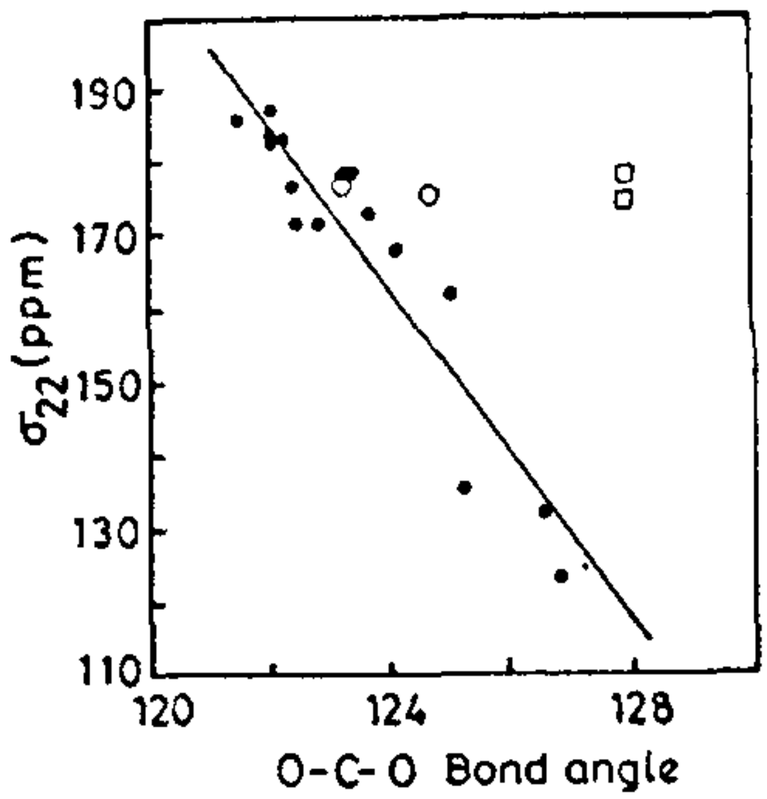

Figure 1. Linear plot showing correlation between the principal element tensor σ_{22} and the O−C−O bond angle for the carboxyl carbons for a group of organic compounds. The compounds included in (●) (solid circle) are: anhydrous oxalic acid, oxalic acid dihydrate, ammonium hydrogen oxalate hemihydrate, benzoic acid, pyromellatic acid dihydrate, succinic acid, glutaric acid, adipic acid, pimelic acid, suberic acid, potassium hydrogensuccinate, ammonium hydrogenfumarate, potassium hydrogenmalonate, potassium hydrogenglutarate. Square (□) represents the experimental σ_{22} value plotted against the O−C−O bond angles of 128° for malonic acid from old X-ray data[6]. Circle (⊙) represents the experimental σ_{22} values plotted for the O−C−O bond angles of malonic acid obtained from new X-ray data[7].

a limit of $\sin\theta/\lambda < 0.6$ Å^{-1}) were collected on an Enraf-Nonius CAD-4 automated computer-controlled diffractometer equipped with a scintillation counter. The structure was solved by direct methods and refined to a final R factor of 0.039 (ref. 7). The O−C−O bond angles for the two carboxyl groups, as determined by the new X-ray data are found to be 123.3° and 124.8°. Substitution of these into the linear equation gives σ_{22} values for the two carboxyl carbons as 173 and 156 ppm respectively, in close agreement with the observed σ_{22} values of 178 and 175 ppm with a margin of ±7 ppm in the experimental measurements[1] (as indicated by a circle (⊙) in Figure 1).

It should be emphasized here that even though the correlation is purely empirical, the present study illustrates clearly, that the chemical shielding tensors (CST) obtained from solid state NMR studies of organic molecules are very sensitive to the local geometry and symmetry of the atoms involved. It may be noted that the principal values of the CST from NMR experiments arise mainly due to the anisotropic nature of the electron density distribution surrounding the nucleus. Since X-ray crystallographic analysis also deals with electron density distribution around atoms, these types of correlations may share a common physical basis. The study of solid state structures of organic and other compounds using the combined techniques of solid state NMR and X-ray crystallography is thus likely to provide us a unique opportunity to correlate chemical shielding effects with the molecular environment of the nucleus of interest.

1. Jagannathan, N. R., *Magn. Reson. Chem.*, 1989, **27**, 941–946.
2. Mehring, M., *High Resolution NMR in Solids*, Springer, Berlin, 1983.
3. Veeman, W. S., *Prog. Nucl. Magn. Reson. Spectrosc.* (eds. Emsley, J. W., Feeny, J. and Sutcliffe, L. H.), Pergamon Press, Oxford, 1984, **16**, 193–235.
4. Facelli, J. C., Grant, D. M. and Michl, J., *Acc. Chem. Res.*, 1987, **20**, 152–158.
5. Etter, M. C., Hoye, R. C. and Vojta, G. M., *Cryst. Rev.*, 1988, **1**, 281–292.
6. Goedkoop, J. A. and MacGillvary, C. H., *Acta Crystallogr.*, 1957, **10**, 125–127.
7. Jagannathan, N. R., Rajan, S. S. and Subramanian, E., *J. Crystallogr. Spectroscop. Res.*, (submitted for publication).

ACKNOWLEDGEMENT. N. R. J. thanks INSA for the award of a research fellowship and the DST, New Delhi for financial assistance.

Received 30 May 1992; revised accepted 15 September 1992

Nonspecific defence mechanisms of *Cirrhinus mrigala* against *Escherichia coli* and *Salmonella typhi*

Debasish Pal and Chanchal Das Gupta*

Institute of Wetland Management and Ecological Design, 15 Darga Road, Calcutta 700 017, India
*Department of Biophysics, Molecular Biology and Genetics, University of Calcutta, 92 A. P. C. Road, Calcutta 700 009, India

As a part of our studies on the microbiology of water and fish in East Calcutta sewage processing oxidation ponds, this paper reports on the phagocytic defense mechanism of the fish *Cirrhinus mrigala* against some sewage-borne bacteria like *Escherichia coli* and *Salmonella typhi*. These bacteria are found in large concentrations in the sewage-fed ponds. They should be potential human pathogens but are non-pathogenic for this fish. The minimum inhibitory titer and minimum bactericidal titer of the fish serum were higher in case of *S. typhi* than *E. coli*. The progress of phagocytosis of both bacteria by fish blood followed similar time course.

SINCE 1930, Calcutta municipal sewage comprising mainly human and animal wastes[1] has been in use for pisciculture in 7,500 acres of water bodies without being subjected to any pretreatment by mechanical and chemical means. In fact the practice of using human and animal wastes for pisciculture has been going on for a long time in Asia[2]. It carries a variety of human pathogens which, if incorporated in fish, may cause health hazards for consumers and handlers[3–7]. In recent years, a hot debate has been going on between

environmental scientists and city planners on the possible health hazards from the East Calcutta sewage-fed fisheries. In order to look at that problem scientifically we have done a number of studies on fish and water from these ponds.

We harvested the Indian major carp Mrigal from sewage-fed and conventional ponds and made comparative measurements of the bacterial content in the fish and in the water[8]. When these fish are placed in bacteria-free water, different bacteria disappear at different rates from the fish depending on various factors like temperature, turbidity, dissolved organic matter etc.[4,7,9]. Concentration of *E. coli* and salmonellae in the muscle of Mrigal appeared to be related to their concentrations in the digestive tract and its contents (DTC)[10]. It is known that bacterial cells penetrate through the digestive tract of fish and are subjected to phagocytosis[7]. Additional phagocytosis takes place in blood stream, lymph stream, and finally in lymphatic organs before the appearance of bacteria in the muscle[7,11]. In this report, we present the results of our experiments on the phagocytic as well as bactericidal effect of fish blood.

Standard laboratory strains of *E. coli* K12 and *S. typhi* which are predominantly found in sewage-fed fisheries of East Calcutta, liquid and plating media used for growth, cell count and biochemical identification of these bacteria etc. were described by Pal and Dasgupta[8].

An Indian major carp, Mrigal (*C. mrigala*), was used in these studies. The collection and disinfection of fish were done following Pal and Dasgupta[10]. About ten fishes were sacrificed to prepare a pool of serum and assay its bactericidal effect. Blood was allowed to clot for one hour after collection at 37° C and serum was separated by centrifugation at 1000 × g for 30 min[12].

To 0.5 ml of the serum diluted in the ratio of 1:1, 1:2, 1:4 etc. in serially arranged tubes, 0.5 ml bacterial suspension at concentrations of 10^3 cells/ml was added[12] and the mixture was incubated overnight at 37° C. A control for each bacterial strain was incubated with buffer[13] only. Then 100 μl of suspension from each tube was spread on three replicas of nutrient agar plates and these were incubated overnight at 37° C. The maximum dilution of serum at which no colony appeared on the plate was taken as the minimum bactericidal titer (MBT). The dilution at which no turbidity due to bacterial growth was observed in the tube but some colonies appeared on the plate was taken as minimum inhibitory titer (MIT). This experiment was repeated three times using different pool of sera. It was observed that MBT as well as MIT of sera against *E. coli* was lower than those in case of *S. typhi* (Table 1). In the control tubes, where bacterial cells were in buffer only, no cell killing was observed.

Table 1. Determination of bactericidal titer of normal Mrigal sera

Bacterial species	Minimum inhibitory titer (In dilution)	Minimum bactericidal titer (In dilution)
E. coli	1:128	1:64
S. typhi	1:16	1:8

To estimate the time course of bactericidal effect of serum, it was isolated from fish blood and diluted to the minimum bactericidal titer for *E. coli* and *S. typhi* respectively. 0.5 ml of each bacterial species at a concentration of 10^3 cells/ml was incubated with 1.0 ml diluted serum. In the control experiment the cells were added to 1.0 ml of diluent. Incubation was done at 25 ± 1° C and at various time intervals, bacterial survival was estimated by plating on nutrient agar in triplicate. In this experiment it was observed that 80% of the *E. coli* and *S. typhi* cells were killed in about 90 minutes following almost identical time courses as shown in Figure 1. But in control tubes bacterial concentrations did not change significantly.

To study phagocytosis in conditions similar to those existing *in vivo*, i.e. in the presence of opsonins complement and natural antibodies, experiments were performed *in vitro* using whole blood[14–18]. For each of the three replicas of this experiment using both species of bacteria, a pool of blood was collected from the

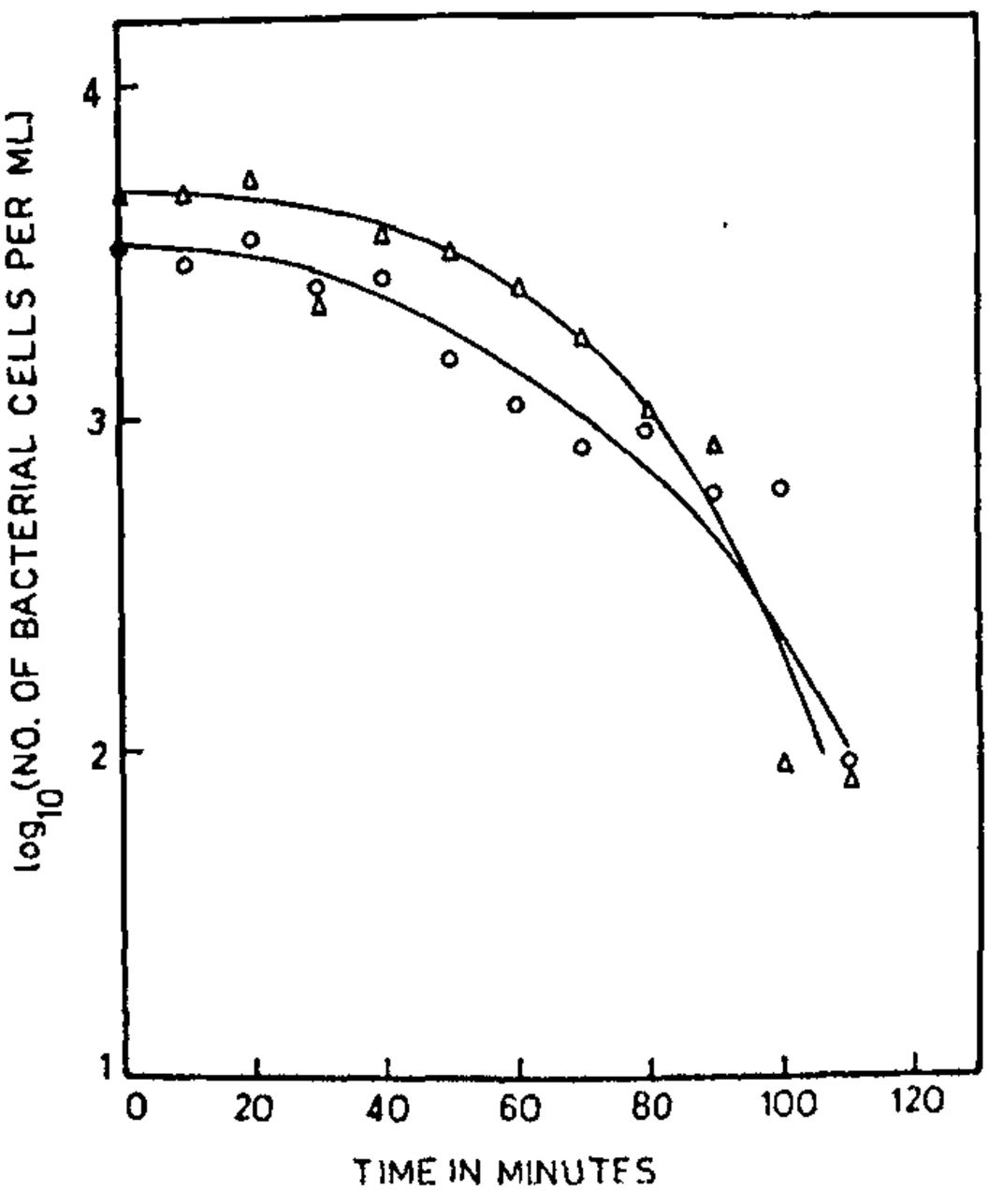

Figure 1. Kinetics of bactericidal effect of Mrigal sera on *E. coli* —— O —— O —— and *S. typhi* —— Δ —— Δ ——.

caudal vein of about 10 fishes. Six hundred units of heparin (Sigma) was added per ml of blood as anticoagulant. The concentration of lymphocytes were about 5×10^7 cells per ml whereas the concentrations of *E. coli* and *S. typhi* varied from 3×10^7 to 5×10^7 per ml in different experiments. After the onset of phagocytosis blood cells were lysed at various times with 5% aqueous solution of saponin (Merck, Germany).

The phagocytic cells of whole blood of Mrigal engulfed *E. coli* and *S. typhi* to the extent of about 5×10^6 and 5×10^7 cells per ml of blood in about 60 minutes at 25°C as shown in Figures 2 and 3 respectively. These numbers represent the viable bacterial counts obtained after plating the lysed blood cell on indicator agar media. The viable bacterial cell counts slowly decreased after 60 min. Perhaps they were killed within the phagocytes. In cell-free control the number of bacteria did not change during 120 min of the experiment. The phagocytic cells were found to

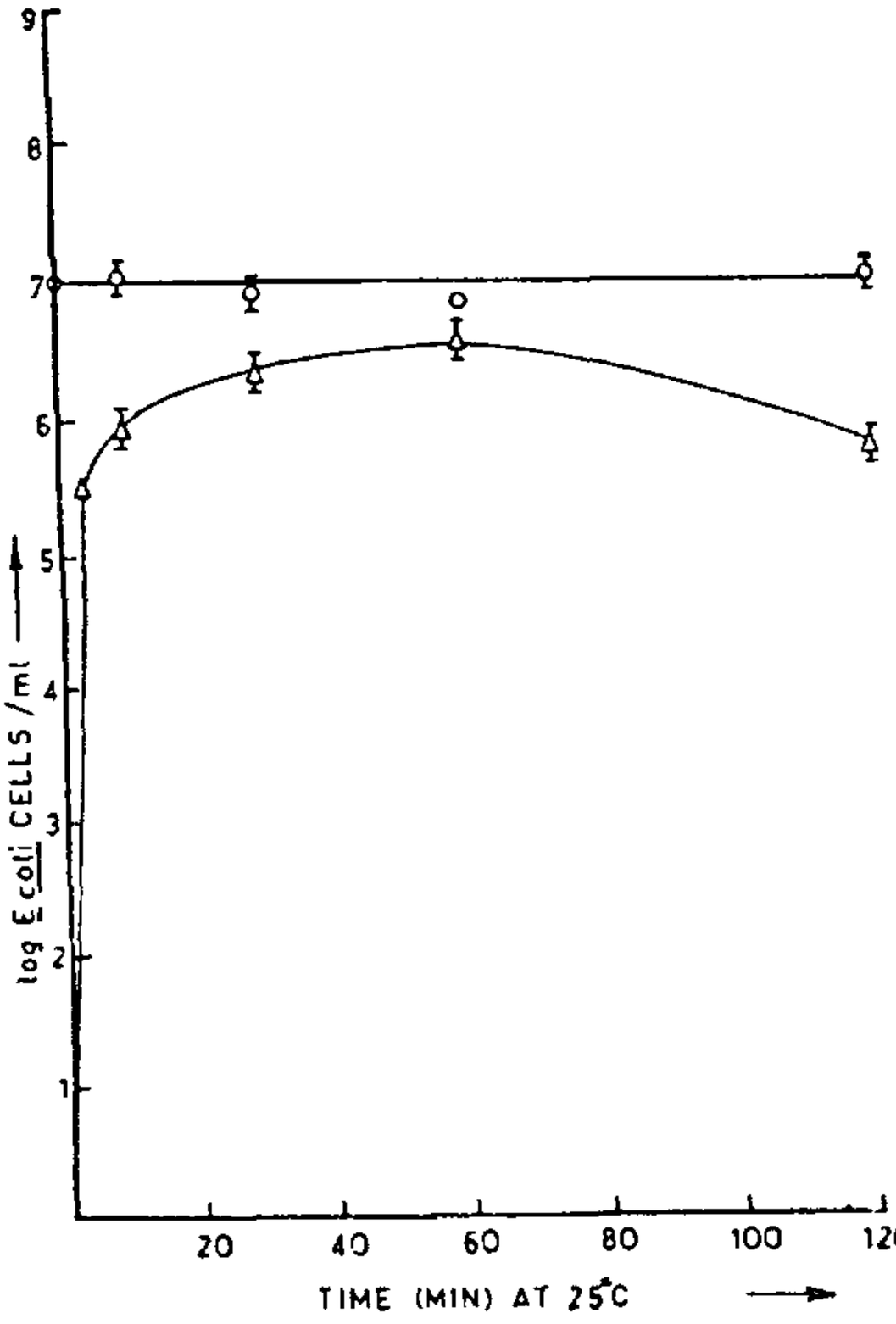

Figure 2. Phagocytosis of *E. coli* by the blood cells of *C. mrigala* at 25 C. —— ○ —— ○ —— Control. Survival of *E. coli* cells when incubated in TC 199 medium plus cell free serum of *C. mrigala*. —— △ —— △ —— Viable *E coli* cells recovered from blood cells of *C. mrigala* after different times of incubation of them together in TC 199 medium.

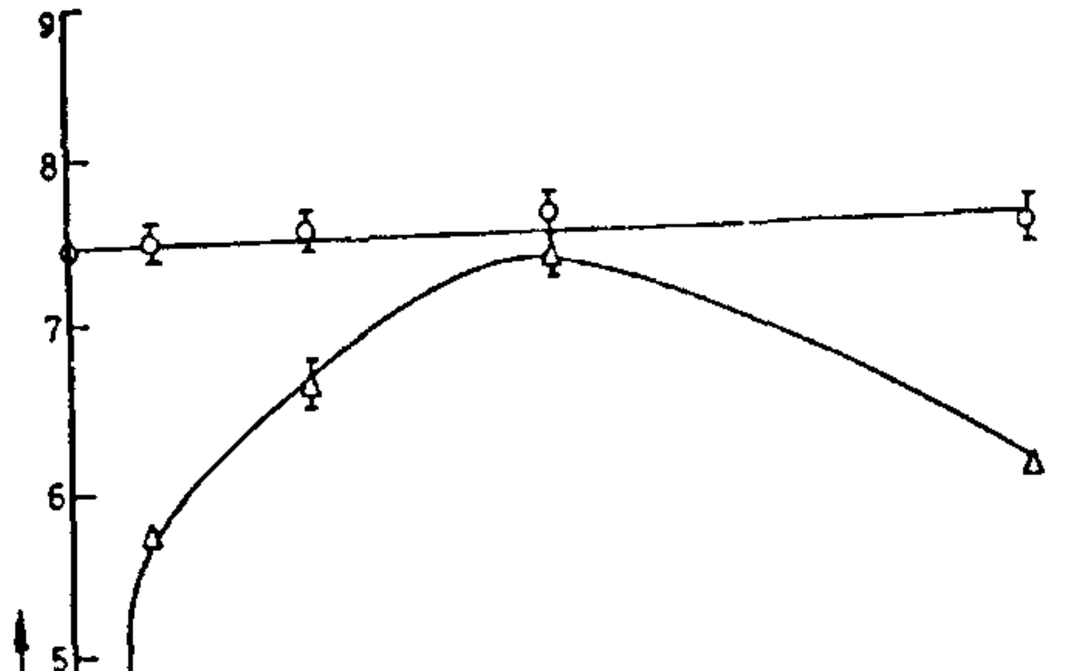

Figure 3. Phagocytosis of *S. typhi* by the blood cells of *C. mrigala* at 25°C. —— ○ —— ○ —— Control: Survival of *S. typhi* cells when incubated in TC 199 medium plus cell free serum of *C. mrigala*. —— △ —— △ —— Viable *S. typhi* cells recovered from blood cells of *C. mrigala* after different times of incubation of them together in TC 199 medium.

contain not more than 100 bacterial cells of *E. coli* and *S. typhi* per ml before *in vitro* experiment.

Although the complement mediated bactericidal activities of mammalian sera have evoked much attention since their first demonstration in the last century, a relatively small amount of data regarding killing effect of sera in poikilothermic animals are available[12–19]. The bactericidal reaction is a sensitive method of detecting circulating antibodies against Gram negative bacteria, serum complement and lysozyme. Like others[20,21] we observed that *E. coli* was more sensitive than *S. typhi* to phagocytosis. The experiment was done at 25°C which is close to the body temperature of fish[18].

In the blood of Mrigal, the number of intracellular bacteria increased rapidly during the first 20 min, then it continued to progress at a slow rate upto 60 min, and thereafter it decreased slowly. The decrease in the number of intracellular viable bacteria indicated that

phagocytic cells of blood were able to kill the bacteria. A number of studies[22–25] suggested that some humoral antibacterial factors normally present in the serum could influence phagocytosis and intracellular killing. In the control, serum became so diluted due to mixing with TC 199 media (Sigma) (used in phagocytosis experiment[18]) that it had no effect on the growth of *E. coli* and *S. typhi* as shown in Figures 2 and 3. In a separate year-long monitoring experiment it was observed that agglutination titer of Mrigal serum against *E. coli* and *S. typhi* were linearly correlated with the same bacterial concentration in their ambient environment (*r* value, *E. coli* = 0.741, *S. typhi* = 0.721). So presumably the natural antibody titer against *E. coli* and *S. typhi* which existed in Mrigal as observed in MIT and MBT was enough to promote the ingestion of bacteria as well as killing to a certain extent as has been seen in the case of mice[26]. Phagocytosis as well as killing of bacteria *in vitro* were also measured at 25°C, the optimum temperature for antibody production in carp[11].

The bacterial cells which escape phagocytosis in different sites especially in blood, reach the muscle[7] and may lead to hygienic problems for handlers and consumers. We have seen that the bacterial cells reached the muscle when their concentrations in the digestive tract exceeded a threshold value[10, 27].

Considering the problems of public health and fish health in pisciculture, bacterial concentration in water should be maintained within the limits of phagocytic and bactericidal ability of the fish. It is known that these bacteria are non-pathogenic for fish. But their presence would cause stress to the defence mechanism of the fish and it may become more prone to infection by fish pathogens[28].

1. Chakraborty, D., Ghosh, D. and Niyogi, S., *Int. J. Environ. Anal. Chem.*, 1987, **30**, 243–253.
2. Tapiador, D. D., Henderson, H. F., Delmendo, H. N. and Tsuitsuy, H., *FAO Fish Tech. Pap.*, p. 168.
3. Guelin, A., in *Fish as Food*, Academic Press, New York, 1962, vol. 2, pp. 480–502.
4. Janssen, W. A., *American Fisheries Society*, special publication, 1970, **5**, 284–290.
5. Lawton, R. L. and Morse, E. V., *J. Environ. Sc. Health*, P-A, 1980, **4**, 339–358.
6. Reichenback-Klinke, H. H., in *Fis. Pathology*, T. F. H. Publications, Neptune City, New York, 1973.
7. Buras, N., Duek, L. and Niv, S., *Appl. Environ. Microbiol.*, 1985, **50**(4), 989–995.
8. Pal, D. and Dasgupta, C. K., *J. Aqua. Anim. Health, USA*, 1992, **4**(1), 32–39.
9. Potter, L. F. and Baker, G. E., *Can. J. Microbiol.*, 1961, **7**, 595-605.
10. Pal, D. and Dasgupta, C. K., *J. Aqua. Anim. Health, USA*, 1991, **3**(2), 124–129.
11. Avtalion, R. R., Wojdani, A., Malik, Z., Sharabani, R. S. and Deuczyminer, M., *Curr. Top. Microbiol. Immunol.*, 1973, **61**, 1–5.
12. Schwab, G. E. and Reeves, P. R., *J. Bacteriol.*, 1966, **91**, 106–112.
13. American Public Health Association, in *Standard Methods for the Examination of Water and Waste-water*, (17th ed. APHA, Inc.), New York, 1975.
14. Nelstrop, A. E., Taylor, H. and Collard, P., *Immunology*, 1968c, **14**, 347–350.
15. Castro, O., Andriole, V. T. and Finch, S. C., *J. Lab. Clin. Med.*, 1972, **80**, 857–860.
16. Charelien, J. H. and Garagusi, V. F., *J. Res. Soc.*, 1972, **11**, 358–360.
17. Glick, B., Sato, K. and Cohenour, F., *J. Res. Soc.*, 1964, **1**, 442–446.
18. Avtalion, R. F. and Shahrabani, R., *J. Immunol.*, 1975, **29**, 1181–1187.
19. Acton, R. T., Weinheimer, P. F., Hildemann, W. H. and Evans, E. E., *Infect. Immun.*, 1971, 160–166.
20. Michael, J. G. and Landy, M., *J. Infect. Dis.*, 1961, **108**, 90–94.
21. Rowley, D., *Br. J. Exp. Pathol.*, 1956, **37**, 223–234.
22. Cohn, Z. A. and Morse, S. I., *J. Exp. Med.*, 1960, **III**, 667–672.
23. Mackaness, G. B., *J. Exp. Med.*, 1960, **112**, 35–39.
24. Shayegani, M. G., Marpel, F. A. and Mudd, S., *J. Immunol.*, 1964, **93**, 88–94.
25. Shayegani, M. G., *Infect. Immun.*, 1970, **2**, 742–747.
26. Benacerraf, B., Sebestyen, M. N and Schlossman, S., *J. Exp. Med.*, 1959, **110**, 27–31.
27. Corbel, H. J., *J. Fish. Biol.*, 1975, **7**, 539–563.
28. Ellis, A. E., in *Stress and Fish* (ed. Pickering, A. D.), Academic Press, 1981, pp. 147–169.

ACKNOWLEDGEMENTS. The authors gratefully acknowledge the help and advice provided by Dr Dhrubajyoti Ghosh, Ex-Director of I. W. M. E. D. and Prof R. K. Poddar, Chairman, I. W. M. E. D. Financial help for the project was obtained from Government of West Bengal.

Received 1 July 1992; revised accepted 30 September 1992

Late Quaternary turbidites of the Arabian Sea abyssal plain, west of Lakshadweep Ridge

K. S. Adiga and V. K. K. Kalluraya

Marine Wing, Geological Survey of India, Mangalore 575 003, India

Sediment cores collected from the Arabian Sea abyssal plain representing late Quaternary column have intercepted deep sea turbidite sequences of composite origin. Based on the study of their coarse fraction, they are grouped into Indus Fan (IF) and calciclastic (CC) turbidites. IF-turbidites are dominated by distal terrigenous minerals and CC-turbidites are essentially composed of coral fragments, the source of which is proximal Lakshadweep Ridge.

TURBIDITES are sediment accumulations deposited by shortlived turbidity currents charged with particles in suspension and are characterized by graded bedding, laminations and other primary sedimentary structures. Gravity-driven underwater currents resulting from factors such as heavily charged rivers, oversteepening of