CHAPTER THREE

OBSERVATIONS

A. GENERAL OBSERVATIONS :-

Both the organophosphorus pesticides, namely malathion and sumithion, at present used widely, for control of various agricultural crop pests. These are also used in public health, control of household and for veterinary pests. The overuse of these pesticides in some agricultural and public health operations may not cause disequilibrium or disturbances in aquatic environment, but it has sometimes adversely affected non-target organisms such as freshwater fishes. On entering the water these pesticides alter the physico-chemical properties of water e.g. depletion in oxygen and alternation of pH which impaire the various physiological activities, leading to death of the fish.

During the present investigation, to study the physico-chemical properties of malathion and sumuthion in aquaria the following parameters were studied; namely, temperature, pH and dissolved oxygen (DO). The $CaCO_3$ hardness of tap water was 51-78 ppm which was not altered after addition of both the pesticides, therefore this parameter was not considered. The results obtained are recorded in Table No. 2 and Table No. 3 and shown in figs, 1, 2, 3, and 4.

Fluctuations in the values of the parameters are evident from the table and figures. The temperature of water varied from 26°C to 28°C during day. The mean temperature being 27°C. The pH of tap water used for

TABLE NO. 2 :- Showing the values of Physico-chemical Parameters of tap water and the same at different concentrations of malathion.

Ob.No.	Concentration	Mean Temperature	pH	DO
1.	0 ppm (Tap water)	27°c	7.35	8.5 ppm
2.	4 ppm malathion	27°c	7.25	6.5 ppm
3.	6 ppm malathion	27°c	7.20	6.0 ppm
4.	8 ppm malathion	27°c	7.15	5.1 ppm
5.	10 ppm malathion	27°c	7.12	4.6 ppm
6.	12 ppm malathion	27°c	7.05	4.2 ppm

Fig. 1 : SHOWING pH VALUES AT DIFFERENT CONCENTRATIONS OF MALATHION.

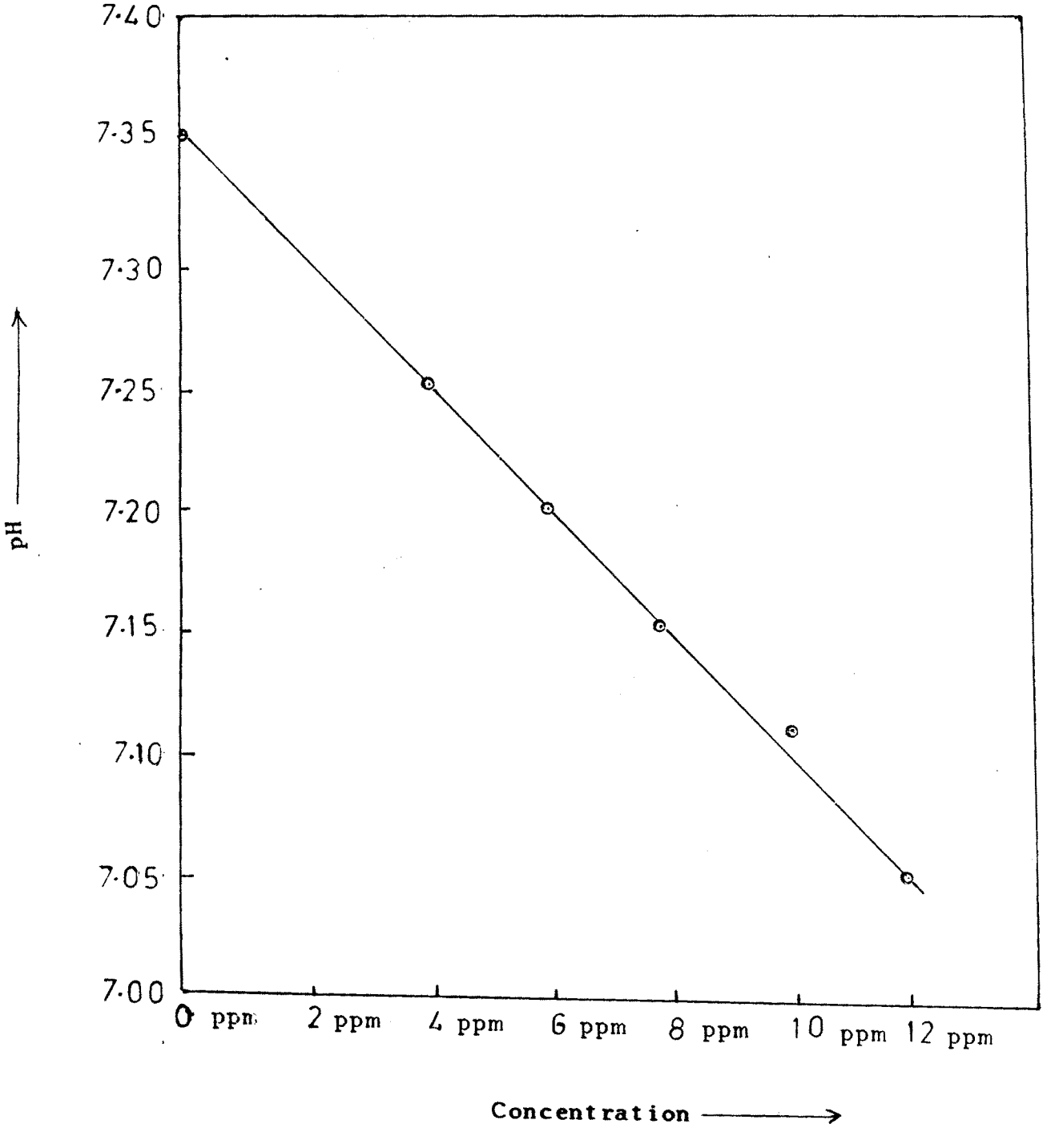


Fig. 2 : SHOWING DISSOLVED OXYGEN VALUES IN ppm AT DIFFERENT CONCENTRATIONS OF MALATHION.

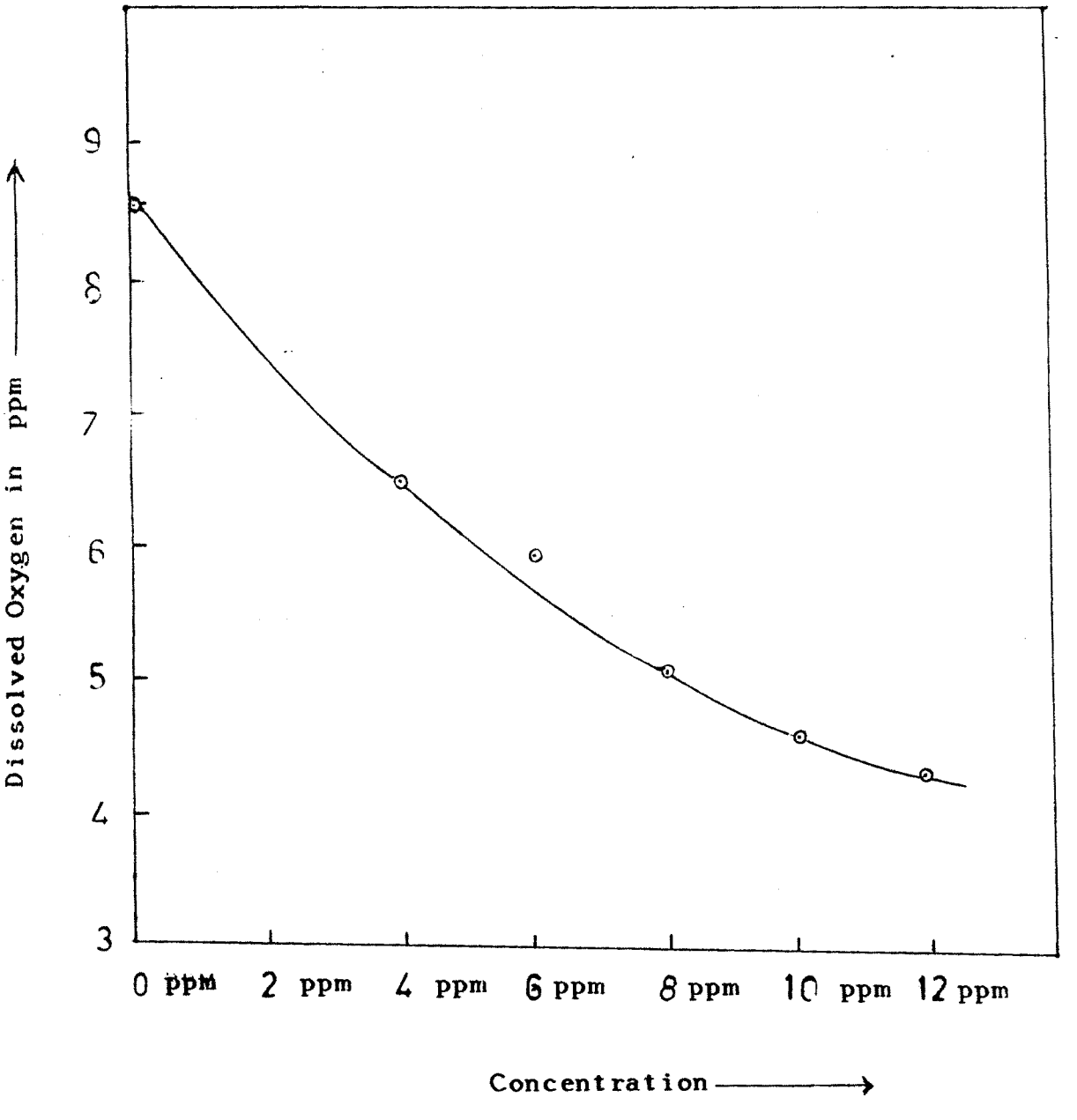


TABLE NO. 3 :- Showing the values of Physico-chemical parameters of tap water the same at different concentration of sumithion.

Ob.No.	Concentration	Mean Temperature	pH	DO
1.	0 ppm (Tap water)	27°c	7.35	8.5 ppm
2.	10 ppm sumithion	27°c	7.32	8.0 ppm
3.	15 ppm sumithion	27°c	7.23	7.5 ppm
4.	20 ppm sumithion	27°c	7.19	7.0 ppm
5.	25 ppm sumithion	27°c	7.15	6.6 ppm
6.	30 ppm sumithion	27°c	7.10	5.5 ppm

Fig. 3 : SHOWING pH VALUES AT DIFFERENT CONCENTRATIONS OF SUMITHION.

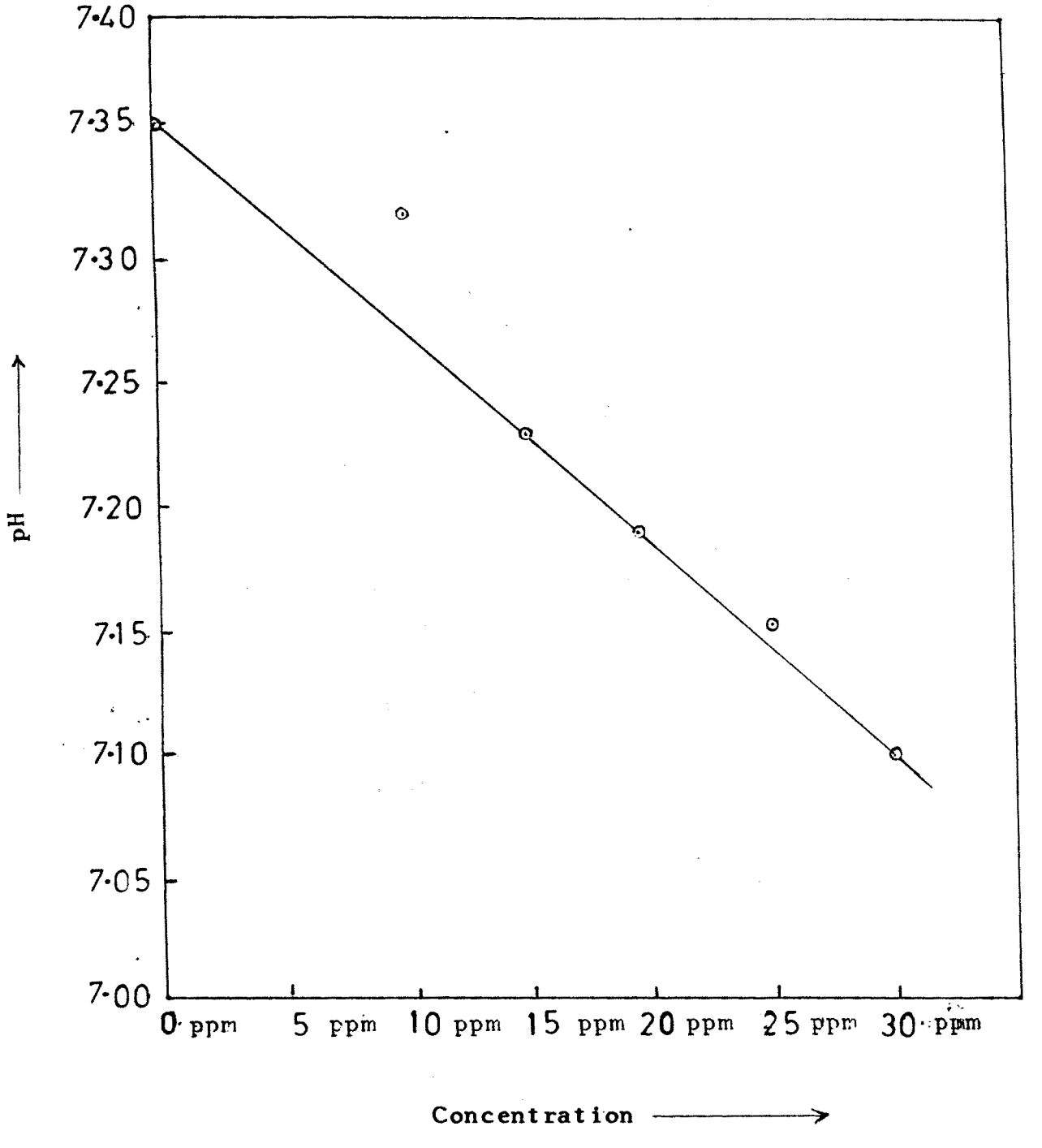
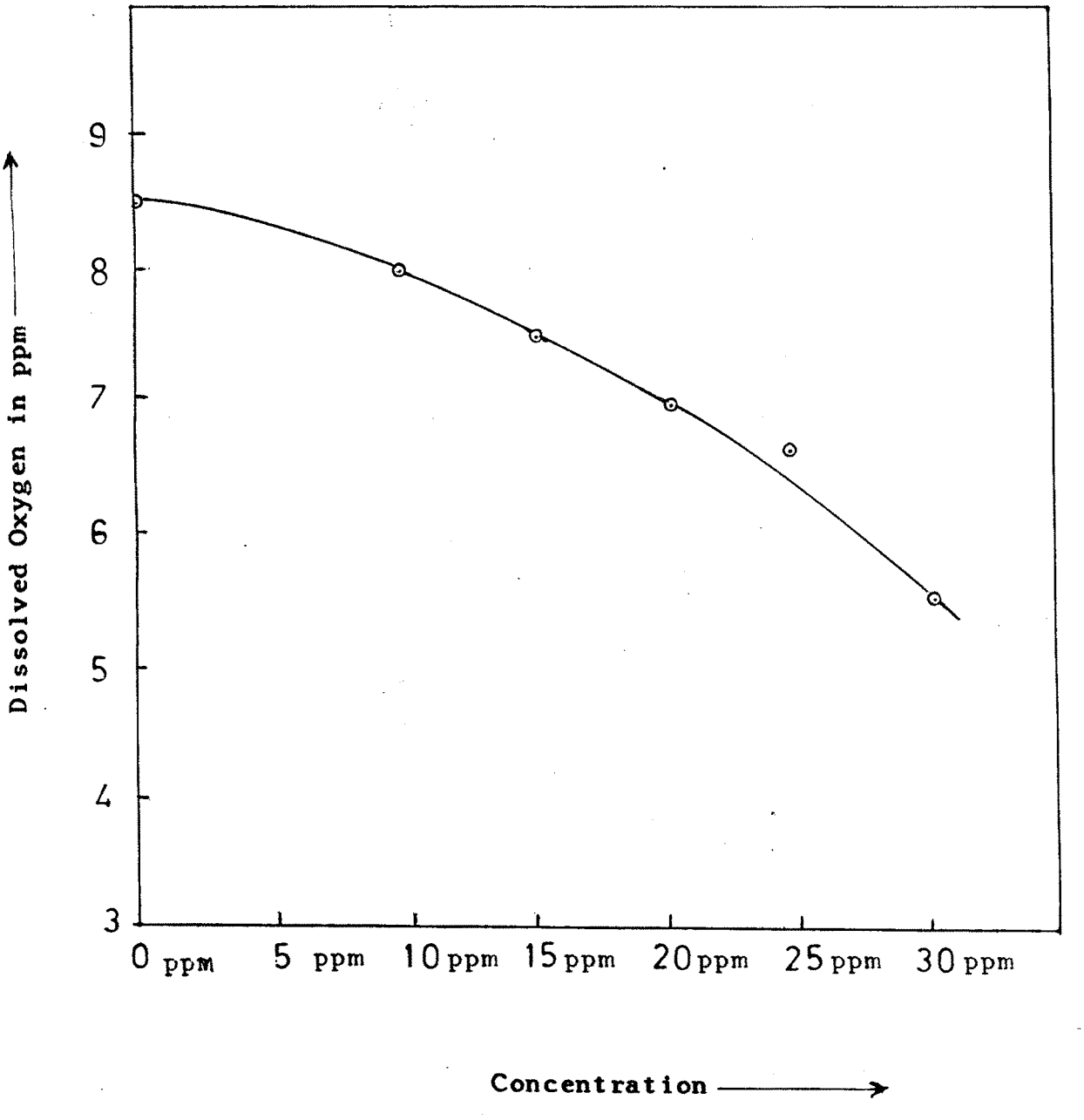


Fig. 4 : SHOWING DISSOLVED OXYGEN VALUES IN ppm AT DIFFERENCE CONCENTRATIONS OF SUMITHION.



acclimatization of fishes was 7.35 which gradually decreased after addition of both the pesticides in it, for making the concentrations of strength 4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm malathion and 10 ppm, 15 ppm, 20 ppm, 25 ppm and 30 ppm sumithion. The corresponding pH values were observed to be 7.25, 7.20, 7.15, 7.12 and 7.05 for malathion and 7.32, 7.23, 7.19, 7.15 and 7.10 for sumithion. The DO values gradually decreased from 8.5 ppm to 4.2 ppm for malathion and 8.5 ppm to 5.5 ppm for sumithion

B. DETERMINATION OF LC 50 VALUES :-

Determination of LC 50 values for present fish was carried out by using different concentrations of malathion and sumithion. The results of the rate of mortality are shown in Table No. 4 and Table No. 5 for malathion and sumithion respectively and are shown in figs. 5 and 6 . They show that 50% mortality of fish occured at 7 ppm malathion concentration and at 17.5 ppm sumithion concentration. Therefore the LC 50 value for C.punctatus is 7 ppm for malathion and 17.5 ppm for sumithion.

Time factor

C. FISH BEHAVIOUR :-

When well acclimatized fishes were transfered to test acqaria , containing different concentrations of

TABLE NO 4 :- Showing mortality record of C.punctatus at different concentrations of malathion.

Concentration of Malathion	No.of fishes used	No. of dead fishes					Mortality at 48 hours.
		12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	
0 ppm	10	Nil	Nil	Nil	Nil	Nil	0.0%
4 ppm	10	Nil	1	1	3	2	20%
6 ppm	10	1	1	2	3	3	40%
8 ppm	10	1	2	3	4	-	60%
10 ppm	10	2	3	4	1	-	90%
12 ppm	10	8	2	-	-	-	100%

Fig. 5 : SHOWING LC 50 VALUES FOR C.punctatus AT DIFFERENT CONCENTRATIONS OF MALATHION.

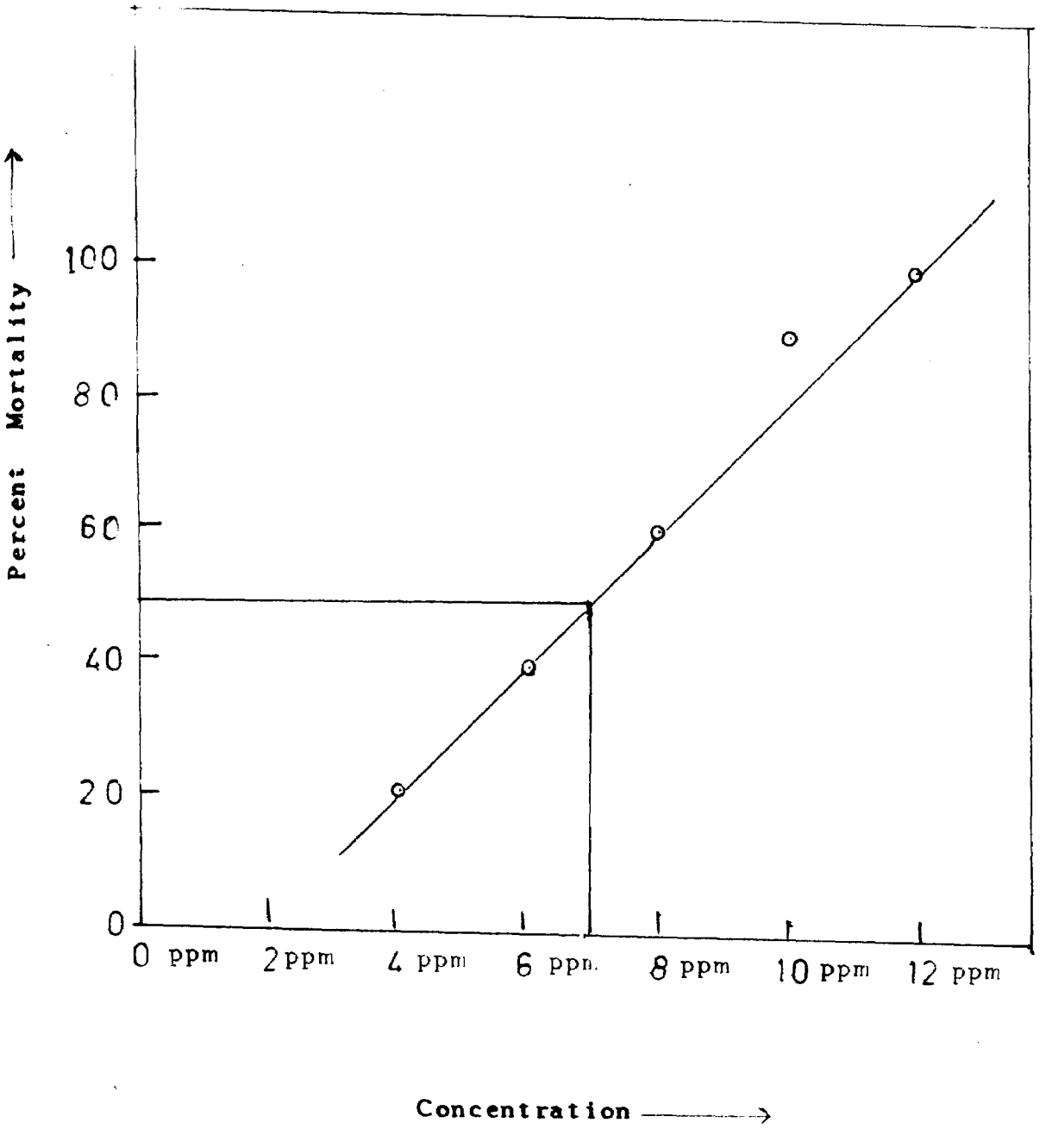
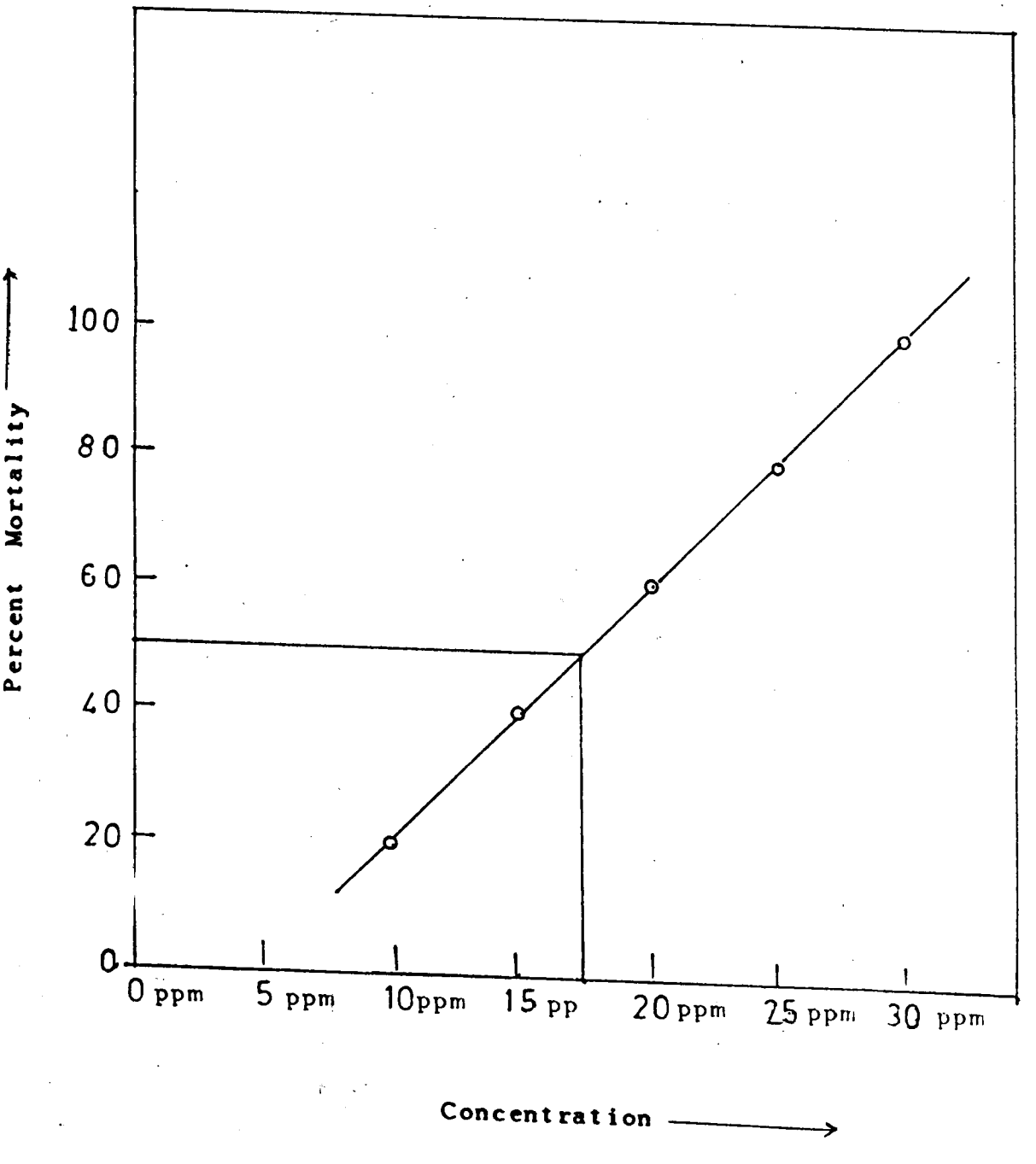


TABLE NO. 5 :- Showing morality record of C.punctatus at different concentrations of sumithions.

Concentration of sumithions	No. of fishes used	No. of dead fishes					Mortality at 48 hrs.
		12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	
0 ppm	10	Nil	Nil	Nil	Nil	Nil	0.0%
10 ppm	10	Nil	Nil	2	2	3	20%
15 ppm	10	Nil	2	2	4	2	40%
20 ppm	10	1	2	3	4	-	60%
25 ppm	10	2	3	3	2	-	80%
30 ppm	10	3	6	1	-	-	100%

Fig. 6 :- SHOWING LC 50 VALUES FOR C.punctatus AT DIFFERENT CONCENTRATIONS OF SUMITHION



malathion and sumithion, showed changes in normal behaviour, which are as follows :

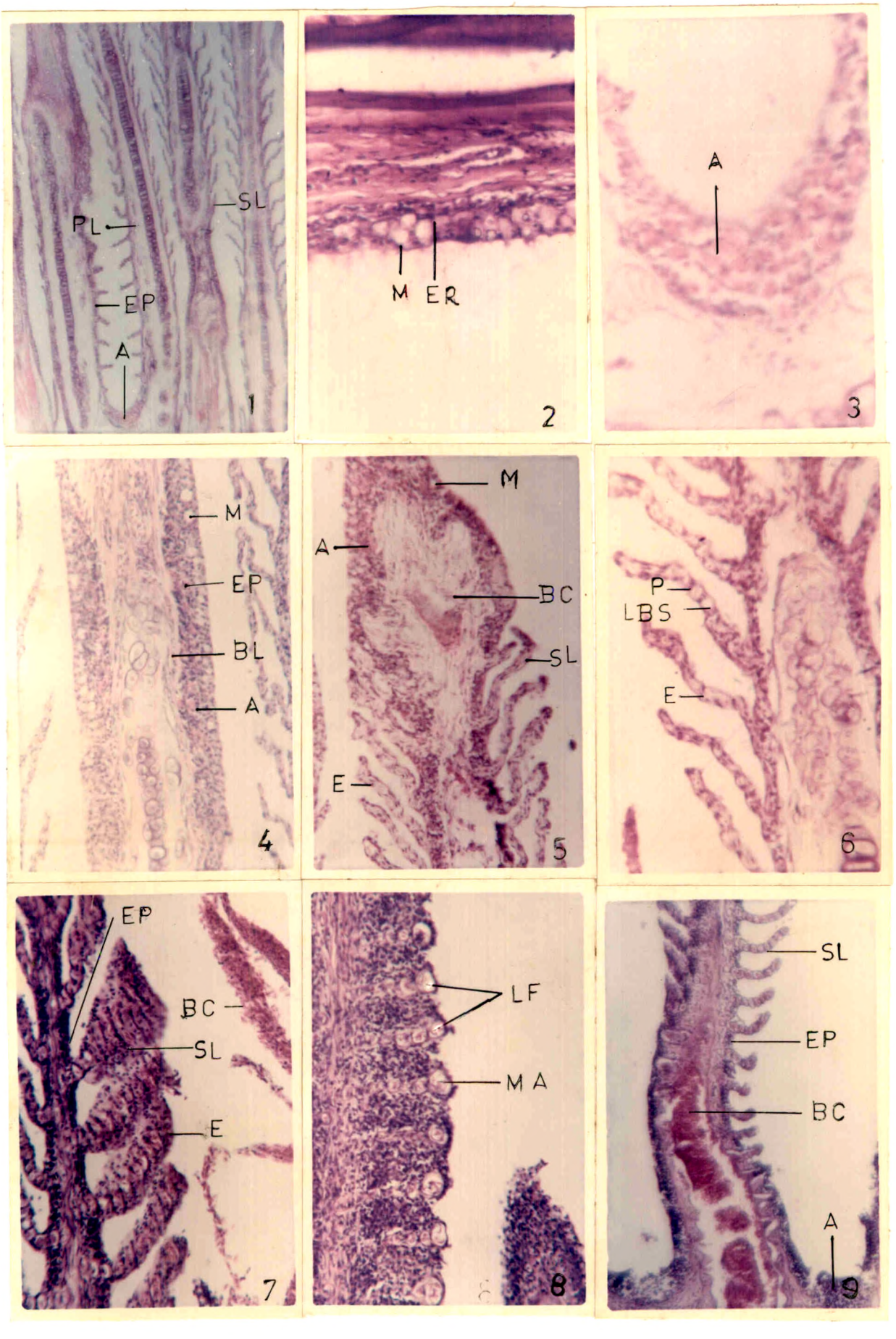
1. At lower concentrations, the fishes initially showed hypersensitivity, they were excited in the begining for some time and they became steady with erratic swimming, spirraling and occasional jerky movements.
2. There was gradual decrease in opercular movement of the fish which was later followed by higher opercular beating, rapid air gulping and surfacing activity.
3. This was followed by the slow movement of the fish towards the surface of water assuming a diagonal position.
4. Loss of equilibrium, sensetivity to touch and sound was noticed .
5. The fish became inverted (belly upwards) and floated near the surface for some time. They finally settled down at the bottom of aquarium.
6. At higher concentrations of both the pesticides, the fishes tried to leap out, to avoid the toxic medium.
7. At the time of death, mouth and opercula of fishes remained open at lower concentrations, however, they closed their mouth and opercula at higher concentrations.
8. The mortality was evident because of the absence of

any motion or respiration, since there was no movement of operculum.

It appears that asphyxiation of the fishes was the ultimate result due to strained respiration, when it was exposed to lethal concentration of malathion and sumithion.

D. HISTOLOGICAL OBSERVATIONS :-

Histomorphologically the gill of Channa punctatus revealed identical structure to that of many fresh water teleosts. There were four pairs of gills. Each gill was supported by gill arch (Fig. 2) formed by bony material. The gill arch was covered with multilayered epithelial cells (Fig. 2). The epithelial cells forming the outermost layer were flat with basophilic cytoplasm and centrelly located nuclei. The epithelial cells at deeper layer were not so much differentiated which were rest on basement lamina. Below this was present a muscle sheath which was followed by a bony element. Elongated to round mucous cells were found in the outer epithelial layer (Fig. 2). The cytoplasm of these cells was homogenous and basophilic. As these cells were covered with great quantity of mucus material the nuclei could not be observed in these cells. Round to oval acidophilic cells were also found in this layer of epithelium.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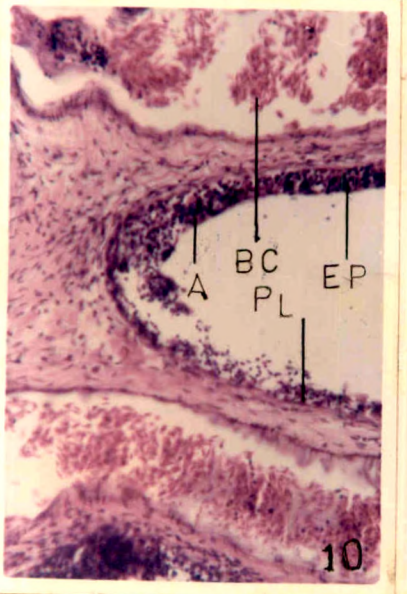
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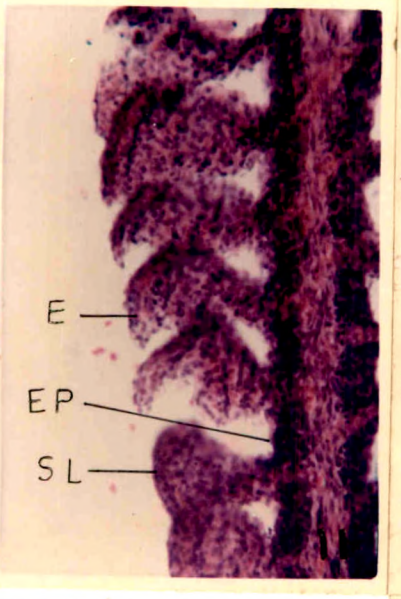
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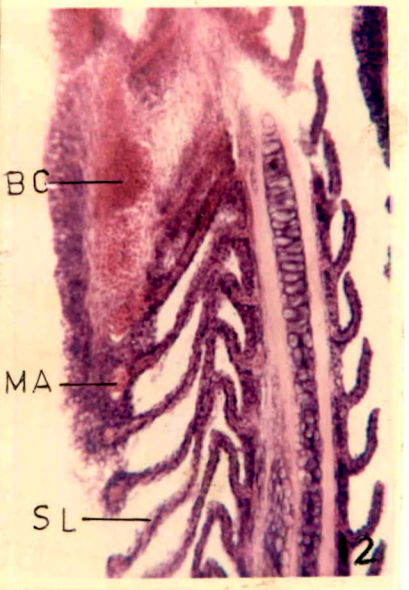
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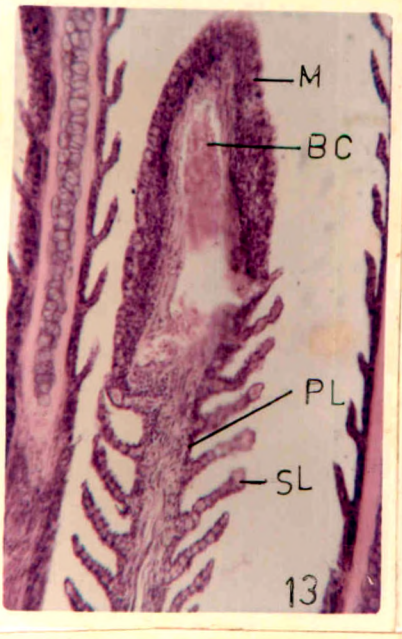
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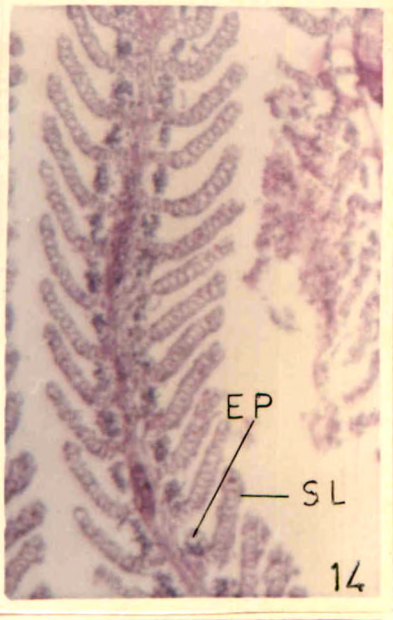
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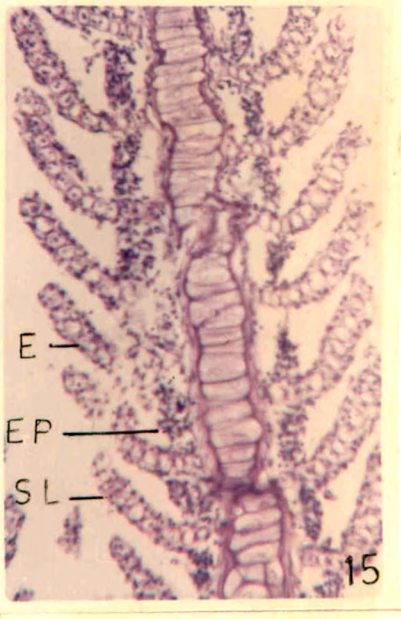
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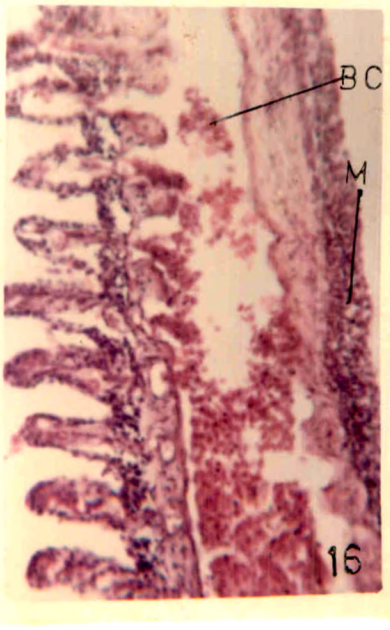
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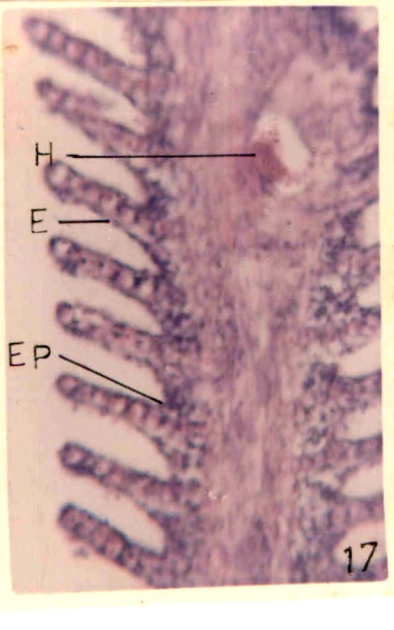
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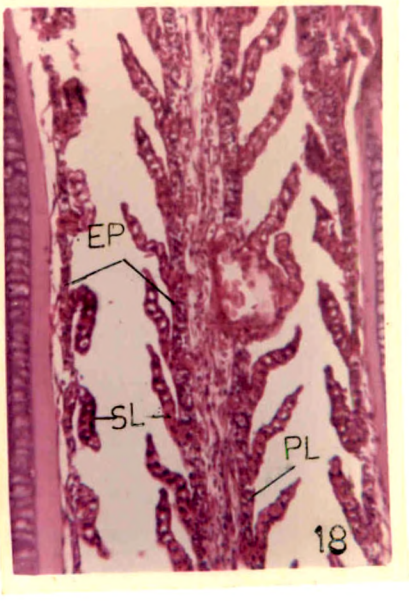
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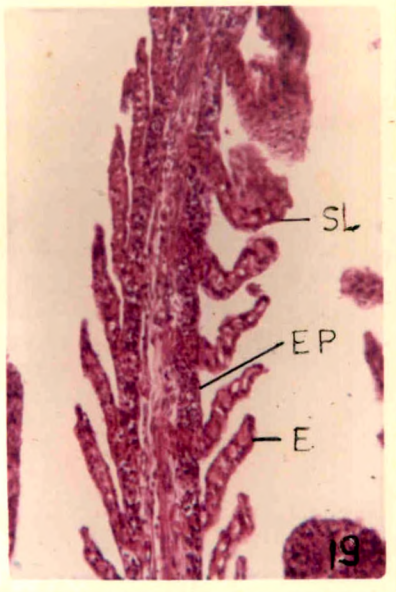
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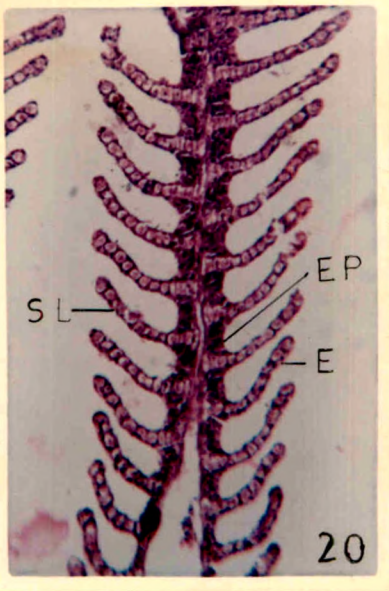
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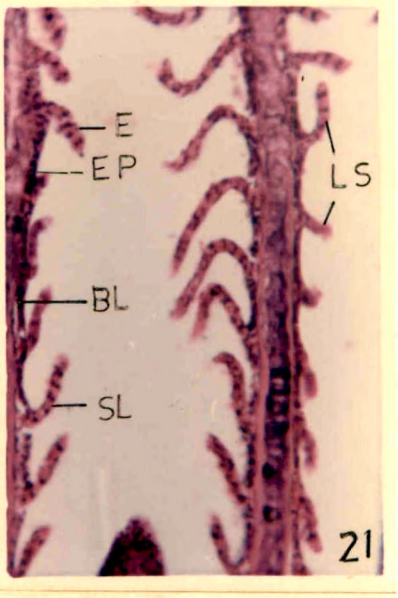
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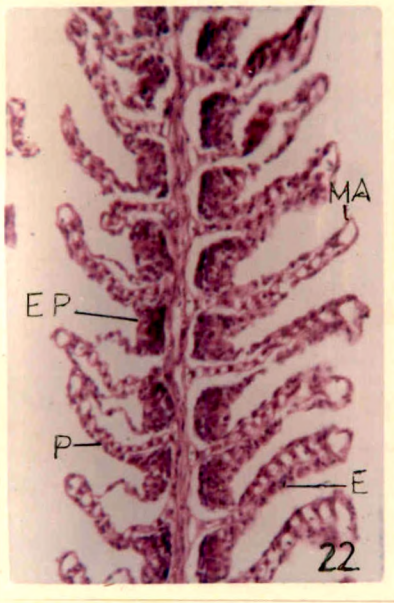
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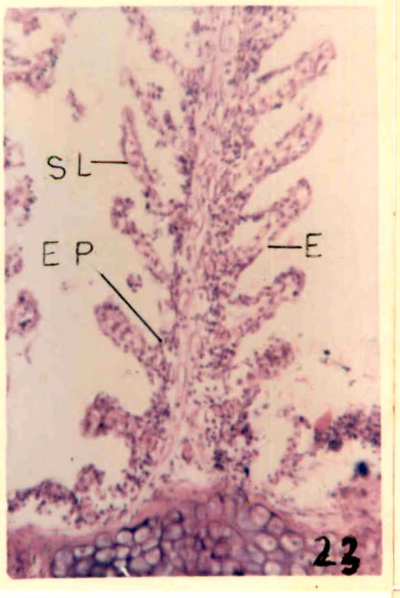
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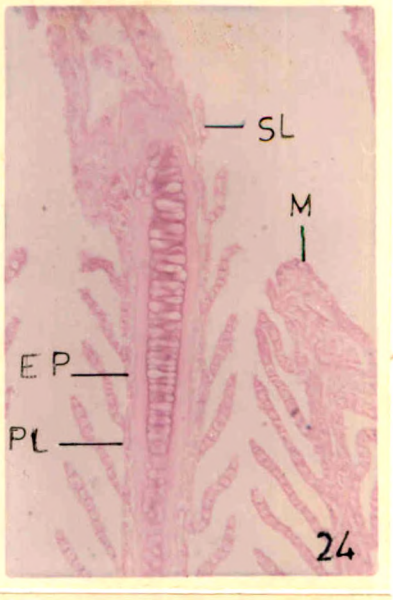
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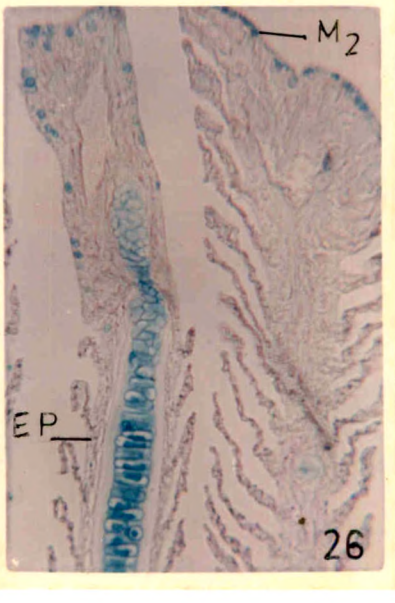
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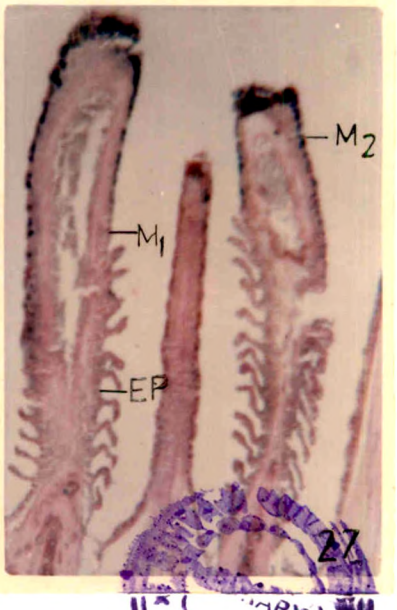
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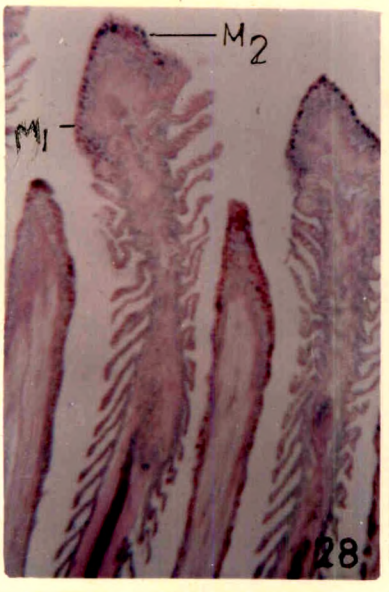

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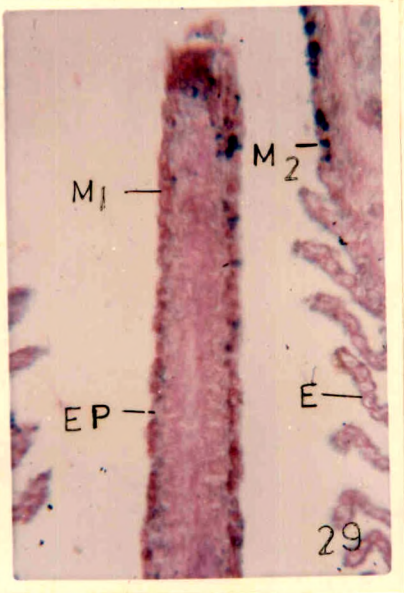

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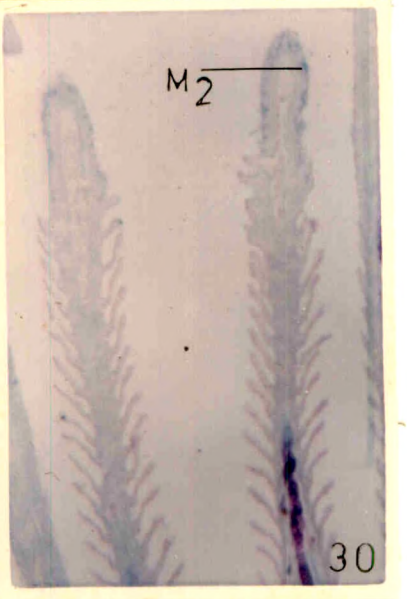

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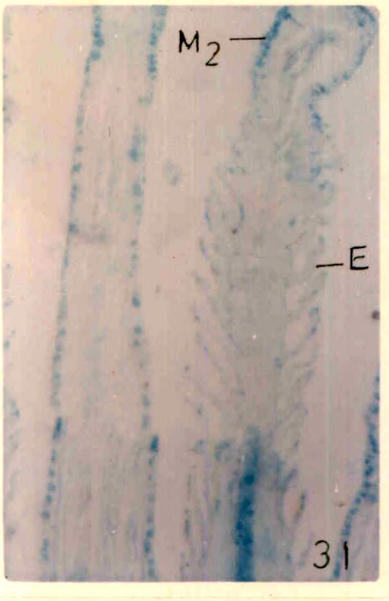

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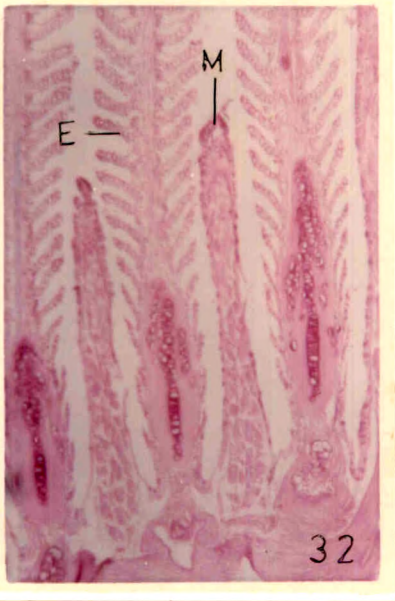

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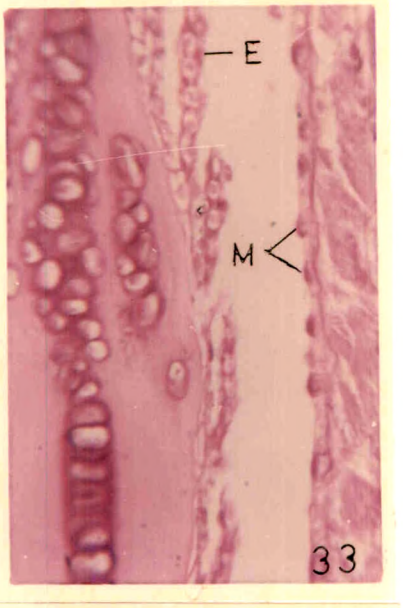

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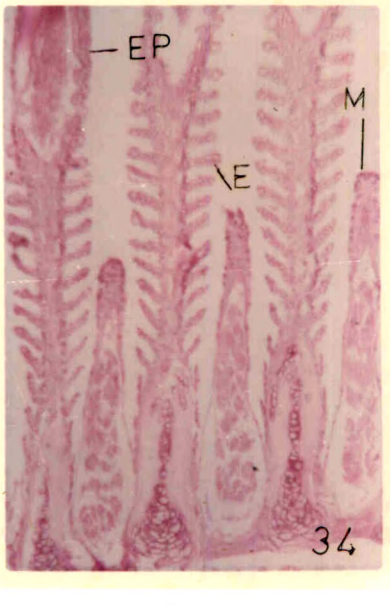

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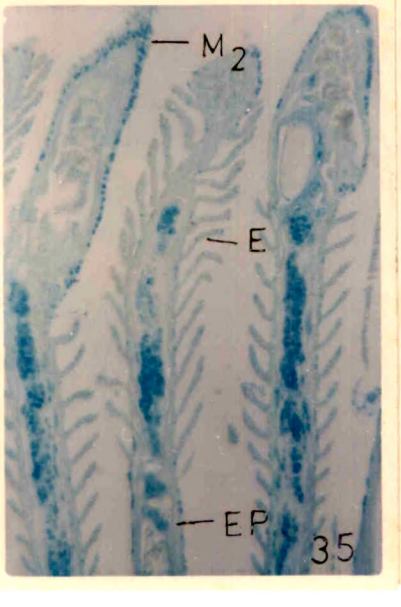

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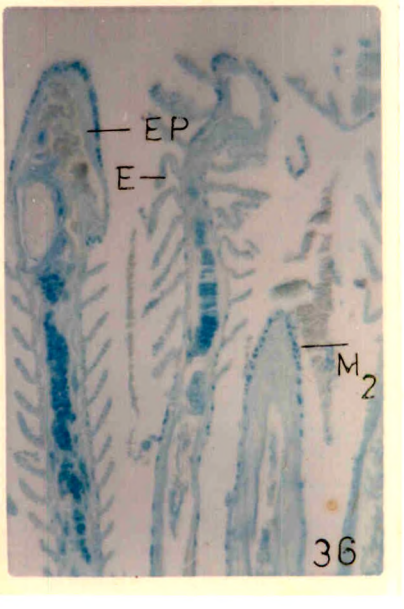

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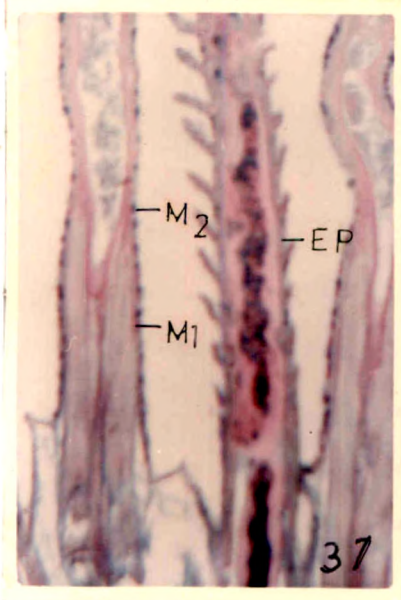
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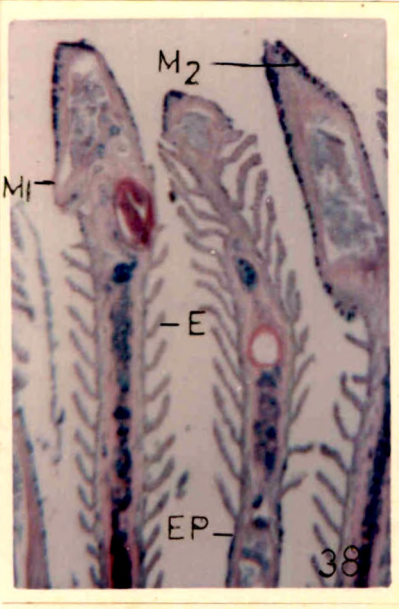
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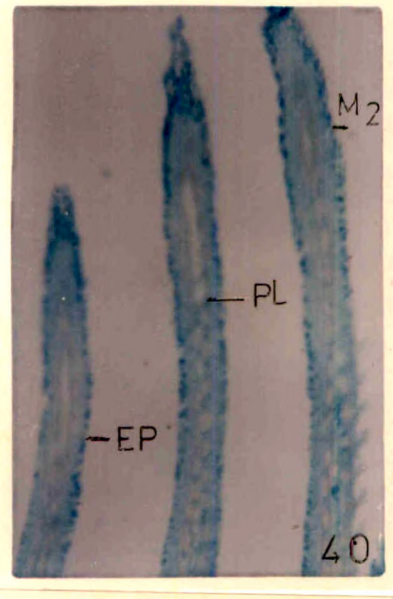
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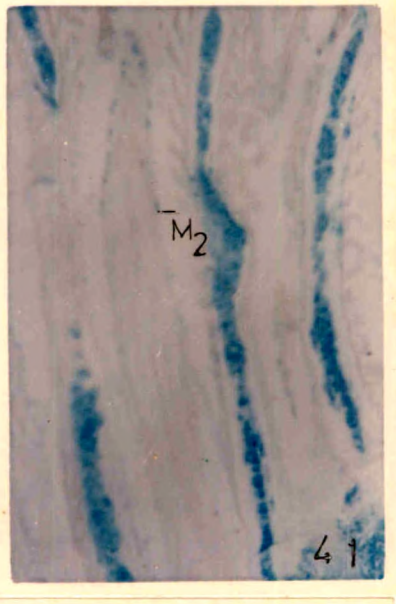
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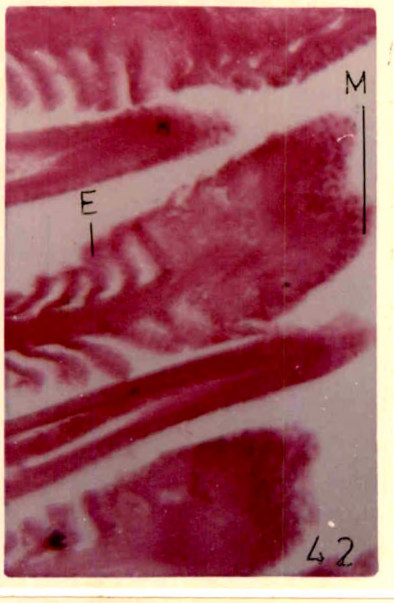
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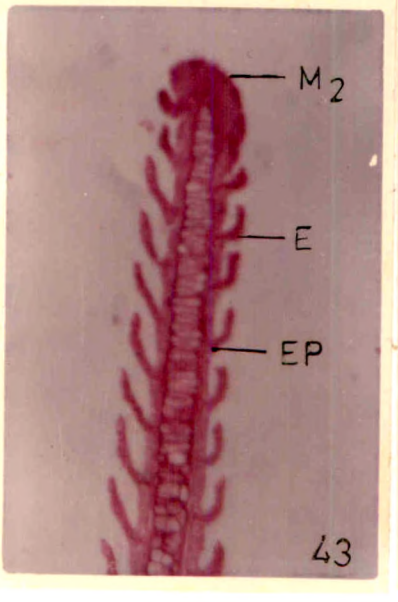
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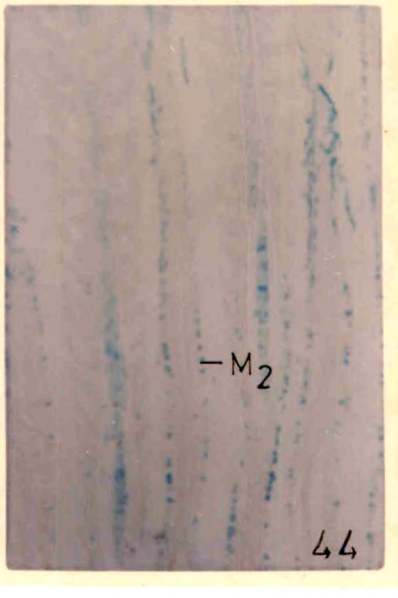
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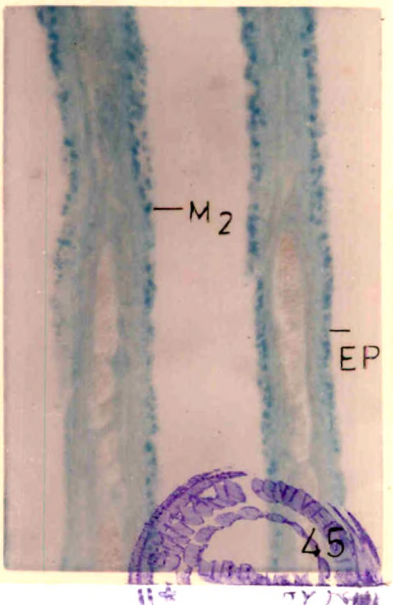
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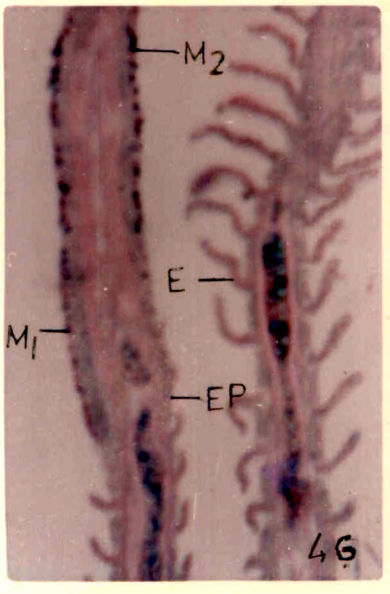

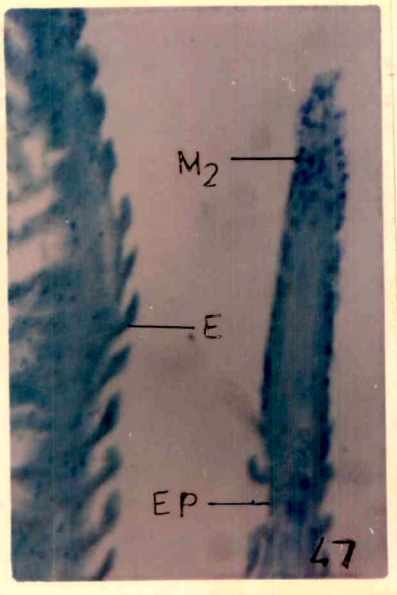

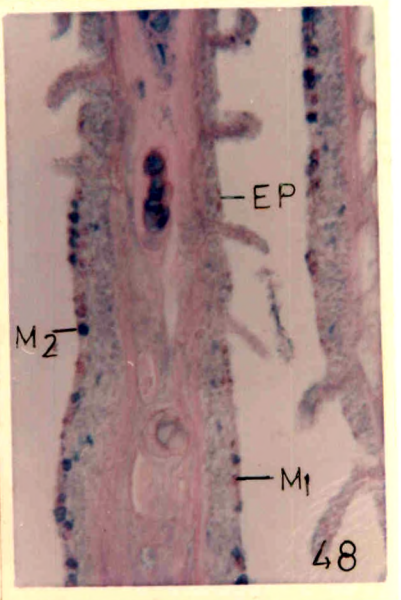

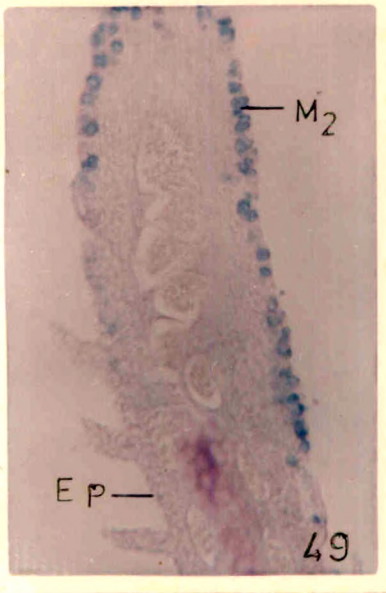

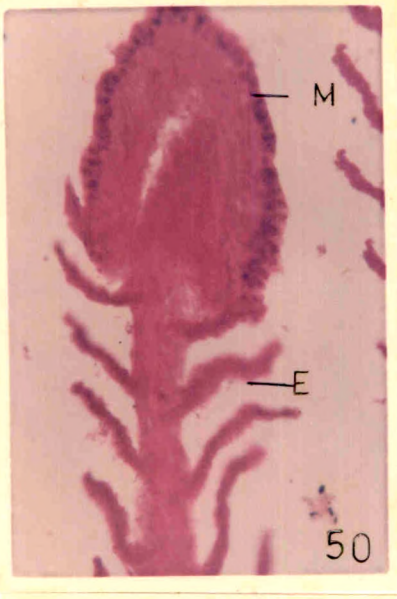

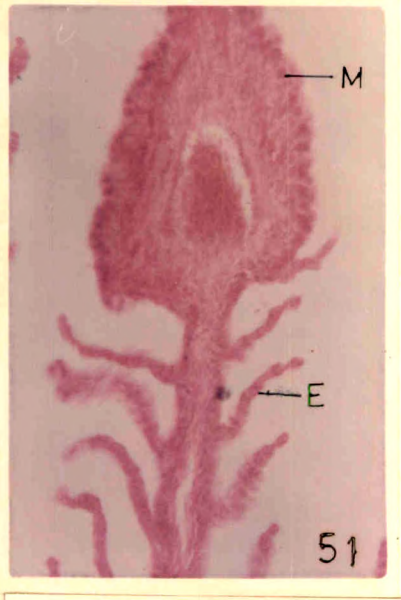

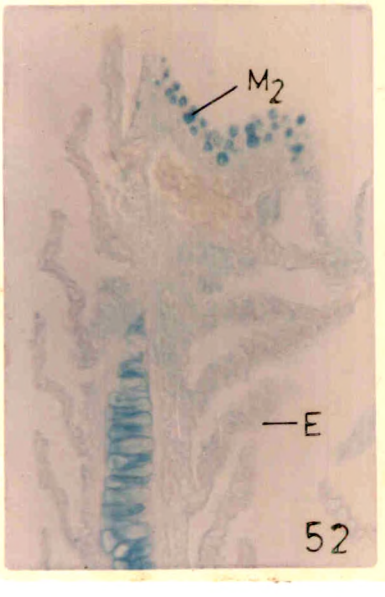

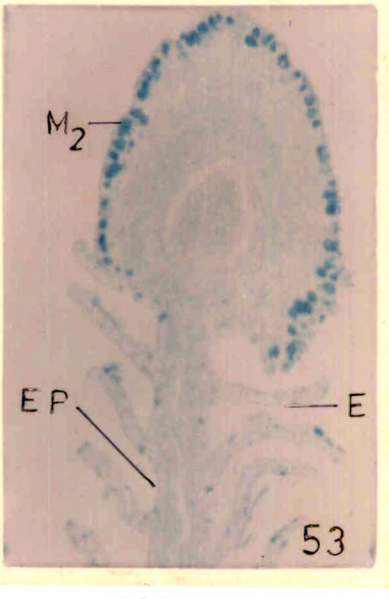

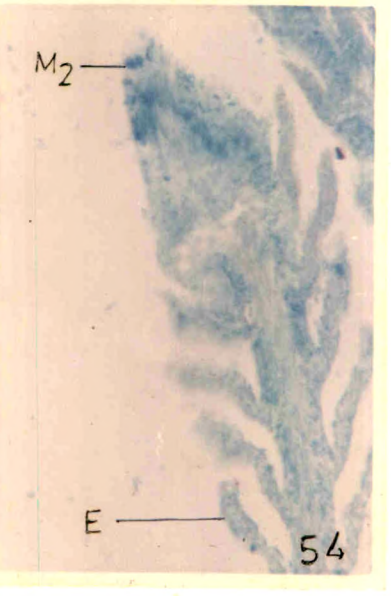

CAPTIONS TO FIGURES

Fig. 1 : L.S. of gill fo control fish. H-E Staining x 100.

Fig. 2 : L.S. of gill arch of control fish. H-E staining x 300.

Fig. 3 : L.S. of gill of control fish, showing inter lamellar region. H-E staining x 450.

Fig. 4 : L.S. of gill of control fish. H-E staining x 300.

Fig. 5 : L.S. of gill of control fish. Showing more mucous cells at the tip of primary gill lamellae H.E. Staining x 300.

Fig. 6 : L.S. of gill of control fish. H-E staining x 450.

Fig. 7 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours, showing thickened epithelium of Secondary gill lamellae. H-E. staining x 300.

Fig. 8 : L.S..of gill of fish exposed to 4 ppm malathion for 72 hours showing fusion of adjacent secondary gill lamellae. H-E staining x 300.

Fig. 9 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours showing progressive degeneration of supporting structure towards basal part of primary gill lamellae. H-E staining x 300.

ABBREVIATIONS

PL	-	Primary gill lamellae.
SL	-	Secondary gill lamellae.
EP	-	Epithelium of primary gill lamellae.
E	-	Epithelium of Secondary gill. Lamellae.
A	-	Acidophil cells.
M	-	Mucous cells.
BL	-	Basement lamina.
LBS	-	Lamellar blood sinuses.
P	-	Pillar cells.
LF	-	Lammellar fusion.
MA	-	Marginal blood cells.
ER	-	Epithelium of gill arch

CAPTIONS TO FIGURES

Fig. 10 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours. H-E staining x 300.

Fig. 11 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours. , showing thickening of epithelium of primary gill lamellae. H-E staining x 300.

Fig. 12 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours., showing dialated primary gill lamellae. H-E staining x 200.

Fig. 13 : L.S. of gill of fish exposed to 8 ppm malathion for 72 hours showing, enlargment of tip of Primary gill lamellae. H-E staining x 200.

Fig. 14 : L.S. of gill of fish exposed to 10 ppm malathion for 48 hours, showing lifting of epithelium of Primary gill lamellae. H-E staining x 150.

Fig. 15 : L.S. of gill of fish exposed to 10 ppm malathion for 48 hours, showing necrosis in epithelial cells of primary and secondary gill lamellae . H-E staining x 300.

Fig. 16. : L.S. of gill of fish exposed to 12 ppm malathion for 12 hours, showing hyperplasia of blood cells. H-E staining x 300.

Fig. 17 : L.S. of gill of fish exposed to 12 ppm malathion for 12 hours H-E staining x 300.

Fig. 18 : L.S. Of gill of fish exposed to 10 ppm sumithion for 96 hours, showing uneven curling of secondary lamellae. H-E staining x 300.

ABBREVIATIONS

PL	-	Primary gill lamellae.
SL	-	Secondary gill lamellae.
EP	-	Epithelium of primary gill lamellae
E	-	Epithelium of secondar gill lamellae.
A	-	Acidophil cells.
BC	-	Blood cells.
MA	-	Marginal blood cells.
M	-	Mucous cells.
H	-	Haematomass

CAPTIONS TO FUGURES

Fig. 19 : L.S. of gill of fish exposed to 10 ppm sumithion for 96 hours, showing hyperplasia of epithelial lining of secondary gill lamellae. H-E staining x 300.

Fig. 20 : L.S. of gill of fish exposed to 15 ppm sumithion for 72 hours, showing degeneration of the supporting material at the base of primary gill lamellae. H-E staining x 200.

Fig. 21 : L.S. of gill of fish exposed to 20 ppm sumithion for 72 hours, showing thining and shortening of secondary gill lamellae. H-E staining x 200.

Fig. 22 : L.S. of gill of fish exposed to 25 ppm sumithion for 48 hours, showing thickening of inter lamellar epithelium. H-E staining x 300.

Fig. 23 : L.S. of gill of fish exposed to 30 ppm sumithion for 24 hours, showing necrosis of interlamellar epithelium of primary gill lamellae. H-E staining x 300.

Fig. 24 : L.S. of gill of control fish. PAS staining x 100.

Fig. 25 : L.S. of gill of control fish. AB pH 1.0 staining x 100.

Fig. 26 : L.S. of gill of control fish. AB pH 2.5 staining x 450.

Fig. 27 : L.S. of gill of control fish AB 1.0-PAS staining x 100.

ABBREVIATIONS

PL	-	Primary gill lamellae.
SL	-	Secondary gill lamellae.
EP	-	Epithelium of primary gill lamellae.
E	-	Epithelium of secondary gill. lamellae.
IS	-	Inter-lammelar space.
BL	-	Basement lamina.
MA	-	Marginal blood channel.
P	-	Pillar cells.
M1	-	M1-Mucous cells.
M2	-	M2-Mucous cells.
M	-	Mucous cells.

CAPTIONS TO FIGURES

Fig. 28 : L.S. of gill of control fish. AB pH 2.5-PAS staining x 100.

Fig. 29 : L.S. of gill of control fish C.I.-PAS staining x 225.

Fig. 30 : L.S. of gill of control fish. AF-AB 2.5 staining x 150.

Fig. 31 : L.S. of gill of control fish. M 37 - AB pH 2.5 staining x 150.

Fig. 32 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours. PAS staining x 100.

Fig. 33 : A magnified view of L.S. of gill of fish exposed to 4 ppm malathion for 72 hours. PAS staining x 450.

Fig. 34 : L.S of gill of fish exposed to 4 ppm malathion for 72 hours. D-PAS staining x 100.

Fig. 35 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours. AB pH 1.0 staining x 100.

Fig. 36 : L.S. of gill of fish exposed to 4 ppm malathion, AB pH 2.5 staining x 100.

ABBREVIATIONS

EP	-	Epithelium of Primary gill lamellae.
E	-	Epithelium of secondary gill. lamellae.
M1	-	M1-mucous cells.
M2	-	M2-Mucous cells.
M	-	Mucous cells.

CAPTIONS TO FIGURES

Fig. 37 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours.
AB pH 2.5-PAS staining x 150.

Fig. 38 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours.
C.I.-PAS staining x 100.

Fig. 39 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours.
AF staining x 150.

Fig. 40 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours.
DM 37 - AB pH 2.5 staining x 100.

Fig. 41 : L.S. of gill of fish exposed to 4 ppm malathion for 72 hours.
Pepsin - AB pH 2.5 staining x 150.

Fig. 42 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
PAS - staining x 150.

Fig. 43 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
D-PAS staining x 150.

Fig. 44 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
AB pH 1.0 staining x 100.

Fig. 45 : L.S. of gill of fish exposed to 6 ppm malathion.AB pH 2.5 staining x 150.

ABBREVIATIONS

Pl	-	Primary gill lamellae.
EP	-	Epithelium of primary gill lamelle.
E	-	Epithelium of secondary gill lamellae.
M1	-	M1-mucous cells.
M2	-	M2-mucous cells.
M	-	Mucous cells

CAPTIONS OF FIGURES

Fig. 46 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
AB pH 2.5 - PAS staining x 150.

Fig. 47 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
C.I. Staining 150.

Fig. 48 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
C.I.-PAS staining x 300.

Fig. 49 : L.S. of gill of fish exposed to 6 ppm malathion for 72 hours.
AF - AB pH 2.5 staining x 300.

Fig. 50 : L.S. of gill of fish exposed to 8 ppm malathion for 72 hours.
PAS staining x 300.

Fig. 51 : L.S. of gill of fish exposed to 8 ppm malathion for 72 hours.
P-PAS staining x 300.

Fig. 52 : L.S. of gill of fish exposed to 8 ppm malathion for 72 hours.
AB pH 1.0 staining x 300.

Fig. 53 : L.S. of gill of fish exposed to 8 ppm malathion. AB pH 2.5 staining x 200.

Fig. 54 : L.S. of gill of fish exposed to 8 ppm malathion for 72 hours.
C.I. staining x 300.

ABBREVIATIONS

Ep	-	Epithelium of primary gill lamellae.
E	-	Epithelium of secondary gill lamellae.
M1	-	M1-mucous cells.
M2	-	M2-Mucous cells.
M	-	Mucous cells.

Each gill arch, on its one side only, was provided with two rows of gill filaments or primary gill lamellae borne on the ceratobranchial and epibranchial segments (Fig. 1) . The primary gill lamellae were having broader base (Fig. 1) and apical portion (Fig. 5) but in the middle part they were slightly narrow (Fig. 1). The bases of primary gill lamellae were supported by bony cones which further extended into the gill rays.

The epithelium of the gill arch at interlamellar region was much thicker in which mucous cells and acidophilic cells were found in plenty. The epithelial cells were mostly oval and possessed centrally located, rounded nuclei and strongly basophilic cytoplasm. The acidophilic cells were oval to round in apperance. These cells provided with homogenous cytoplasm and excentrically located nuclei. The mucous cells were distributed randomly in the deeper as well as superficial layer of epithelial cells in this region. Their nuclei were not visible .

The primary gill lamellae consisted of a vascular layer in the middle with an envelope of epithelial cells and thin layer of connective tissue inbetween. These were supported by gill rays which were connected with gill arch and with each other by fibrous ligament. The wall of the primary gill lamellae cosisted of epithelial cells arranged in three to four

layers, however, many layered epithelium was observed towards the tip. The epithelial cells were oval to flat in shape. They contained centrally placed nuclei and homogenous basophilic cytoplasm. The cells in the deeper part of epithelium were not well differentiated. They also possessed round, centrally placed nuclei and homogenous basophilic cytoplasm.

These cells rested upon the basement lamina formed of connective tissue (Fig. 4). The good many number of mucous cells and acidophil cells were found among the epithelial cells forming the wall of the primary gill lamellae (Fig. 4). The number of mucous cells was more in the epithelium lining the tips of the primary gill lamellae (Fig. 5). These cells showed similar histological features as mentioned earlier.

The primary gill lamellae possessed two rows of secondary gill lamellae, one row on either side of their axis (Fig. 1, 4, 6) . The secondary gill lamellae developed as outfoldings from the primary gill lamellae as thin fillamentous structures more or less right angle to the axis of primary gill lamellae. Secondary gill lamellae towards the apical part of primary gill lamellae were elongated than towards the basal part of primary gill lamellae.

The secondary gill lamellae consisted of a single layer of squamous to low cuboidal epithelial cells

(Fig. 6) which were supported by basement lamina. These cells contained centrally placed, oval nuclei and basophilic cytoplasm. Occasionaly mucous cells were also observed in this layer. Internal to the epithelium secondary lamellae possessed a central core, consisted of blood sinuses lined and spanned by pillar cells (Fig. 6). The pillar cells are oval to irregular in outline with homogenous basophilic cytoplasm and centrally placed nuclei. Acidophilic cells were observed at the bases of secondary gill lamellae.

E. HISTOPATHOLOGICAL OBSERVATIONS :-

Histopathological changes in the gills of C.punctatus produced due to different concentrations of malathion are shown in plate Nos. 1 and 2 (Figs. 7-17) and of sumithion in the plate Nos. 2 and 3 (Figs. 18-23).

I. MALATHION :

1. 4 ppm :-

The fishes exposed to this concentration showed mostly thickened epithelium of the secondary gill lamellae (Fig. 7). The enlargement of the tips of the secondary gill lamellae due to excessive multiplication of cells at that region and their fusion in some secondary gill lamellae were also noticed (Fig. 7). Necrosis of the superficial epithelial cells of secondary

gill lamellae was noticed (Fig. 7) . Enlargement of the marginal channel (Fig. 8) was observed. Dilation of lamellar blood sinuses were also evident (Fig. 8). Vascular congestion in some secondary gill lamellae was noticed (Fig.-8). Swelling of pillar cells occured.

Enlargement of the central supporting structure of the primary gill lamellae occured towards their apical part while progressive degeneration of the supporting structure initiated towards the basal part of the primay gill lamellae (Fig. 9, 10), leaving very larger spaces. Accumulation of the blood cells occured in these intralamellar gaps.

Fusion of the adjacent secondary gill lamellae occured at some part of primary gill lammellae (Fig. 8) . The mucous cells increased in their number. The large number of blood cells were noticed outside the secondary gill lamellae (Fig. 7) which may be due to rapture of epithelial cells.

2. 6 ppm :

Here, the changes are more pronouched than in the earlier concentration. The supporting structure of the gill rachis was damaged and invaded by blood cells. A slight thickening of the interlamellar epithelium of primary gill lamellae was noticed (Fig. 11). The blood spaces at the distal ends of the primary gill lamellae

were dilated (Fig. 12) and were filled with large number of blood cells.

Hyperplasia of the epithelium of some secondary gill lamellae was evident (Fig. 11). The fusion of some secondary gill lamellae only at their ends was noticed (Fig. 11). Thinning and elongation of few secondary gill lamellae and their uneven curling was noticed (Fig. 12). The distal secondary gill lamellae appeared to fuse at the tips and they were merged with each other (Fig. 12). Marginal blood channels dilated and filled with blood (Fig. 12). Blood filled spaces appeared at the bases of secondary lamellae (Fig. 11). Blood filled spaces also appeared at the bases of primary gill lamellae. Mucous cells hypertrophied. Secondary gill lamellae entangled with mucus. Damaged epithelial cells, pillar cells and blood sinuses in secondary gill lamellae were noticed.

3. 8 ppm :-

The histopathological results at this concentraction showed enlargement of the tips of primary gill lamellae (Fig. 13). The blood spaces at the distal ends of primary gill lamellae dilated and filled with blood (Fig. 13). Thickening of epithelium on primary gill lamellae was also noticed. Enlargement of tips of secondary gill lamellae and their fusion at the tips only

was noticed. Slight enlargement of marginal blood channels and blood sinuses of the secondary gill lamellae was observed. The rupture of epithelial cells, blood sinuses and pillar cells of some secondary gill lamellae was evident that led to alteration of total structure of secondary gill lamellae. Increased number of mucous cells was observed at the tips of primary gill lamellae. The mucous cells hypertrophied.

4. 10 ppm :-

In the gills the fishes exposed to this concentration of malathion the histopathological lesions were more pronounced than in the earlier. Accumulation of mucus at the gill opercula and over the general gill surface was significant (Fig. 14) . Thickening of interlamellar epithelium and lifting of epithelial cells from the interlamellar zones of the primary gill lamellae was noticed (Figs. 14, 15). The initiation of histolysis was evident at the bases of secondary gill lamellae (fig. 15). Necrosis in epithelial cells of secondary and primary gill lamellae was much more evident at this concentration. Rupture of lamellar epithelium and bleeding in to pharynx at some region was observed. All these changes led to loss of respiratory surface.

The secondary gill lamellae were separated from the primary gill lamellae leaving space between them.

The blood sinuses of secondary lamellae were much more dialated (Figs 14,15). A slight thickening in the epithelium of the secondary gill lamellae occured. The pillar cells were also slightly bulged. Thickening of central supporting material of primary gill lamellae was also evident.

5. 12 ppm :-

Acute changes in the histomorphological structure of gill lamellae were observed at this concentration.

The primary lamellae showed no change at the basal region. The histological changes, however, were evident in their apical portion. (Fig. 16) . The blood spaces at the apical ends of primary gill lamellae was dilated and was filled with blood cells. The swelling of epithelium of only some secondary gill lamellae was observed. The lifting of epithelium from the basement lamina of some secondary gill lamellae was also evident (Fig. 16). The secondary gill lamellar blood sinuses dilated. The organization of secondary lamellae was not identical all arround the primary gill lamellae. The loss of respiratory epithelium due to necrosis, damaged pillar cells and red blood cells, reduction in the length of secondary gill lamellae and hematomas were extensive (Fig. 16) . At some places the blood was found to be diffused in the secondary lamellae, thus indicating

capillary damage and haemorhage. Vascular congestion was also evident. Very few secondary gill lamellae remained intact and most of them were destroyed.

II. SUMITHION :-

1. 10 ppm :-

At this concentration of sumithion the primary gill lamellae remained largely unaffected. There was no any evidence of damage to the capillary at this concentration. The central supporting structure showed no any alterations.

Slight thickening of the interlamellar epithelium was noticed (Fig. 19). The epithelium of the primary gill lamellae showed slight detachment from the basement membrane (Fig. 18). The blood spaces at the distal ends of primary gill lamellae was unaffected.

An uneven curling of the secondary gill lamellae was noticed (Fig. 18). Some of the secondary gill lamellae showed thickening and shortening characters (Fig. 18). Hyperplasia of the epithelial lining of the secondary gill lamellae was found only towards the tips, that led to the fusion of only tips of secondary gill lamellae (Fig. 19) . The apical secondary gill lamellae were more affected than the basal ones. (Fig. 19). The dilation of blood sinuses in secondary gill lamellae was also observed. (Figs. 18, 19) . Blood filled lamellar

aneurism (clavate lamellae) was also noticed at some part of primary gill lamellae (Fig. 18).

2. 15 ppm :

At this concentration of sumithion the primary gill lamellae also exhibited histomorphological changes, which were not evident at earlier concentration. Severe reduction in the central supporting material of primary gill lamellae was noticed. (Fig. 20). Degeneration of the supporting material occured at the bases of primary gill lamellae (Fig. 20.). Tips of the primary gill lamellae showed swellings. The blood spaces at the tips of primary gill lamellae dilated and filled with large number of blood cells.

The secondary gill lamellae remained more or less unaffected at this concentration. No any change was evident in the epithelium lining the secondary gill lamellae, pillar cells and marginal blood channels of secondary gill lamellae. However, slight dilation of secondary gill lamellar blood sinuses was observed. Blood cells diffused into and accumulated in blood spaces of secondary gill lamellae. Mucous cells increased in number and were hypertrophied.

3. 20 ppm :

Here the changes were more pronounced than in

the earlier case. Accumulation of mucus at the gill opercula and over the general gill surface was significant. An uneven curling occured in the secondary lamellae. Some of the secondary lamellae showed thining and shortening characters. (Fig. 21). The basal secondary gill lamellae were more affected than the apical ones. (Fig. 21). There was in increase in the inter-lamellar space. Thining of pillar cells was evident. Lamellar blood sinuses constricted. The epithelium of primary gill lamellae showed thinning and slight detachment from the basement lamina (Fig. 21) . The blood spaces at the distal ends of primary gill lamellae were dilated. Blood filled aneurism was noticed (fig. 89).

4. 25 ppm :

The histopathological changes at this concentration of sumithion were much drastic in primary gill lamellae, secondary gill lamellae and blood spaces.

The supporting rod of the primary gill lamellae became very thin. Thickening of interlamellar epithelium was more pronounced. Slight detachment of epithelium from the basement lamina of the primary gill lamellae was observed.

The changes were more drastic in the secondary lamellae (Fig. 22). Hyperplasia of the epithelium at the bases of secondary lamellae was noticed. The secondary

lamellae showed degenerative changes at their bases. The epithelium of secondary gill lamellae was found to be shifted considerably from the basement membrane which led into curling of secondary gill lamellae (Fig.22). The blood sinuses of secondary gill lamellae dilated considerably. The marginal blood channels also dilated. Swelling of pillar cells was also seen.

5. 30 ppm :-

The primary gill lamellae showed progressive degenerative changes in the central supporting structure. Necrosis of interlamellar epithelium of primary gill lamellae was noticed. Lifting of epithelial cells from the basement lamina of the secondary gill lamellae and their necrosis occured. Pillar cells and blood sinuses of secondary gill lamellae get damaged.

Accumulation of blood cells in the intercellular space occured due to degeneration of septum which was more pronouced. Most of the blood cells showed swelling and haemolysis. This process led to the formation of hematomass.

Acute changes were seen in the secondary gill lamellae at this concentration. Almost all of them showed shortening due to loss of pillar cells (Fig. 23), leaving very small gaps in the intercellular region. These gaps were filled with blood cells (fig. 23). In certain region of the secondary gill lamellae formation

of hematomass occured. At the basal part of the primary gill lamellae the secondary gill lamellae were found to entangeled in the mucous material. Their epithelium was found to be merged with the epithelium of the primary gill lamellae. These alterations clearly indicated the degenerative changes in the gill structure of the fish.

F. HISTOCHEMICAL OBSERVATIONS :-

The histochemical analysis of mucosubstances was carried out in the epithelial cells and mucous cells in the gills of control fish as well as in fishes exposed to various concentrations of malathion and sumithion. The histochemical reactivities of mucosubstances in the epithelial cells and mucous cells of control and of fishes exposed to various concentrations of malathion and sumithion are recorded in Table Nos, 6, 7, 8 and 9, according to the visually estimated intensities and shades with ++++ representing the intense activity. The histochemical distribution of mucosubstances in the gills of fishes investigated is illustrated in photomicrographs (Control fish - Figs. 24-31 ; fishes exposed to different concentrations of malathion - Figs. 32-72 and fishes exposed to different concentrations of sumithion - Fig. 73-99). The results of various histochemical reactions with their interpretations are described in some details hereafter.

a. EPITHELIAL CELLS :-

1. Control fish :-

The gill epithelial cells of the control fish showed poor PAS reactivity (Fig. 24) which was resistant to diastase or α - amylase digestions but could completely be blocked after pheynlhydrazine treatment, indicating the absence of glycogen and any acidic mucosubstances but presence of only neutral mucins.

The epithelial cells failed to show positive reactions for acidic mucosubstances with AB PH 1.0 (Fig. 25) , AB pH 2.5 (Fig. 26) , C.I. and AF. This was also confirmed by pepsin digestion which did not exhibit any alcianophilia.

The presence of only neutral mucins in the epithelial cells was also substantiated by their only PAS reactivity in sequential staining procedures such as AB pH 1.0-PAS (Fig. 27), AB pH 2.5 - PAS (Fig. 28) and C.I. - PAS (Fig. 29) and metachromatic staining only after sulfation of the sections.

Thus, the above mentioned histochemical observations revealed the absence of acidic mucosubstances and confirmed the presence of only neutral mucins in poor amount in the epithelial cells in gills of control fish.

2. Fish exposed to 4 ppm malathion :

The gill epithelial cells of the fish exposed

to 4 ppm malathion showed poor to weak PAS reactivity (Figs. 32,33) which was resistant to diastase or α-amylase digestions (Fig. 34). However, the PAS reactivity was blocked (it was retained in trace amount) by prior phenylhydrazine treatment. These initial histochemical reactivities indicated the absence of glycogen but probable presence of acidic and neutral mucosubstances.

Moreover, the epithelial cells exhibited trace alcianophilia at pH 1.0 (Fig. 35), indicating the presence of acidic mucosubstances which were sulfomucins. The absence of carboxymucins in these cells was evidenced by the fact that there was no enhancement in their alcianophilia at pH 2.5 (Fig. 36) and C.I. than at pH 1.0.

The above conclusion that the epithelial cells of gills of fish exposed to above concentration of malathion contained trace amount of sulfomucins was further supported by purple staining (pink tinge was more) with AB pH 1.0-PAS (Fig. 37) and C.I. - PAS (Fig. 38) sequential staining techniques, purple staining with AF alone (Fig. 39) or with AB pH 2.5 step onwards, metachromatia with azure A at all the pH levels even without sulfation indicating the presence of sulfomucins. In CEC techniques, the sulfomucins were also confirmed, since these cells exhibited alcianophilia only at 0.2 M Mg^{++} concentration. The results with methylation saponification procedures also confirmed the presence of

sulfomucins. These sulfomucins resisted mild methylation (Fig. 40) but active methylation effected in complete blockade of the alcianophilia and subsequent saponification failed to restore it. The alcianophilia was resistant to acid hydrolysis and sialidase, hyaluronidase and pepsin digestions. (Fig. 41).

The presence of neutral mucins was also evidenced from the fact that, these epithelial cells showed bluish-purple staining(purple tinge was more) with AB pH 1.0-PAS, AB pH 2.5-PAS (Fig. 37) and C.I.-PAS (Fig. 38) sequential stainings.

Therefore, it was conclued that these epithelial cells in gills of fish exposed to 4 ppm malathion contained a mixture of neutral mucins (poor) and sulfomucins (trace).

3. Fish exposed to 6 ppm malathion :

In fish exposed to 6 ppm malathion, the gill epithelial cells exhibited weak PAS reactivity (Fig. 42). The PAS staining intensity could not be altered by diastase or α-amylase digestions (Fig. 43), indicating the absence of glycogen. However , the PAS reactivity was blocked partially by phenylhydrazine pretreatment indicating the partial presence of neutral and acidic mucosubstances. The epithelial cells in this fish showed negative staining reactivity with AB pH 1.0 (Fig. 44) indicating the absence of sulfomucins. However, these cells showed alcianophilia at AB pH 2.5

(Fig. 45) and C.I. (Fig. 47) indicating the presence of carboxymucins. The presence of carboxymucins was also inferred by negative staining with AF alone and only blue staining with AF-AB pH 2.5 (Fig. 49) sequence, metachromatia with azure A at and above pH 3.0, reduction in alcianophilia in CEC technique with 0.2 M Mg^{++} concentration than with 0.1 M Mg^{++} concentration and restoration of alcianophilic staining after saponification of previously methylated (both mild and active methylations) sections. The carboxymucins further confirmed as sialmucins as their alcianophilia was reduced following acid hydrolysis and sialidase digestion.

The presence of neutral mucins in the gill epithelial cells of this fish was also substantiated by their only PAS reactivity (magenta colouration) in sequential staining procedure with AB pH 1.0-PAS and bluish-purple staining with AB pH 2.5-PAS (Fig. 46) and C.I.-PAS sequential stainings.

Thus, the aforementioned histochemical observations revealed the presence of neutral mucins poor to weak) and sialomucins (trace) ,

4. Fish exposed to 8 ppm malathion :-

The gill epithelial cells of the fish exposed to above concentration of malathion showed weak to

oderate PAS reactivity. (Fig. 50). The PAS reactivity of these cells was resistant to diastase or α - amylase digestions and the intensity of PAS was diminished by phenylhydrazine (Fig. 51) pretreatment. These histochemical results revealed the absence of glycogen but presence of neutral mucosubstances.

The gill epithelial cells of the fish exposed to this concentration showed only trace alcianophilia at pH 1.0 (Fig. 52) and the alcianophilic blue staining was slightly enhanced at AB pH 2.5 (Fig. 53) and C.I. (Fig. 54) staining. From these observations it was concluded that these cells contained sulfomucins and carboxymucins.

The presence of sulfomucins was further characterised by only slight purple staining with AF and blue purple staining with AF-AB pH 2.5 sequence, metachromatic pink staining with azure A even at lower pH levels (pH 1.5) and alcianophilic blue staining in CEC techniques in presence of graded concentrations with Mg^{++} up to 0.2 M. The presence of sulfomucins was also confirmed by active methylation which removed alcianophilia and subsequent saponification failed to restore it. These sulfomucins were resistant to mild methylation (Fig. 57) and hyaluronidase digestion and there was no enhancement in the alcianophilia by prior pepsin digestion.

Together with suflomucins, carboxymucins were also identified in the gill epithelial cells of fish

posed to above concentration. The presence of carboxymucins were inferred from slight increase in alcianophilia at pH 2.5 (Fig. 53) than at pH 1.0 (Fig. 52), blue staining with C.I. (Fig. 54), blue purple staining with AF followed by AB pH 2.5 staining, enhanced metachromatic pink staining with Azzure A at pH 3.0 and above, partial loss of alcianophilia in CEC technique by addition of 0.1 M Mg^{++} concentration and only trace restoration of alcianophilia by both mild and active methylations followed by saponification. These carboxymucins were further identified as sialomucins since acid hydrolysis (Fig. 58) and sialidase digestion slightly reduced the alcianophilia in these cells.

The presence of neutral mucosubstances in these cells was inferred by reduction in the PAS reactivity by phenylhydrazine pretreatment (Fig. 51), blue purple staining with AB pH 1.0-PAS, AB pH 2.5-PAS (Fig. 55) and C.I.-PAS (Fig. 56) sequential staining procedures and increased metachromatic pink staining with azure A pH 1.5 following sulfation.

These aforementioned histochemical reactivities lead to the conclusion that the epithelial cells of gill in fish exposed to 8 ppm malathion contained neutral mucins (poor to weak), sulfomucins (trace) and sialomucins (trace).

5. Fish exposed to 10 ppm malathion :

The gill epithelial cells in fish exposed to

above concentration of malathion showed weak to moderate PAS reactivity (Fig. 59) . The PAS staining was unaffected by diastase or α - amylase digestions and the intensity of the PAS was diminished by phenylhydrazine pretreatment. These initial histochemical results revealed the absence of glycogen but presence of neutral mucosubstances.

Poor alcianophilia with AB pH 1.0 (Fig. 60) which was not enhanced at pH 2.5 (Fig. 61) indicated the presence of acidic muconsubstances which were sulfomucins but absence of carboxymucins. The presence of sulfomucins in these cells was also characterised by blue-purple staining (latter being predominant) with AB pH 1.0-PAS (Fig. 62), poor purple staining with AF and AF-AB pH 2.5 (Fig. 64), combined histochemical procedure, poor pink metachromatic staining with azure A even at lower pH level (1.5) which was not enhanced with increasing pH levels and poor alcianophilic staining in CEC technique in presence of only 0.2 M Mg^{++} concentration. These sulfomucins were resistant to mild methylation (Fig. 65) but active methylation saponification procedures effected in irriversible loss of alcianophilia in these cells. These sulfomucins were hyaluronidase resistant and there was no enhancement in the alcianophilia by prior pepsin digestion.

The reduction of PAS staining intensity by

phenylhydrazine pretreatment indicated the presence of neutral mucosubstances in the epithelial cells of this fish. This conclusion was further strengthened by blue purple combined staining (with purple tinge more) with AB pH 1.0-PAS (Fig. 62), AB pH 2.5-PAS and C.I.-PAS (Fig. 63) sequential staining procedures and enhancement in metachromatia with azure A (Weak to moderate staining) at pH 1.5 after saponification.

The results obtained with histochemical staining techniques, thus revealed the presence of neutral mucosubstances (Poor to weak) and sulfomucins (poor) in the gill epithelial cells of fish exposed to 10 ppm malathion.

6. Fish exposed to 12 ppm malathion :-

The gill epithelial cells in fish exposed to above concentration of malathion resembled in their staining reactivities (Figs. 66-72) similar to those described for epithelial cells in gills of fish exposed to 4 ppm malathion. Therefore, it was concluded that these epithelial cells contained neutral mucosubstances (poor) and sulfomucins (trace).

7. Fish exposed to 10 ppm sumithion :-

The histochemical results of the gill epithelial cells of fish exposed to above concentration

of sumithion were practically similar (Figs. 73-80) to those described for epithelial cells in gills of fish exposed to 4 ppm malathion. Thus, it was concluded that the gill epithelial cells in this fish contained neutral mucosubstances (poor ~~to weak~~) and sulfomucins (trace).

8.Fish exposed to 15 ppm sumithion :

The gill epithelial cells in above fish showed weak staining with PAS (Fig. 81). The PAS reactivity in these cells could not be blocked by diastase or α-amylase digestions but could be blocked partially by phenylhydrazine pretreatment. (Fig. 82). These histochemical results revealed the absence of glycogen but presence of neutral mucosubstances.

The epithelial cells in this fish reacted poorly towards AB pH 1.0 (Fig. 83) and similar poor blue staining was also observed with AB pH 2.5 (Fig. 84) and C.I. These histochemical results indicated the presence of acidic mucosubstances which were sulfomucins but absence of carboxymucins. The presence of sulfomucins in these cells was also characterised by blue purple staining with AB pH 1.0-PAS, poor purple staining with AF alone and with AF-AB pH 2.5 combined histochemical procedure, poor pink methachromatic staining with azure A even at lower pH level (pH 1.5) which was not enhanced with increasing pH levels, ~~trace~~ alcianophilic staining

in CEC technique in presence of graded Mg^{++} concentration up to 0.2 M. These sulfomucins were resistant to mild methylation but active methylation could block alcianophilia completely which could not be restored even after saponification. These sulfomucins were hyaluronidase resistant and there was no enhancement in the alcianophilia by prior pepsin digestion (Fig. 86).

Thus, the above discussed histochemical reactivities lead to the conclusion that the gill epithelial cells of fish exposed to the 15 ppm sumithion contained neutral mucins (poor) and sulfomucins (poor).

9. Fish exposed to 20 ppm sumithion :-

The gill epithelial cells of fish exposed to above concentration of sumithion resembled in their histochemical reactivities (Figs. 87-90) to those exhibited by epithelial cells in gills of fish exposed to ~~8 ppm and~~ 10 ppm malathion. Therefore, it was concluded that these epithelial cells contained neutral mucosubstances (poor to weak) and sulfomucins (poor).

10. Fish exposed to 25 ppm sumithion :-

The histochemical results of the gill epithelial cells of fish exposed to this concentration of sumithion were practically similar (Figs. 91-95) to those epithelial cells in gill of fish exposed to 8 ppm ~~and 10 ppm~~ malathion. Therefore, it was concluded that the

epithelial cells in fish exposed to above concentration of sumithion contained neutral mucosubstances (poor to weak), sulfomucins (trace) and sialomucins (trace).

11. Fish exposed to 30 ppm sumithion :-

The histochemical staining reactivities observed in the epithelial cells of above fish were practically identical (Figs. 96-99) to that observed in gill epithelial cells of fish exposed to 15 ppm sumithion. Hence, it was concluded that the gill epithelial cells of this fish contained neutral mucosubstances (poor) and sulfomucins (poor).

b. .MUCOUS CELLS :-

The mucous cells were found distributed in the epithelium of the gill arch, primary gill lamellae and secondary gill lamellae. These were more numerous at the tip of primary gill lamellae. On the basis of the results obtained, the gill mucous cells could be divided into following types, each elaborating its own peculiar type of mucosubstances. These were M1-cells, M2-cells and M3-cells.

1. Control fish :-

In the gills of control fish two types of mucous cells were identified which were M1 and M2 types of mucous cells.

i) M1-Mucous cells :-

The M1 mucous cells in the gills of controlled fish reacted weakly towards PAS (Fig. 24) and their PAS reactivity was resistant to diastase or α- amylase digestions and could completely be blocked by prior treatment with phenylhydrazine. These histochemical reactivities indicated the presence of only neutral mucosubstances rich in Vic-glycols but absence of glycogen. The absence of acidic mucosubstances in these cells was inferred by their negative reactivity towards AB at pH 1.0 (Fig. 25), pH 2.5 (Fig. 26) C.I. and AF and only pink staining with AB pH 1.0-PAS (Fig. 27), AB pH 2.5-PAS (Fig. 28) and C.I.-PAS (Fig. 29). Moreover, these cells exhibited only orthochromatic blue staining with azure A at pH 3.0 and above, the intensities increased with the higher pH levels. These cells exhibited weak metachromatia after sulfation and these results could not be modified by acid hydrolysis, hyaluronidase digestion and pepsin digestion.

Thus, the aforementioned results indicated the presence of only weak quantities of only PAS reactive neutral mucosubstances in the M1 mucous cells in gills of control fish.

ii) M2 -Mucous cells :-

These mucous cells of control fish exhibited weak to moderate PAS reactivity (Fig. 24) which could not be blocked by prior phenylhydrazine treatment and

their PAS reactivity was resistant to diastase or α - amylase digestions. These initial histochemical reactivities indicated the absence of neutral mucins and glycogen in these cells of control fish.

Moreover, these cells exhibited weak to moderate alcianophilia at pH 1.0 (Fig. 25) which was not enhanced at pH 2.5 (Fig. 26) indicating the presence of acidic mucosubstances in them which were sulfomucins. The absence of carboxymucins in these cells was evidenced by the fact that there was no enhancement in their alcianophilia at pH 2.5 (Fig. 26) than at pH 1.0 (Fig. 25).

The above conclusion that the M2-cells contained only sulfomucins was further supported by their only blue staining with C.I., AB pH 1.0-PAS (Fig. 27), AB pH 2.5-PAS (Fig. 28) and C.I.-PAS (Fig. 29) sequential staining procedures, purple staining with AF alone. These cells exhibited weak to moderate metachromatia with azure A at all the pH levels even without sulfation indicated the presence of sulfomucins. In CEC techniques the sulfomucins in these cells were also confirmed, since these cells exhibited alcianophilia at pH 5.6 in presence of 0.2 M Mg^{++} concentration and it was resistant up to 0.6 to 0.8 M Mg^{++} concentration. The mucosubstances in these cells resisted mild methylation (Fig. 31) but active methylation could eliminate alcianophilia in them which could not be reversed following saponification.

Their alcianophilia could not be altered by hyaluronidase digestion indicating that the sulfomucins were resistant to hyaluronidase digestion. These results could not be modified by acid hydrolysis, sialidase and pepsin digestions. Therefore, it was concluded that the M2-mucous cells in control fish contained only sulfomucins (weak to moderate).

2. Fish exposed to 4 ppm malathion :-

In fish exposed to above concentration of malathion also, M1 and M2 - mucous cells were observed in its gills.

i) M1-Mucous cells :-

These cells exhibited staining reactivities similar to those exhibited by M1-mucous cells in control fish, the only difference was in the staining reactivity (Figs. 33-34) which was weak to moderate. Therefore, it was concluded that the M1-mucous cells in gills of fish exposed to 4 ppm malathion contained only neutral mucins in weak to moderate amount.

ii) M2-mucous cells :-

These cells also showed practically identical staining reactivities (Figs. 33-41) with the M2-Mucous cells in control fish. The only difference that was observed in these cells was these cells reacted

moderately. Therefore, it was concluded that the M2 cells in gills of fish exposed to 4 ppm malathion contained only sulfomucins (moderate).

3. Fish exposed to 6 ppm malathion :-

In the fish exposed to 6 ppm malathion, also, two types of mucous cells were observed which were M1 and M2-mucous cells.

i) M1-Mucous cells :-

The histochemical observations (Figs. 42-49) in these mucous cells were practically similar to those described in M1-mucous cells in control and in fish exposed to 4 ppm malathion which revealed only neutral mucosubstances rich in Vic-glycols. The only difference was that of staining reactivities which were moderate.

ii) M2-Mucous cells :-

These cells also showed identical staining reactivities (Figs. 42-49) to those described earliar for M2-Mucous cells of fish exposed to 4 ppm malathion. Thus, it was concluded that the M2-mucous cells in fish exposed to 6 ppm malathion also contained sulfomucins (Moderate).

4. Fish exposed to 8 ppm malathion :-

In the gills of fish exposed to above

concentration of malathion, two types of mucous cells were investigated which were M1-mucous cells and M2-mucous cells.

i) M1-Mucous cells :-

The histochemical staining reactivities (Figs. 50-58) of these cells were practically identical to such type of cells observed in the gills of fish which was exposed to 6 ppm malathion. Thus, it was concluded that the M1-mucous cells in this fish contained only moderate amount of neutral mucosubstances.

ii) M2-Mucous cells :-

The M2-Mucous cells in gills of fish exposed to above concentration of malathion showed practically identical staining reactivities (Figs. 50-58) to those cells described earliar in gills of fishes exposed to 4 ppm and 6 ppm malathion. The only difference was that the histochemical reactivities were moderate to intense. Hence it was concluded that the M2-mucous cells in gills of fish exposed to 8 ppm malathion contained only sulfomucins (moderate to intense).

5. Fishes exposed to 10 ppm and 12 ppm malathion :

In the gills of fish exposed 10 ppm and 12 ppm malathion only one type of mucous cells were observed

which were M2-mucous cells. These cells showed practically identical staining reactivities (Figs. 59-65 and Figs. 66-72) to those described for such cells in gills of fishes exposed to 4 ppm and 6 ppm malathion. Therefore,it was concluded that the M2-cells in gills of above two types of fishes contained only moderate amount of sulfomucins.

6. Fish exposed to 10 ppm sumithion :-

The gills of fish exposed to 10 ppm sumithion also exhibited M1 and M2 types of mucous cells.

i) M1-Mucous cells :-

These cells in fish exposed to 10 ppm sumithion showed practically identical staining reactivities (Figs. 73-80) to those cells described earliar in gills of fish exposed to 4 ppm malathion. Hence,it was concluded that these cells in gills of fish exposed to 10 ppm sumithion elaborated only weak to moderate amount of neutral mucins.

ii) M2-Mucous cells :-

The histochemical results of these type of mucous cells in gills of fish exposed to 10 ppm sumithion (Figs. 73 to 80) were practically similar to those cells present in gills of control fish . The only difference

was that these cells reacted moderately. Therefore, it was concluded that these M2-cells in the gills of fish exposed to above concentration of sumithion also elaborated only moderate amount of sulfomucins.

7. Fish exposed to 15ppm sumithion :

In the gills of above fish only one type of mucous cells have been recognised which reacted moderate to intensely with PAS (Fig. 81). The PAS reactivity in these cells was resistant to diastase or α-amylase digestions and the intensity of PAS was slightly diminished by phenylhydrazine pretreatment (Fig. 82). These histochemical results revealed the absence of glycogen but partial presence of neutral mucosubstances.

Weak to moderate alcianophilia with AB pH 1.0 (Fig. 83) which was not enhanced at pH 2.5 (Fig. 84) indicated the presence of acidic mucosubstances which were sulfomucins but absence of carboxymucins in these cells of above fish exposed to 15 ppm sumithion. The presence of sulfomucins in these cells was also characterised by purple-blue staining (the later tinge being predominant) with AB pH 1.0-PAS, weak to moderate purple staining with AF and Af-AB pH 2.5 combined histochemical procedure weak to moderate metachromatic staining with azure A even at lower pH levels (pH 1.5) which was not enhanced with increasing pH levels and

persistant alcianophilic weak staining in CEC technique in presence of 0.8 M Mg^{++} concentration. These sulfomucins were resistant to mild methylation but active methylation-saponification procedures effected in irreversible loss of alcianophilia. These sulfomucins were hyaluronidase resistant and there was no enhancement in the alcianophilia by prior pepsin digestion (Fig. 86).

The presence of neutral mucosubstances in these cells was inferred by slight reduction in the PAS reactivity by phenylhydrazine pretreatment (Fig. 82), purple-blue staining with AB pH 1.0-PAS , AB pH 2.5-PAS and C.I.-PAS (Fig 85) sequential staining procedures and enhanced metachromatic pink staining with azure A at pH 1.5 following sulfation.

These aforementioned histochemical reactivities lead to the conclusion that these cells in the gills of fish exposed to 15 ppm sumithion elaborated sulfomucins (weak to moderate) and neutral mucosubstances (poor).

Hence, on the basis of mucosubstances elaborated by these mucous cells, these cells had refered as M3-mucous cells.

8. Fish exposed to 20 ppm, 25 ppm, and 30ppm sumithion :-

The gills of fishes exposed to above three concentrations of sumithion showed only one type of mucous cells which were M2 type of mucous cells. The

histochemical staining reactivities (Figs. 87-90, 91-95, 96-99) of these cells in fishes exposed to above three concentrations of sumithion were identical to those exhibited by such type of cells in gills of fish exposed to 10 ppm sumithion. Therefore, it was concluded that these mucous cells in gills of fishes exposed to above three concentrations of sumithion elaborated only sulfomucins in moderate amount.