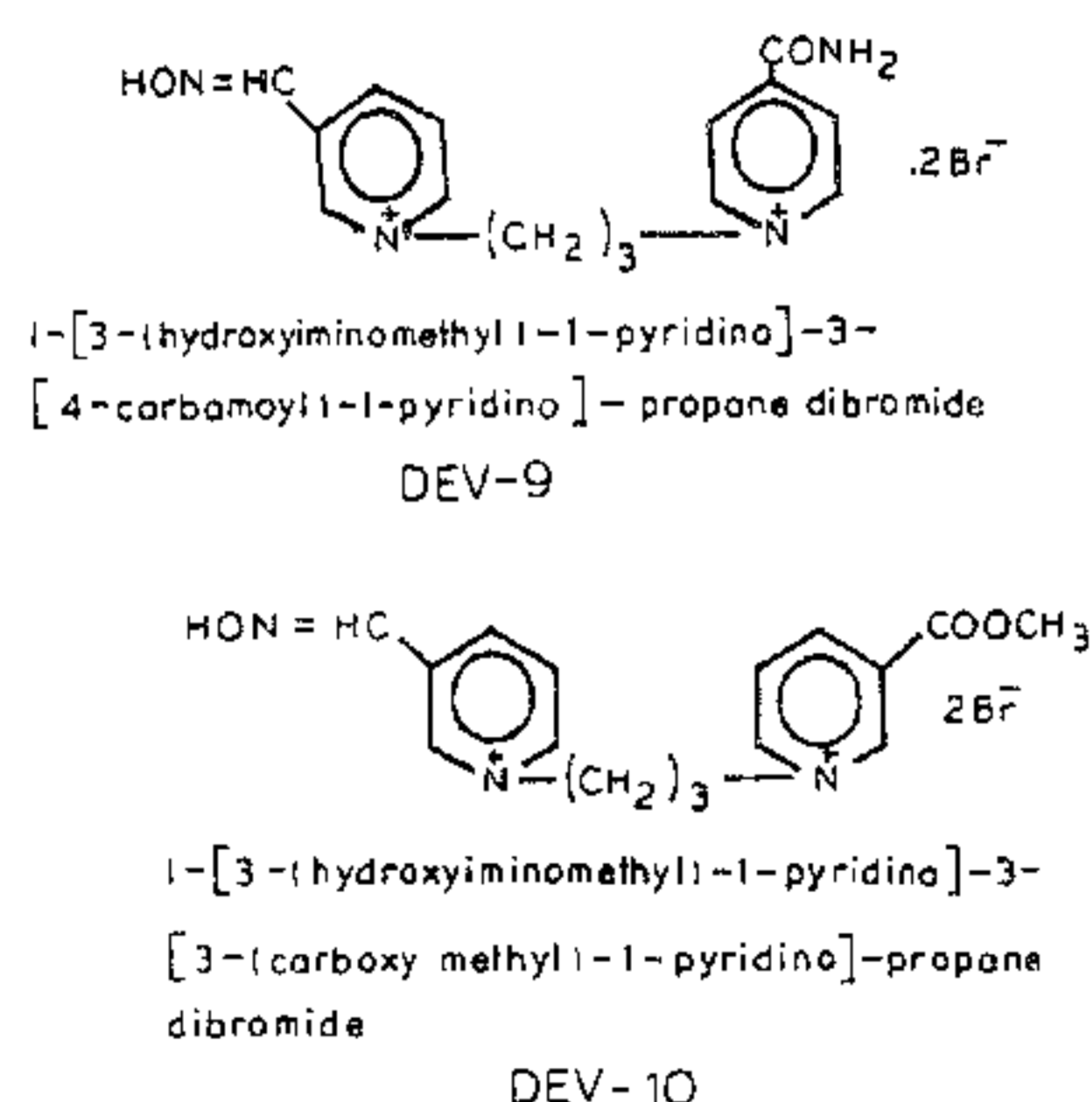


Figure 1. The new oximes DEV-9 and DEV-10.

divided into five groups of six animals each. They were provided food and water *ad libitum* except for a 12-h fasting before their use in the experiments. The animals in group 1 were treated with normal saline and served as control whereas those in group 2 were injected DFP (in normal saline) intraperitoneally (2 mg/kg = 0.5 LD<sub>50</sub>). Animals in groups 3, 4 and 5 were first treated with DFP, then atropine (10 mg/kg, i.m.), followed immediately by respectively, pralidoxime (2-formyl-1-methylpyridinium oxime chloride, 2-PAM), DEV-9 and DEV-10, each at 50 mg/kg i.p. Atropine was given to protect the animals from the acute, lethal effects of DFP. Animals were sacrificed 1 h later and the blood was collected in heparinized tubes. Brain was dissected into two parts, cerebral cortex and corpus striatum, according to the method of Glowinski and Iverson<sup>4</sup>. Blood and brain tissue AChE activity was assayed as described by Ellman *et al.*<sup>5</sup> using acetylthiocholine as substrate. Protection index (PI) was determined by the method of Das Gupta *et al.*<sup>6</sup> Data were analysed statistically using Student's *t* test.

The inhibition of AChE in blood was 84% while in cortex and striatum it was 66% and 76% respectively 1 h after DFP administration (Table 1), indicating more binding of DFP to blood AChE and penetration into deeper structures of the brain. However, the inhibition was more in striatum than in cortex. It is suggested that even a small change in AChE activity can be detected in this brain region, which has been reported to be involved in the control of motor activity<sup>7</sup> and also rich in AChE enzyme<sup>8</sup>. Further the results indicate that all three oximes significantly reactivated AChE inhibited by DFP in the blood but DEV-9 and DEV-10 did so to a smaller extent than 2-PAM. However, 2-PAM failed to reactivate DFP-inhibited cortical and striatal AChE activity, while DEV-9 and DEV-10 both reactivated cortical but not striatal AChE activity (Table 1). It has been previously reported that pyridine aldoximes have the disadvantage of limited tissue distribution. Being