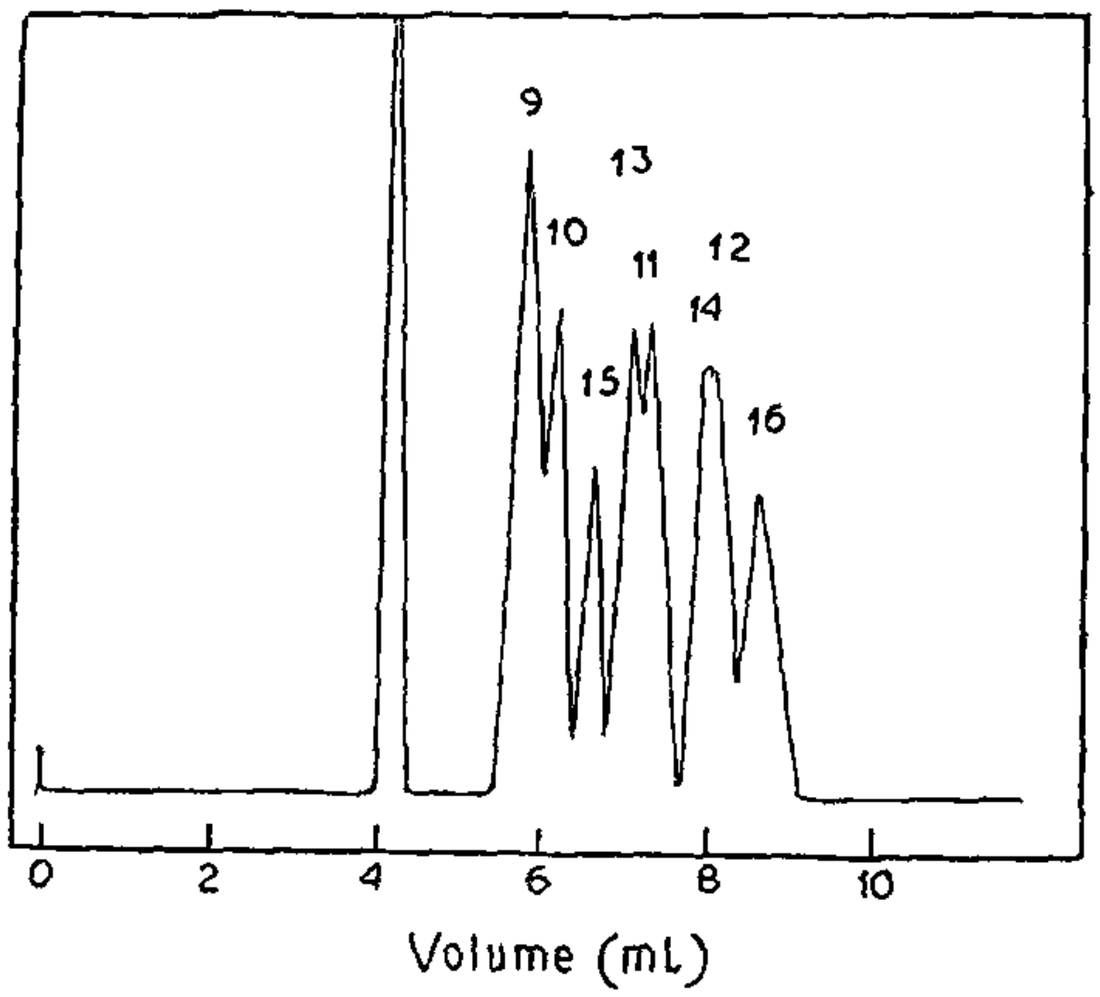

FIG. 2. Separation of partially methylated glucosides.

methyl glucosides the same solvents were used in the ratio 30 : 70. Flow rate in all the experiments was 0·5 ml/min.

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EFFECT OF LETHAL (Lc 50/48 HRS) CONCENTRATION OF METASYSTOX ON SELECTED OXIDATIVE ENZYMES, TISSUE RESPIRATION AND HISTOLOGY OF GILLS OF THE FRESH WATER AIR-BREATHING FISH, *CHANNA STRIATUS* (BLEEKER)

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ABSTRACT

Lethal exposure (Lc 50, 5 mg/litre) of metasystox decreased succinate dehydrogenase activity and tissue respiration, while lactate dehydrogenase activity increased in gill, brain, liver, muscle and kidney tissues. Histological observations indicated extensive damage to gill epithelium, particularly secondary lamellae and chloride secreting cells. Possible reasons for these changes are discussed.

INTRODUCTION

THE mode of action of oganophosphorus insecticides has been studied by many workers. Primarily, they appear to inhibit the working of the enzyme cholinesterase and so act on the nervous system[15,6]. Secondarily, they are also responsible for a number of physiological and biochemical disturbances[16,17]. Recently, several experimental evidences indicate that carbonic anhydrase and adenosine triphosphatase enzymes in channel catfish, *Ictalurus punctatus*[3] were severely inhibited following O, O, diethyl O isopropyl 4-methyl 6 pyrimidyl phosphorothionate intoxication. But unfortunately very little information is available on the toxic effects of locally used insecticides on economically important fresh water fishes. Metasystox is probably the most widely used insecticide against sucking aphids, spider mites, saw flies etc. It is primarily a neurotoxicant inhibiting the working of the enzyme acetylcholinesterase[12]. Metasystox exposure (ME) also inhibited bimodal respiration and some blood parameters in air-breathing fishes[14]. The present paper deals with the effect of metasystox on some selected oxidative enzymes, tissue respiration and histology of gills of ubiquitous major fresh water fish, *Channa striatus*. The fish, abundantly available locally, is extensively cultured in ponds and rice-fields and is frequently

TABLE I

Effect of lethal (Lc 50/48 hrs) concentration of metasystox on succinate dehydrogenase and lactate dehydrogenase activities of selected organs of C. striatus

Sl. No.	Tissue	SDH activity*		LDH activity*	
		Control	Experimental	Control	Experimental
1.	Gill	0·051±0·001	0·024±0·003 −92 $P < 0·001$	0·007±0·001	0·013±0·002 +86 $P < 0·001$
2.	Brain	0·396±0·021	0·112=0·004 −72 $P < 0·001$	0·056±0·001	0·230±0·007 +318 $P < 0·001$
3.	Liver	0·153±0·001	0·036±0·010 −70 $P < 0·001$	0·063±0·002	0·100±0·010 +59 $P < 0·05$
4.	Muscle	0·617±0·014	0·161±0·010 −64 $P < 0·05$	0·010±0·004	0·050±0·002 +400 $P < 0·001$
5.	*Kidney*	*0·198±0·012*	*0·141±0·009* −29 $P < 0·001$	*0·157±0·001*	0·178±0·003 +13 $P < 0·001$

* μ mole formazan/mg protein/hr

Each value is the mean of 6 individual observations. ± indicates SD. The signs + or − indicate per cent increase or decrease over control.

TABLE II

Effect of lethal (Lc 50/48 hrs) concentration of metasystox on tissue respiration of C. striatus

Sl. No.	Tissue	Control	Experimental	% change	*P*
1.	Gill	1530±25·32	720±30·10	−54	0·001
2.	Brain	704±19·50	368±28·40	−48	0·001
3.	Liver	625±21·62	319±33·72	−33	0·001
4.	Muscle	216±13·05	188±40·18	−13	0·05
5.	Kidney	1362±42·01	1211±46·25	−11	0·05

Values (μ l of oxygen/gram/hour) are mean±SD of 6 individual observations.
The sign − indicate per cent decrease over control.

exposed to metasystox during agricultural operations.

Materials and Methods

Collection, maintenance and weight range of fish used have been described earlier[14]. The technical grade of metasystox (Demeton : O-Methyl sulphoxide = O, O-dimethyl S-2 (Sulphenyl) ethyl phosphorothioate of 95% purity was obtained from Bayer LTD, Bombay. A stock solution of metasystox was made in acetone (1 mg/ml) and suitable quantities of this solution were added to the aquaria to obtain the desired concentrations. Fishes in batches of 10 each were exposed to 5 ppm (lethal concentration) for 48 hr after due standardisation by probit analysis[7]. On completion of ME, the fish were stunned to death and the gill, brain liver, kidney and some skeletal muscle from the anterodorsal region of the trunk were dissected out from each fish in a sterilised cold room at 15° C. For enzyme assays, the tissues were homogenized in 0·25 M cold sucrose solution using a hand-operated ground glass tissue grinder and centrifuged at 3500 g, for 10 min to remove cell debris. The succinate dehydrogenase (SDH) (succinate ; acceptor oxidoreductase EC·1·3·99·1) was estimated by the method of Nachlas *et. al*[13]., and lactate dehydrogenase (*LDH*) (L. *Lactate ; NAD* oxidoreductase EC1·1·1·27) by the method of Srikantan and Krishnamurthy[18]. The protein content was determined by Folin's reagent[10]. Prior to these estimations, the excised organs were allowed 10 min for equilibration. Oxygen consumption was measured in Warburg constant volume respirometer with fish ringer fluid at 7·5 pH (Ekenberg[6]) as liquid phase and air as gas phase (*Umbriet et. al.*[21]). The gills from control and ME fishes were fixed in a saline formol solution (formaldehyde 10%, acetic acid 5% and NaCl 0·85%) for 24 hr. Sections were cut at 6 μ and stained with haematoxylin and eosin.

Results and Discussion

It is evident from Table I that SDH activity is significantly inhibited in all the tissues of ME fishes. Within the tissues, the decrease in SDH was as follows : gill > brain > liver > muscle > kidney. Maximal inhibition in gill tissue indicates its greater vulnerability than other tissues. Being a key enzyme in the TCA cycle, it is logical to assume that with the inhibition of SDH activity, the metabolic pathway might have shifted towards anaerobic side to meet the increased energy demands during pollution stress. Since this enzyme also play an important role in osmoregulation[1], its depression would have disrupted the osmoregulatory machinery. Histological observations corroborates this assumption. Similar inhibition of SDH activities have been reported in *Tilapia mossambica* for sumithion treatment[16]. Following ME, the LDH activity is significantly enhanced (Table I) in all the tissues analysed. The decrease indicated the following trend : muscle > brain > gill > liver > kidney. Interestingly, this enzyme also occupies an important place in metabolic pathways. It is quite likely that the metabolic system might have turned towards anaerobic side. Although pyruvic dehydrogenase enzyme was not measured, it is possible that inhibition of SDH was directly related to anaerobic metabolism. Earlier, Koundinya and Ramamurthy[16] favoured a similar conclusion for sumithion intoxication in *T. mossambica*.

ME decreased (Table II) the O_2 consumption of all the tissues experimented. Within the tissues, the inhibition was in the following order: gill > liver > brain > muscle > kidney. Again the target organ for maximal inhibition was gill. It was followed by brain, liver, muscle and kidney. Greater inhibition

Figs 1–3. Photomicrographs of longitudinal sections of a gill filament of *C. striatus* (× 800). H and E stained.

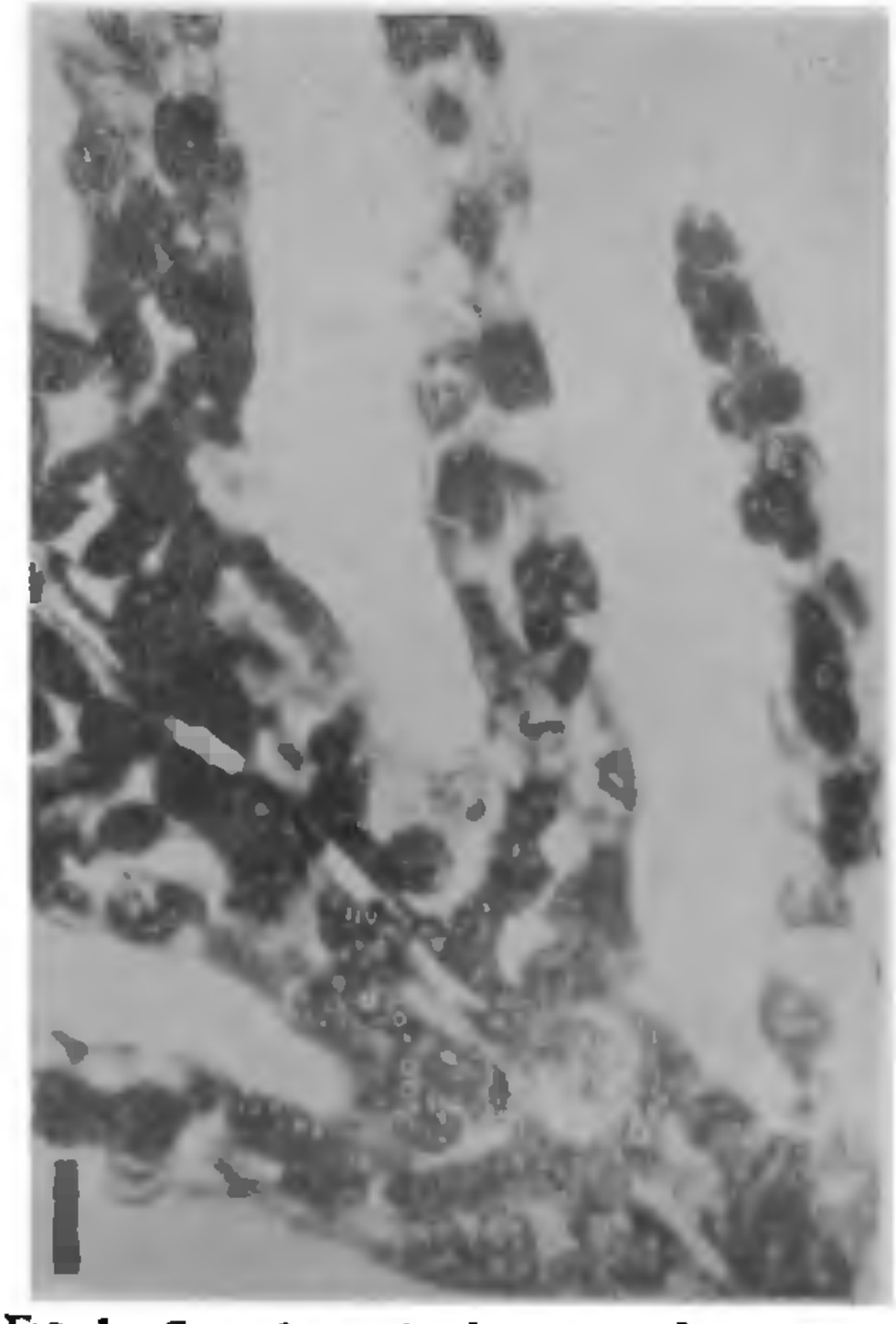

Fig. 1. Secondary gill lamellae of control fish, showing regularly arranged compact filament epithelium.

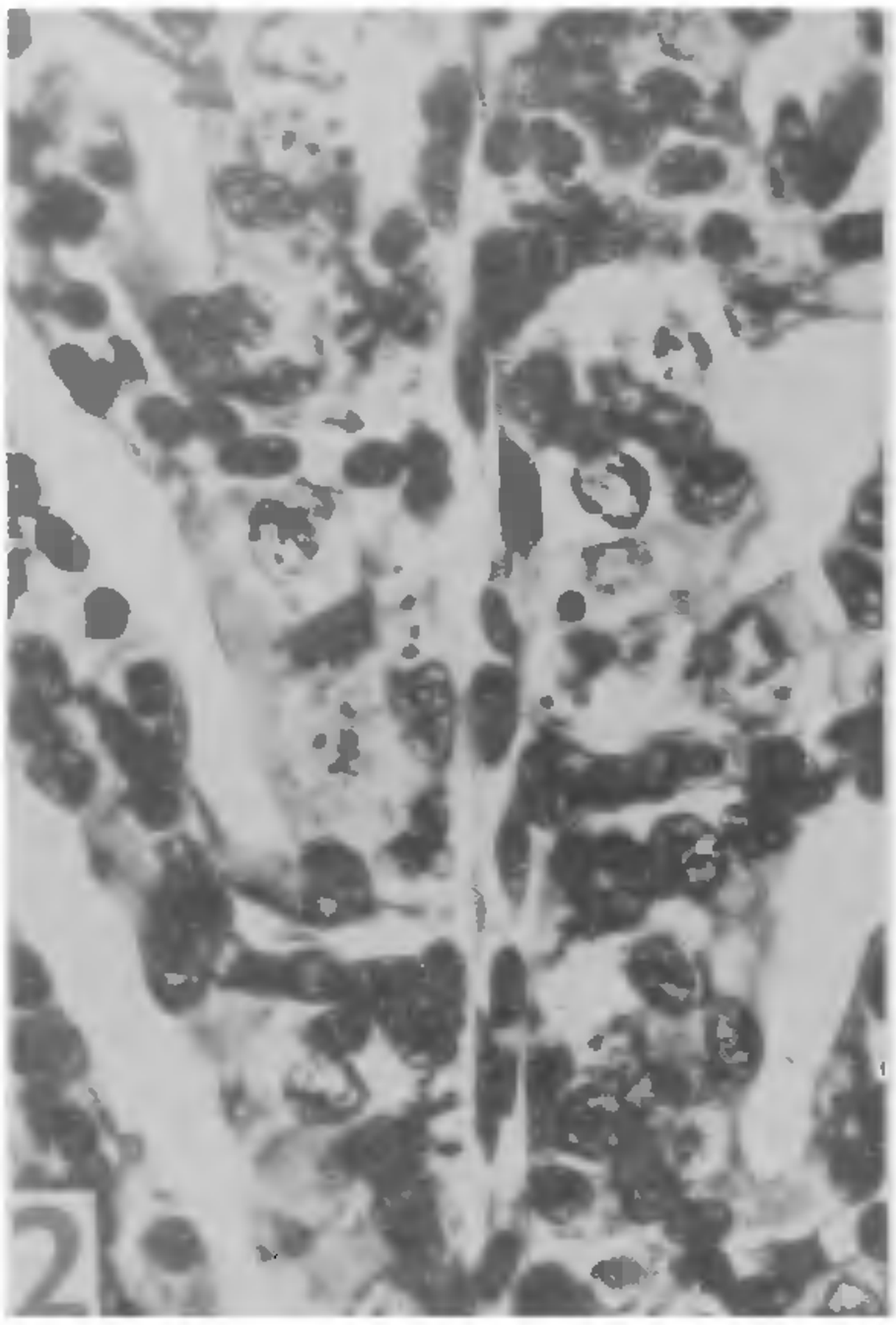

FIG. 2. ME fish : A portion of the gill filament showing separation of epithelial cells from the basement membrane with increased size of nuclei. Swelling of chloride cells (arrow) at the basis of secondary lamellae is also seen.

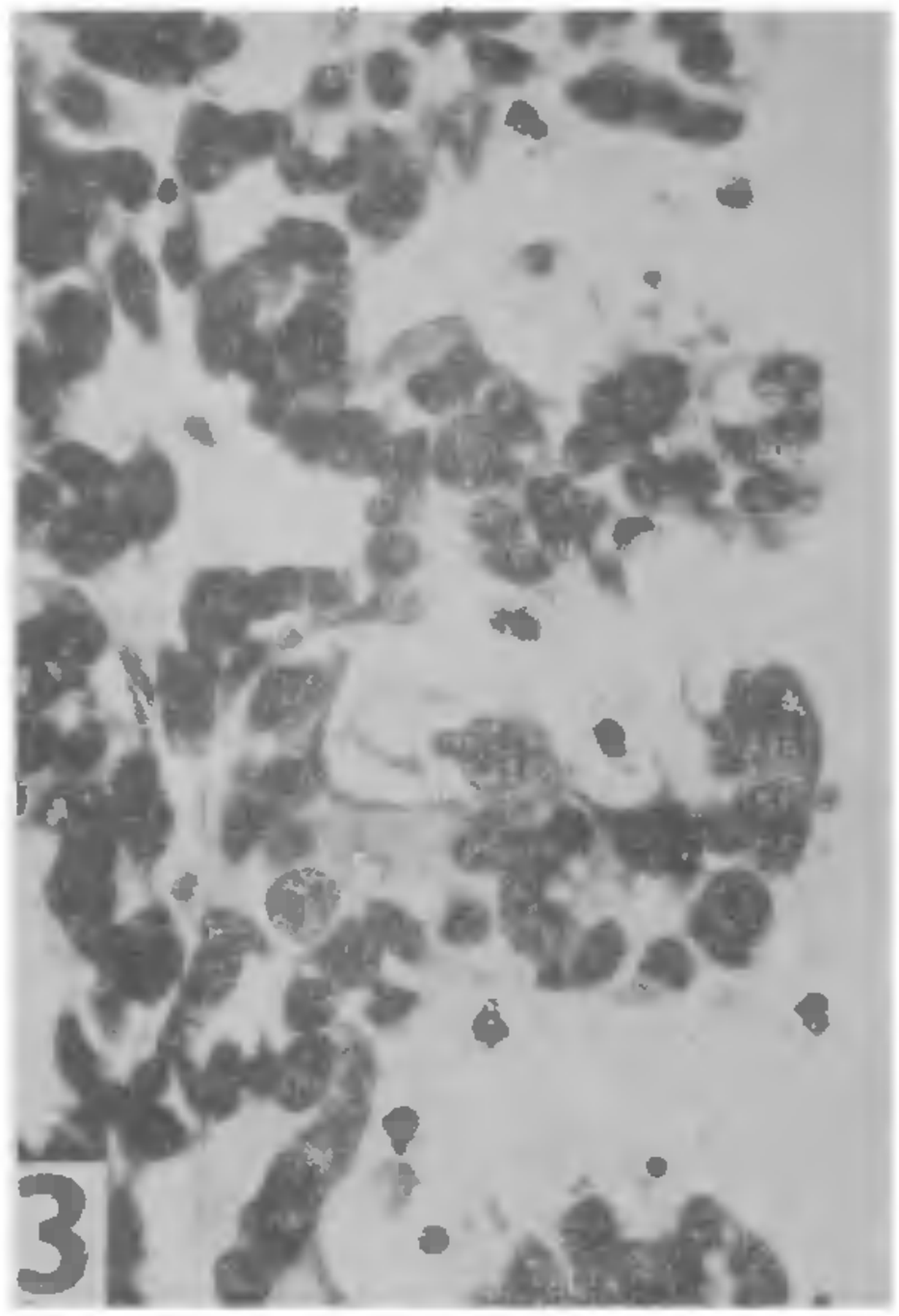

FIG. 3. ME fish: A portion of the gill filament showing extensive vacuolisation and fusion of secondary lamellae.

of O_2 consumption in the gill tissues may be due to the disintegration of respiratory epithelium. It may also be due to the "Coagulation film anoxia[11,15] in which mucus is lost from the gill, as a result of which absorption of O_2 from the surrounding is adversely affected. Exposure of *Lebistes reticularis* to 3·4 mg malathion inhibited respiration by 65%[18]. *In vitro* studies of Hiltibran[8] indicate minimal O_2 uptake by blue gill liver mitochondria in the presence of organophosphate insecticides.

As with a number of toxicants, gill damage was the obvious and acute toxic effect of metasystox. The gill epithelium was completely separated (Fig. 2) from the basement membrane and pillar cells and there was a swelling of the secondary lamellae and dilation of vessels. The chloride secreting cells were enlarged and their nuclei increased in size (Fig. 2). Marked vacuolisation of secondary lamellae was also seen (Fig. 3). Many of these changes can be compared with the observations of Chliamovitch and Kuhn[2] describing the effects of molluscicide bis (tri-*n*-butyltin) oxide on the gills of *Salmo gairdneri*. Khangarot and Somani[9] reported extensive damage to secondary gill filaments following mercury intoxication.

It is well established[11] that secondary gill lamellae play an important role in the transport of respiratory gases. The damage done to the lamellae might have reduced the O_2 transport which in turn would have influenced the metabolic systems of fish. Likewise, the osmoregulatory function was also disrupted in the ME fishes.

The enlargement of chloride secreting cells support the above assumption. Hence it is reasonable to conclude that metasystox intoxication caused severe anaerobic stress and disruption of chloride secreting cells in *C. striatus*. However, a detailed analysis of ion transport during ME is necessary to warrant the latter conclusion. Further work on these aspects is in progress.

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Dr. S. Ramaseshan, Director of Indian Institute of Science, Bangalore has been elected as Vice-President of International Union of Crystallography at the recent Ottawa Congress.