SUMMARY

The present paper dealt with "Studies on the bacterial involvement in the ulcerative disease of fishes", consisting of, (i) Ulcerative disease in air-breathing fishes and isolation of bacteria from ulcer tissues, (ii) Experimental induction of ulcers in *Channa punctatus* and *Anabas testudineus* by bacterial culture, (iii) Histopathological observations of liver, kidney and spleen of experimentally infected *Clarias batrachus* and *Channa punctatus*, (iv) Effect of bacterial culture on Haemoglobin content and Erythrocyte count in *Channa punctatus*, (v) Evaluation of the role of bacteria in causing ulcer disease, (vi) Drug sensitivity testing and preliminary observation on vaccination by formalin killed bacteria in mixed condition in fish *Anabas testudineus*, (vii) Ulcerative disease in Indian major carps and isolation of bacteria from ulcer tissues of *Cirrhinus mrigala*.

The ulcerative fish disease in epizootic form occurred for the first time in May 1988 in some areas of Eastern Indian states such as Tripura, Meghalaya and Assam. Subsequently, the disease spread to West Bengal. The disease severely affected almost all the districts of West Bengal except Purulia in 1989 and

recurred there after. In 1990, the disease spread to some areas of other states of India such as Orissa, Bihar, Uttar Pradesh, Sikkim, Manipur and Nagaland. In 1991, the disease was reported from Kerala. The diseases affected various types of fishes, wild and cultured.

In case of fishes without scales, such as Clarias batrachus and Heteropneustes fossilis , the symptoms of the disease first appeared as a red spot on the skin of the body. Gradually the red spot increased in size and ulcer developed in the affected area. Ultimately the muscle layer became affected. In scaly fishes, such as Anabas testudineus, the mucous layer covering the scales was first affected. Red spots appeared in some regions of the body. Scales were sloughed and the ulcer became deep and necrotic. All three species of naturally infected fishes (C. batrachus, H. fossilis and A. testudineus) showed various histopathological changes in the liver, kidney and spleen. In all cases sections of liver showed necrosis and vaculations of hepatocytes. Microscopic observations of the naturally infected kidney of A. testudineus showed tubular breakage, tubular degeneration and vaculation of tubular cells. Necrosis in the haematopoietic region were also observed with occasional haemorrhages in certain regions of the kidney. Vaculation, degeneration

and necrosis of the tubular cells were also observed in the kidney of infected C. batrachus and H. fossilis. Sections of the spleen of the three species of fish showed vaculation and necrosis in some regions of the spleen. Smear preparations of the ulcer tissues and with the blood of the infected fishes showed presence of bacteria, rod and coccus. Infected fishes showed vaculation in the nucleus and cytoplasm of erythrocytes, perinuclear hallow and nuclear shadows of erythrocytes (degenerating erythrocytes). Bacteria were detected in the sections of liver, kidney and spleen of infected fishes stained with Carbol fuchsin. Bacteria were also detected in the nutrient broth incubated with ulcer tissues of all the fishes. Four types of bacteria, two fluorescent pseudomonads, one aeromonad (Aeromonas caviae) and one coccus (Micrococcus varians) were isolated from the skin lesions of all the three species of fishes. The cultures of all the bacteria were routinely maintained.

Healthy fish, C. punctatus and A. testudineus were inoculated with mixed and pure cultures of four bacteria, two fluorescent pseudomonads, one aeromonad (A. caviae) and one coccus (M. varians). The mixed culture induced severe ulcer, A. caviae induced ulcer

but not severe as caused by the mixed culture, and two pseudomonads induced superficial ulcer in both the fishes. Mortalities were high in mixed culture treated groups compared to aeromonad and pseudomonad treated groups. No ulcer formation and mortality were recorded in the coccus treated and control groups of fishes. It was concluded that no one bacterium was responsible for induction of ulcer in C.punctatus, and A. testudineus; the coccus, M. varians was non pathogenic; two fluorescent pseudomonads were slightly pathogenic; the aeromonad (A. caviae)was more pathogenic than two pseudomonads.

Healthy fish C. batrachus and C. punctatus were inoculated with saline suspensions of two fluorescent pseudomonads (R_1 and R_2) and A. caviae (R_3) in mixed and pure conditions by intramuscular injection. Histopathological studies showed vaculation and necrosis in the liver of C.batrachus and C. punctatus treated with three bacteria in mixed and pure conditions. In kidney changes were degeneration, tubular vaculation and necrosis in the haemotopoietic region in both types of fishes treated with three bacteria in mixed and pure conditions. In spleen necrosis and vaculation were found in both types of fishes treated with the bacteria in mixed condition. Inoculation with aeromonad (A.caviae) showed necrosis in the spleen of C. punctatus only. Inoculation with R_1 and R_2 (two pseudomonads) showed no detectable changes in the spleen of both fishes.

Presence of bacteria were detected in the smear preparation with the blood and impression smear preparations of liver, kidney and spleen. Bacteria were also detected in the sections stained with Giemsa and Carbol-fuchsin of liver, kidney and spleen in all groups of treated fishes. It was considered that one way of bacterial infection in ulcerative disease of air-breathing fishes was that bacteria could enter into the muscle through injured or damaged skin. The bacteria multiplied in the muscle causing ulceration and necrosis and through blood disseminated to different organs such as liver, kidney and spleen. Degenerative and necrosis changes in liver, kidney and spleen were probably due to toxins produced by these bacteria.

Healthy fish C. punctatus were inoculated with intraperioneal injections of mixed and pure cultures of two pseudomonads, aeromonad (A.caviae) and coccus (M.varians). Fishes treated with mixed culture of four bacteria showed significant decrease in erythrocyte count and haemoglobin content after 48 hrs. ($P < 0.05$) and 24 hrs. ($P < 0.05$) of inoculation respectively. Significant decrease ($P < 0.05$) in erythrocyte counts in fishes treated with R_3 (aeromonad) and R_2 and R_1

(two pseudomonads) were observed at 24, 72 and 120 hrs. after inoculation. Similarly significant decrease ($P < 0.05$) in haemoglobin content in fishes treated with R_3 (aeromonad), R_2 and R_1 (two pseudomonads) were observed at 48, 72 and 144 hrs. after inoculation respectively. No significant changes in erythrocyte count and haemoglobin content were observed in fishes treated with coccus (C) and in control fishes upto 168 hrs. It was considered that initial decrease in erythrocyte count and haemoglobin count in fish C.punctatus was due to disintegration of erythrocytes as a result of bacterial infection and ultimately haemopoiesis was affected.

Healthy air-breathing fish A. testudineus, C. punctatus and C. batrachus were first inoculated by intramuscular injection of mixed and pure cultures of two pseudomonads and aeromonad (A. caviae). After 72 hrs. of first treatment with pure culture of two pseudomonads and one aeromonad survived fishes were inoculated with a second bacterium by intramuscular injection at the same site of previous injection. Mixed culture induced severe ulcer and highest percent of mortality in all three species of fishes. Aeromonad induced ulcer but not severe as caused by mixed culture and two pseudomonads induced superficial ulcer

formation. Aeromonad induced higher percent of mortality than that caused by two pseudomonads. After the second treatment with the aeromonad (A. caviae) in fishes initially treated with R_1 and R_2 (pseudomands) the ulcer became severe in A. testudineus and C. batrachus and in C. punctatus change was there in the ulcer but was not severe. In all other experimental groups no change in the ulcer was detected. Though there was no change in the ulcer but percent of mortalities were higher due to second treatment. It was considered that aeromonad (A. caviae) was the chief etiological agent of the ulcerative disease of air-breathing fishes and the pseudomonads were acting as predisposing agent or coagent.

From the drug sensitivity testing it was indicated that the drug trimithoprim/sulphamethoxizole and antibiotic streptomycin were effective to all bacteria and could be used for controlling the disease. Formalin-inactivated mixed bacterial vaccination showed slight protection upto six weeks.

Affected Indian major carps Catla catla, Labeo rohita and Cirrhimus mrigala showed infection of different stages of development, that is, from multiple haemorrhagic spots to deep, haemorrhagic or necrotic ulcer. Some infected fishes showed abnormal swimming behaviour and occasional jumping out of the water. Accumulation of fluid was found in the intestine of one of the infected C. mrigala. Three forms of the disease, acute, chronic and nervous form were detected. One aeromonad (= Aeromonas sp.), two fluorescent pseudomonads, (different from those isolated from air-breathing fishes) and one micrococcus (Micrococcus varians) were isolated from the ulcers of C. mrigala.