mixed and in pure conditions, were given to healthy C. punctatus weighing 30 - 40 gms. Control fishes received only 0.2 ml of sterile nutrient broth. Water temperature was maintained at 29 ± 1°c. After 0, 24, 48, 72, 96, 120, 144 and 168 hrs. of inoculation blood from five fishes was collected by cutting caudal region of the fishes by using EDTA (Ethylenediamine tetra acetic acid) as anticoagulant (1 mg/ml). Enumeration of total erythrocyte count (TEC) and Haemoblogin (Hb) content of blood were estimated by usual haematological methods (Lucky,1977).

Observations and Results

Total erythrocyte count and haemoglobin content of the control and treated Channa punctatus obtained during the present investigation are given in Table (5 & 6) and Figure (82 & 83).

Fishes experimentally infected with mixed culture of four bacteria showed a significant decrease in erythrocyte count and haemoglobin content after 48 hrs. ($P < 0.05$) and 24 hrs. ($P < 0.05$) of inocu-

Table 5: Total Erythrocyte count (TEC) (X $10^6/mm^3$) of control and experimentally infected fishes, Channa punctatus with mixed and pure culture of R_3, R_2, R_1 and C bacteria. (Mean ± SD)

Hours after inoculation	Mixed	R_3	R_2	R_1	C	Control
0	3.11 ± 0.20	2.95 ± 0.15	3.16 ± 0.24	3.01 ± 0.27	2.84 ± 0.38	3.01 ± 0.32
24	3.05 ± 0.18	2.91 ± 0.18	3.04 ± 0.34	2.96 ± 0.22	2.81 ± 0.24	3.07 ± 0.19
48	2.29 ± 0.42[a]	2.48 ± 0.10[a]	2.73 ± 0.36	2.57 ± 0.30	2.74 ± 0.26	2.96 ± 0.23
72	2.19 ± 0.32[a]	2.37 ± 0.24[a]	2.63 ± 0.11[a]	2.63±0.22	2.78±0.25	3.01 ± 0.21
96	2.08±0.31[a]	2.26±0.40[a]	2.32±0.16[a]	2.57±0.29	2.65±0.37	2.91 ± 0.14
120	2.01 ± 0.37[a]	2.36 ± 0.11[a]	2.27 ± 0.22[a]	2.56±0.23[a]	2.54±0.23	2.99±0.27
144	2.01±0.04[a]	2.28±0.17[a]	2.29±0.19[a]	2.24±0.14[a]	2.56±0.26	3.01±0.25
168	1.84±0.32[a]	2.05±0.27[a]	2.19±0.23[a]	2.02±0.12[a]	2.53±0.19	2.9 ± 0.21

Data represent the mean ± SD values of five replications of each treatment.

[a]Values showing significant ($P < 0.05$) difference with respect to 0 hr. inoculation of each treatment.

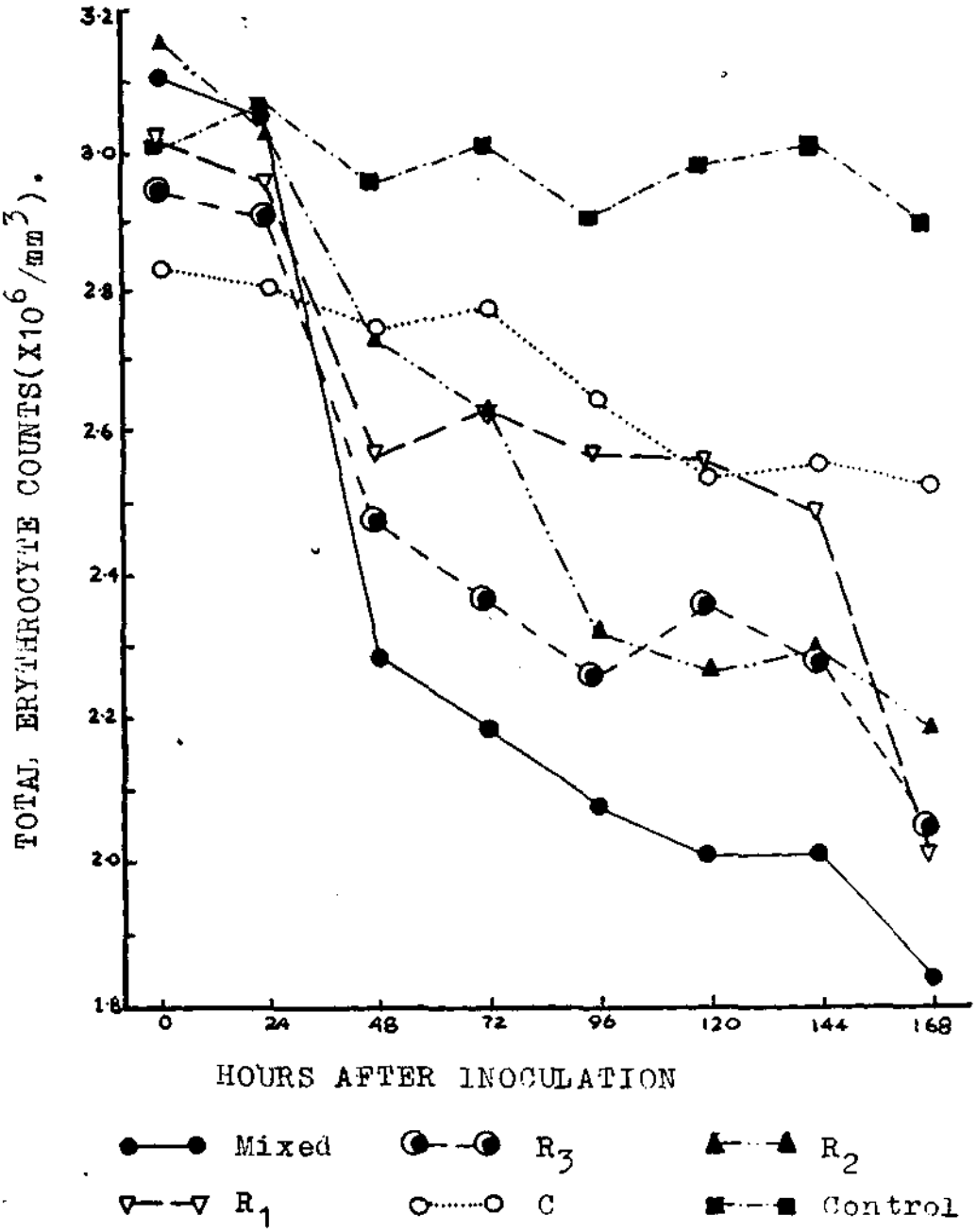

Fig. 82. Total erythrocyte counts in C.punctatus inoculated with mixed and pure cultures of R_3, R_2, R_1 and C, and nutrient broth (Control). Data represent the mean of five replication of each inoculum.

Table 5: Total Erythrocyte count (TEC) (X $10^6/mm^3$) of control and experimentally infected fishes, Channa punctatus with mixed and pure culture of R_3, R_2, R_1 and C bacteria. (Mean ± SD)

Hours after inoculation	Mixed	R_3	R_2	R_1	C	Control
0	3.11 ± 0.20	2.95 ± 0.15	3.16 ± 0.24	3.01 ± 0.27	2.84 ± 0.38	3.01 ± 0.32
24	3.05 ± 0.18	2.91 ± 0.18	3.04 ± 0.34	2.96 ± 0.22	2.81 ± 0.24	3.07 ± 0.19
48	2.29 ± 0.42[a]	2.48 ± 0.10[a]	2.73 ± 0.36	2.57 ± 0.30	2.74 ± 0.26	2.96 ± 0.23
72	2.19 ± 0.32[a]	2.37 ± 0.24[a]	2.63 ± 0.11[a]	2.63±0.22	2.78±0.25	3.01 ± 0.21
96	2.08±0.31[a]	2.26±0.40[a]	2.32±0.16[a]	2.57±0.29	2.65±0.37	2.91 ± 0.14
120	2.01 ± 0.37[a]	2.36 ± 0.11[a]	2.27 ± 0.22[a]	2.56±0.23[a]	2.54±0.23	2.99±0.27
144	2.01±0.04[a]	2.28±0.17[a]	2.29±0.19[a]	2.24±0.14[a]	2.56±0.26	3.01±0.25
168	1.84±0.32[a]	2.05±0.27[a]	2.19±0.23[a]	2.02±0.12[a]	2.53±0.19	2.9 ± 0.21

Data represent the mean ± SD values of five replications of each treatment.

[a]Values showing significant ($P < 0.05$) difference with respect to 0 hr. inoculation of each treatment.

lation respectively (Table 5 & 6). Erythrocyte count in mixed culture treated fishes was 3.11 x 10^6 ± 0.20/mm^3 at 0 hr., after 48 hrs. of inoculation it reduced to 2.29 x 10^6 ± 0.42/mm^3 ($P < 0.05$), and after 168 hrs. of inoculation it reduced to 1.84 x 10^6 ± 0.32/mm^3 ($P < 0.05$). The haemoglobin content in mixed culture treated fishes was 17.38 ± 0.81 gm/100 ml. at 0 hr. after 24 hrs. of inoculation the haemoglobin content was reduced to 16.06 ± 0.56 gm/100 ml ($P < 0.05$) and finally to 13.5 ± 0.93 gm/100 ml ($P < 0.05$) after 168 hrs. of inoculation.

Decrease in erythrocyte counts and haemoglobin content were also noted in experimentally infected fishes with pure cultures of R_1, R_2 and R_3 bacteria (Table 5 & 6). Significant decrease ($P < 0.05$) in erythrocyte counts in fishes treated with R_3, R_2 and R_1 bacteria were observed at 48, 72 and 120 hrs. after inoculation respectively. Similarly significant decrease ($P < 0.05$) in haemoglobin content of fishes treated with R_3, R_2 and R_1 bacteria were noted at 48, 72 and 144 hrs. after inoculation respectively. Total erythrocyte count in fishes treated with pure culture of C-bacteria and sterile nutrient broth were 2.84 x 10^6 ± 0.38/mm^3 and 3.01 x 10^6 ± 0.32/mm^3

respectively at 0 hr. and it showed 2.53 x 10^6 ± 0.19/ mm^3 and 2.9 x 10^6 ± 0.21/mm^3 after the 168 hrs. of inoculation. Similarly, haemoglobin content of Coccus (C) and nutrient broth treated fishes were 16.10 ± 0.86 gm/100 ml and 16.40 ± 0.59 gm/100 ml respectively at 0 hr., and 14.92 ± 0.87 gm/100 ml and 15.86 ± 0.80 gm/100 ml after 168 hrs. of inoculation respectively.

Discussion

Total erythrocyte counts and haemoglobic content of fishes treated with bacterial cultures except coccus showed decreasing trends. Erythrocyte counts of mixed culture and R_3 treated fishes showed significant decrease ($P < 0.05$) after 48 hrs. of treatment and R_1 and R_2 treated fishes showed significant decrease ($P < 0.05$) after 72 hrs. and 120 hrs. of treatment respectively. However, coccus and nutrient broth treated fishes did not show any significant changes in erythrocyte count even after 168 hrs. of treatment.

Similarly haemoglobin level of mixed culture and R_3 treated fish showed significant decrease ($P < 0.05$) after 24 hrs. and 48 hrs. of treatment respectively and R_2 and R_1 treated fish showed significant decrease after 72 hrs. and 144 hrs. of treatment respectively. Coccus and nutrient broth treated fishes did not show any significant decrease even after 168 hrs. of treatment.

Schäperclaus and Mann (1939) showed decrease in number of erythrocytes in infectious dropsy of carp. Takahashi and Kusuda (1979) reported that the erythrocyte count in induced scale protrusion disease by Aeromonas liquefaciens in coloured carp was decreased with lapse of time. They concluded that the cause of decrease in number of erythrocytes was due to enormous disintegration of erythrocytes by the infection. Takahashi (1984 a,b) found marked decline in erythrocyte counts, haemoglobin levels and haematocrit values in experimentally infected carp treated with A. hydrophila and suggested that the erythrocytes were destroyed by hemolysin produced by Aeromonas entering the blood and there was a decrease in hematopoietic function of kidney and spleen due to necrosis in those organs.

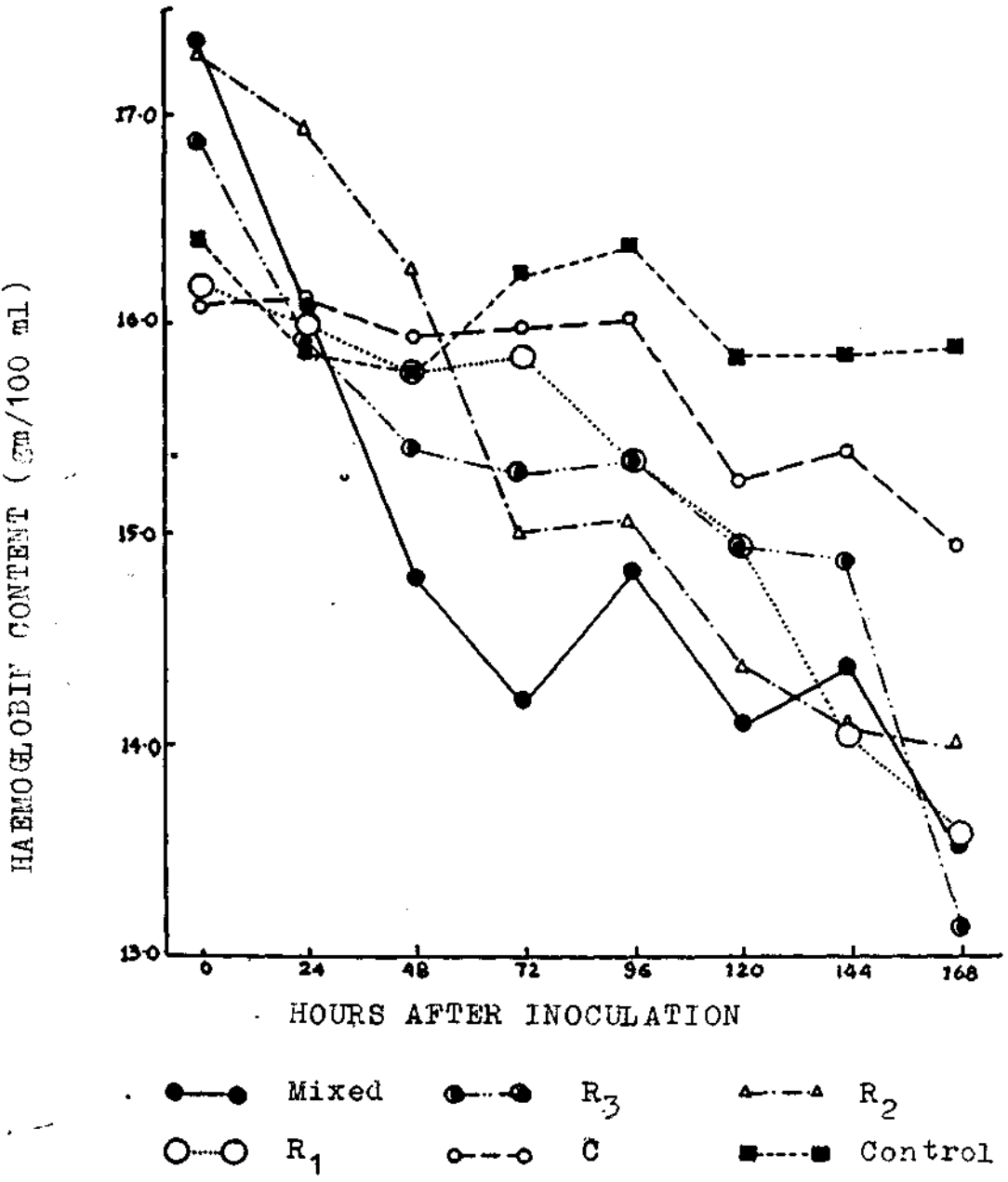

Fig. 83. Haemoglobin contents in C.punctatus inoculated with mixed and pure cultures of R_3, R_2, R_1 and C, and nutrient broth (Control).
Data represent the mean of five replication of each inoculum.

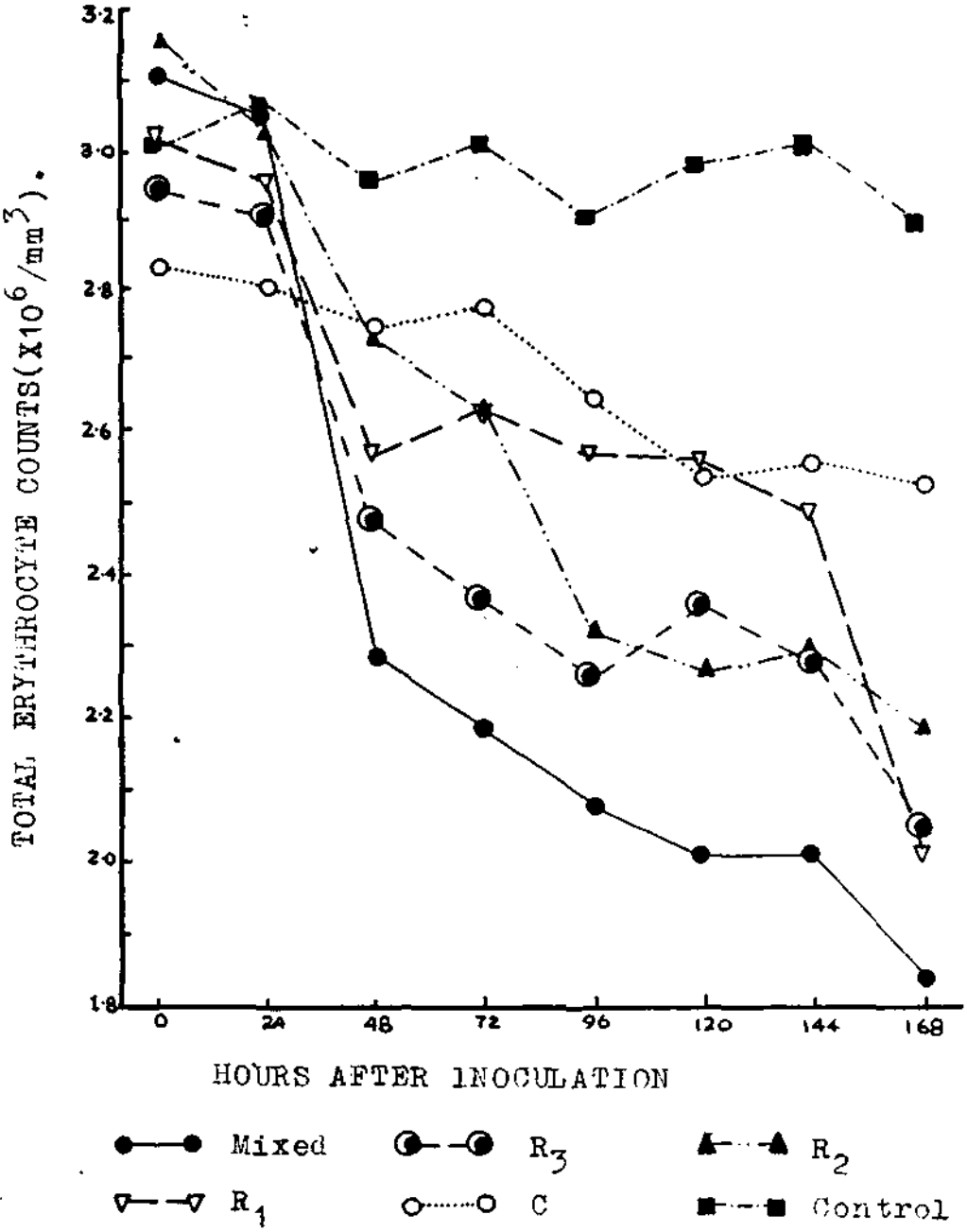

Fig. 82. Total erythrocyte counts in C.punctatus inoculated with mixed and pure cultures of R_3, R_2, R_1 and C, and nutrient broth (Control). Data represent the mean of five replication of each inoculum.

Table 6: Haemoglobin content (gm/100 ml) of control and experimentally infected fishes, *Channa punctatus* with mixed and pure cultures of R_3, R_2, R_1 & C bacteria (Mean ± SD)

Hours after inoculation	Mixed culture of four bacteria.	R_3 bacteria	R_2 bacteria	R_1 bacteria	C bacteria	Control
0	17.38±0.81	16.86±0.49	17.30±0.75	16.16±0.60	16.10 ± 0.86	16.40 ± 0.59
24	16.06±0.56[a]	15.94±1.16	16.94±0.50	16.0±0.66	16.10±1.16	15.88 ±0.59
48	14.72±1.4[a]	15.40±0.96[a]	16.28±0.60	15.72±1.35	15.94±0.26	15.78± 0.53
72	14.19± 1.5[a]	15.28±0.61[a]	15.0±1.08[a]	15.82±0.56	15.98±0.62	16.22 ± 0.61
96	14.80±0.54[a]	15.34±0.62[a]	15.06±0.96[a]	15.32±0.82	16.02±1.08	16.36 ± 0.55
120	14.10±0.092[a]	14.92±0.48[a]	14.38±0.89[a]	14.96±0.99	15.22±0.66	15.82±0.52
144	14.36±0.35[a]	14.86±0.63[a]	14.10± 1.15[a]	14.02±0.78[a]	15.38 ± 0.55	15.82 ± 0.47
168	13.50±0.93[a]	13.14±0.8[a]	14.0 ± 0.94[a]	13.52 ±0.67[a]	14.92 ± 0.87	15.86 ± 0.80

Data represent the mean ± SD values of five replication of each treatment.

[a]Values showing significant ($P < 0.05$) difference with respect to 0 hr. inoculation of each treatment.

Several workers also reported decrease in haematocrit and haemoglobin levels in both experimentally and naturally infected with Bacterial kidney disease (BKD) in coho salmon and brook trout (Wedemeyer and Ross, 1975; Suzumoto et al., 1977; Aldrin et al., 1978; Kimura, 1978). Bruno and Munro (1986) showed significant decline in red cell count, haematocrit and haemoglobin in rainbow trout and Atlantic salmon experimentally infected with Renibacterium salmoninarum.

Barham et al.,(1979) showed fairly severe infection of spleen of diseased fishes, rainbow trout. Barham et al., (1980) noted most of the haematological parameters; Haematocrit values, haemoglobin and plasma protein concentrations etc. were depressed due to bacterial infection and suggested that haemopoiesis was effected severely and this was reflected in the reduced count and haematocrit as well as in the reduced haemoglobin concentration.

It has been observed that necrotic changes in the kidney and spleen of naturally infected fishes Clarias batrachus, Heteropneustes fossilis and

Anabas testudineus (Chapter 1) and also in experimentally infected fishes C. batrachus and Channa punctatus (Chapter 3). Disintegrated erythrocytes were also observed in large number in naturally infected fishes (Chapter - 1).

So it can be concluded that initial decrease in erythrocyte count and haemoglobin content in the experimental fishes C.punctatus is due to disintegration of erythrocytes as a result of bacterial infection and ultimately haemopoiesis is effected as there is necrosis in the main haemopoietic organs e.g. kidney and spleen.