

CHAPTER V

Effect of Experimental Fluoride Poisoning on the Haemogram of Growing Chicks of Domestic Fowl, *Gallus gallus domesticus*.

Variations in the constituents of blood cells are useful in the diagnosis of ailments affecting the liver, pancreas, bone and other organs or systems. Therefore, to assess the extent of damage caused by a pollutant, haematological parameters are frequently examined. Several studies in this regard have been carried out in recent years. However, results of such studies present an array of differences depending upon the experimental model and the pollutant in question. Textile and paper mill effluents have been shown to cause reduction in erythrocyte and leucocyte numbers and an increase in haemoglobin content in fishes (Murugesan and Haniffa, 1984; Haniffa *et al.*, 1984). Exposure of fish to heavy metals for a short duration caused increase in red blood corpuscle (RBC) count and haemoglobin content (Strik *et al.*, 1975; Fernando and Raja, 1984) and a decrease when exposed for a longer duration of time (Sastry, 1984). It has been shown that pesticide intoxication leads to an initial increase and then a decrease in the RBC count in the fish *Clarias batrachus* (Qayyum *et al.*, 1982). Decline in blood cell populations has also been reported in studies with pesticides such as DDT, Dieldrin, Sevin and Sumithion (Toft, 1955; Lone and Javaid, 1976). There have been some studies on the effect of fluoride in this regard in fish (Chitra and Rao, 1980; Shaikh, 1985), in lizard (Suresh, 1989) and in mammals (Susheela and Jain, 1983; Karram and Ibrahim, 1992).

These studies indicate definite effects of fluoride on erythrocyte composition of an animal. In the present context an attempt is made to link the fluoride influence on growth and development of chicks of domestic fowl. The extraembryonic development in domestic fowl itself is marked by attendant changes in blood profiles (Bell and Freeman, 1971; Farner and King, 1972; Sturkie, 1986). Hence, it would be pertinent to examine the haematological indices in fluoride administered growing chicks, to evaluate the role of this element and its mode of action in altering progress of development. The present study concerns with the number of RBC per unit volume of blood, iron (Fe), haemoglobin (Hb), haematocrit (Hc), mean cor-

puscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in chicks administered with 15.4 mg F⁻/kg b.w. for 30 days.

MATERIAL AND METHODS

One day old female Rhode Island Red chicks were obtained from Government Hatchery Baroda. Prior to the commencement of experiment six birds were sacrificed and the haematological picture was analysed as described later. The chicks were then divided into two groups of thirty each and maintained in metal cages (150 x 100 x 75 cm) under a conventional lighting regimen of 14 h light and 10 h darkness. The birds were maintained on a standard starter diet. Water was available *ad libitum* for both the groups. Birds in the first group were provided through intragastric route 1 ml of fluoridated water of appropriate concentration, so as to make a dose of 15.4 mg F⁻/kg b.w. (1/5 of LD₅₀) daily. The pollutant was administered at early morning hours. Food and water were withdrawn for 1 h following fluoride administration. Chicks in the other group were treated similarly with distilled water and were considered as controls.

Birds from experimental and control groups were bled at days 1, 5, 10, 20 and 30 after the initiation of experiment. They were bled between 07.00 and 08.00 h. Each bird was bled only once. Six birds from each group were selected at random for bleeding and the data have been pooled to get the mean. The chicks were killed by decapitation until 10 d and thereafter birds were bled from basilic vein using sterile disposable plastic syringes and needles (21 gauge). The blood (1.0 to 1.5 ml) was dispelled immediately into vials containing dipotassium salt of ethylene diamine tetra-acetic acid (EDTA, 1.5 mg/ml of blood) as anticoagulant.

Analytical Methods

Iron and haemoglobin were estimated by the method of Wong (1928).

The blood sample was diluted in the red blood cell pipette using erythrocyte diluting fluid. The RBC counts were made using a haemocytometer with improved Neubauer ruling (Germany) and expressed per cubic millimeter.

The packed cell volume (PCV) was determined by the microhaematocrit method with a microhaematocrit centrifuge (Schlam, 1979). The following indices were calculated (Dacie and Lewis, 1975); mean cell haemoglobin, mean cell haemoglobin concentration and mean cell volume.

Statistics

The statistical significance of the data was evaluated by Student's 't' test.

RESULTS

The mean erythrocyte number in control chicks remained unchanged till day 5 of experiment and then the number increased gradually upto day 20. The RBC count, however, had declined towards the end of experimental regimen (Table I). The total RBC (TRBC) in fluoride treated birds also followed a similar course, but as compared to their controls, the TRBC in experimental birds declined significantly ($p < 0.001$) on day 20 of fluoride administration. A further reduction in RBC number was observed in the treated birds on day 30 of experiment (Figure 1).

The average PCV value of the post-hatched developing chicks fluctuates in correspondence to that of TRBC (Table I). By day 20 of fluoride administration Hc value in the experimental birds recorded a substantial drop compared to that of control chicks. The Hc value in

TABLE I : Haematological indices in growing chicks of domestic fowl, subjected to sublethal dose of sodium fluoride

Parameters studied	Duration of Treatment (Days)											
	0	1	5	10	20	30	Con.	Exp.	Con.	Exp.	Con.	Exp.
RBC (millions/mm ³)	1.49±0.02 ^a	1.49±0.02	1.49±0.02	1.48±0.02	1.73±0.02	1.75±0.03	1.86±0.02	1.70±0.02	1.68±0.02	1.70±0.02	1.68±0.02	1.33±0.05
PCV (%)	30.17±0.75	30.17±0.48	29.67±0.92	29.17±0.83	30.50±0.43	30.17±0.54	33.33±0.33	28.83±0.48	31.33±0.61	28.83±0.48	31.33±0.61	20.17±0.87
Fe(mg/dl)	28.81±0.40	27.46±0.42	27.56±0.40	27.21±0.65	31.49±0.43	31.33±0.40	37.15±0.28	28.11±0.27	33.04±0.31	28.11±0.27	33.04±0.31	22.92±0.49
Hb (g/dl)	8.44±0.15	08.42±0.22	08.35±0.20	08.46±0.20	09.43±0.20	09.57±0.24	11.16±0.20	08.24±0.29	10.62±0.18	08.24±0.29	10.62±0.18	05.65±0.26
MCV (μ ³)	203.72±1.92	202.79±3.35	201.50±4.31	202.09±3.08	175.21±1.64	172.89±3.20	180.98±1.80	165.46±3.18	186.22±2.71	165.46±3.18	186.22±2.71	154.76±2.92
MCH (Pg)	56.35±0.73	55.95±0.94	56.29±0.98	55.97±1.40	54.32±0.67	54.32±1.25	59.94±1.00	48.16±1.92	63.47±1.04	48.16±1.92	63.47±1.04	43.32±1.73
MCHC(%)	27.00±0.68	27.83±0.75	28.07±0.76	28.12±0.93	30.12±0.59	30.52±0.70	33.08±0.69	27.55±1.19	33.36±0.51	27.55±1.19	33.36±0.51	28.65±1.45

@ Values are expressed as mean ± SEM of six experiments. ** p < 0.02; *** p < 0.01; **** p < 0.001

Fig. 1. Total Erythrocyte Count

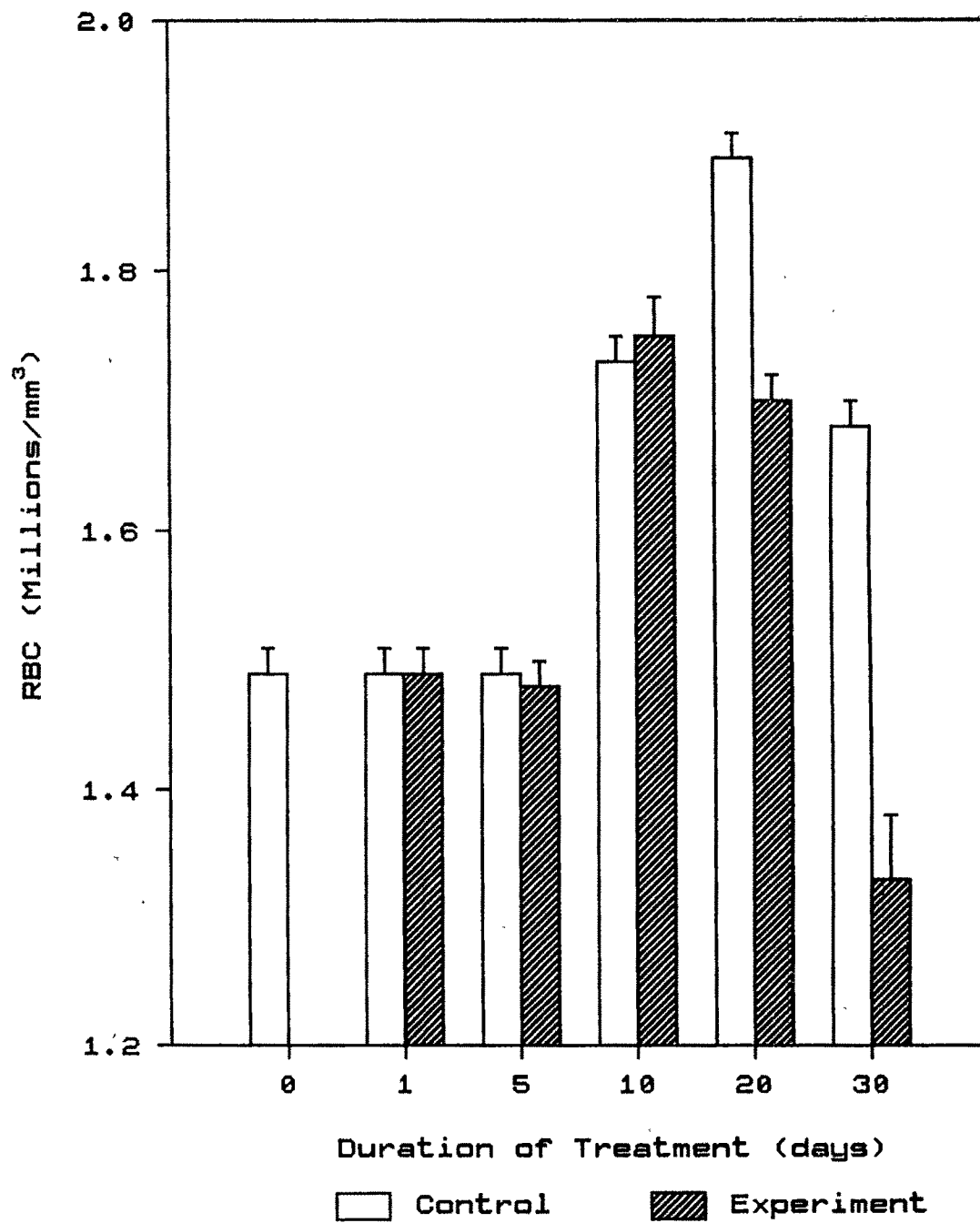


Fig. 2. Packed Cell Volume

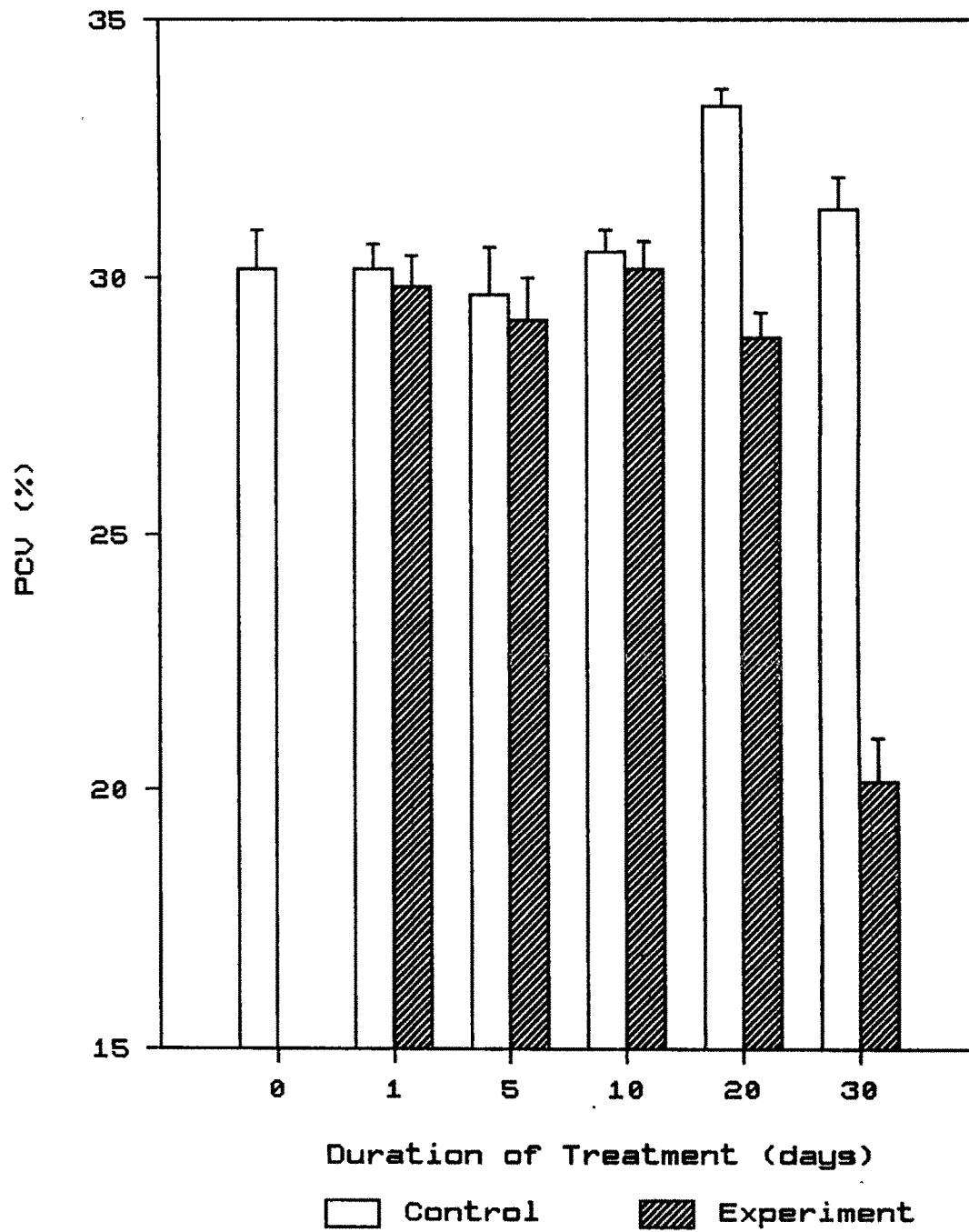


Fig. 3. Iron Content in Blood

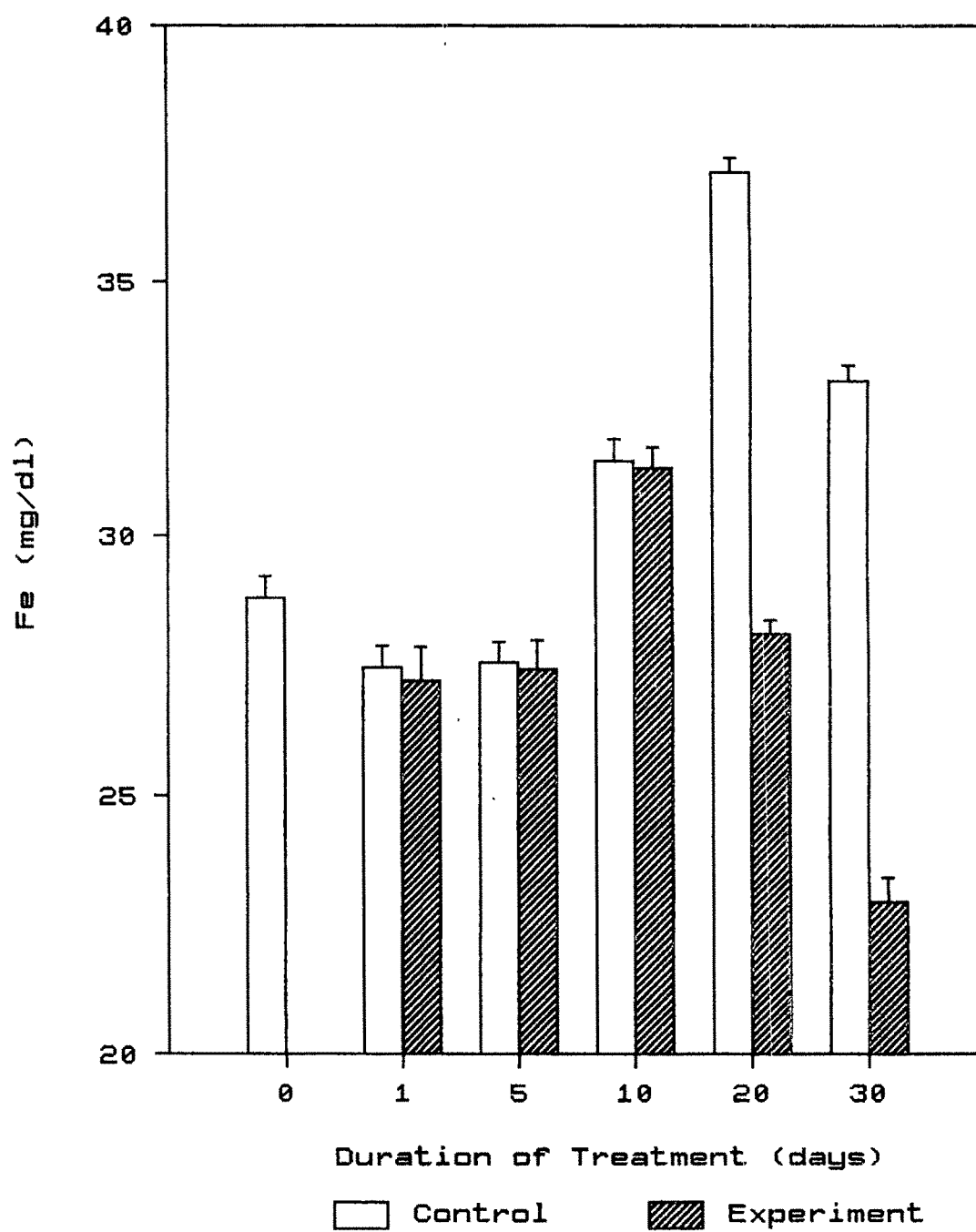


Fig. 4. Haemoglobin Content in Blood

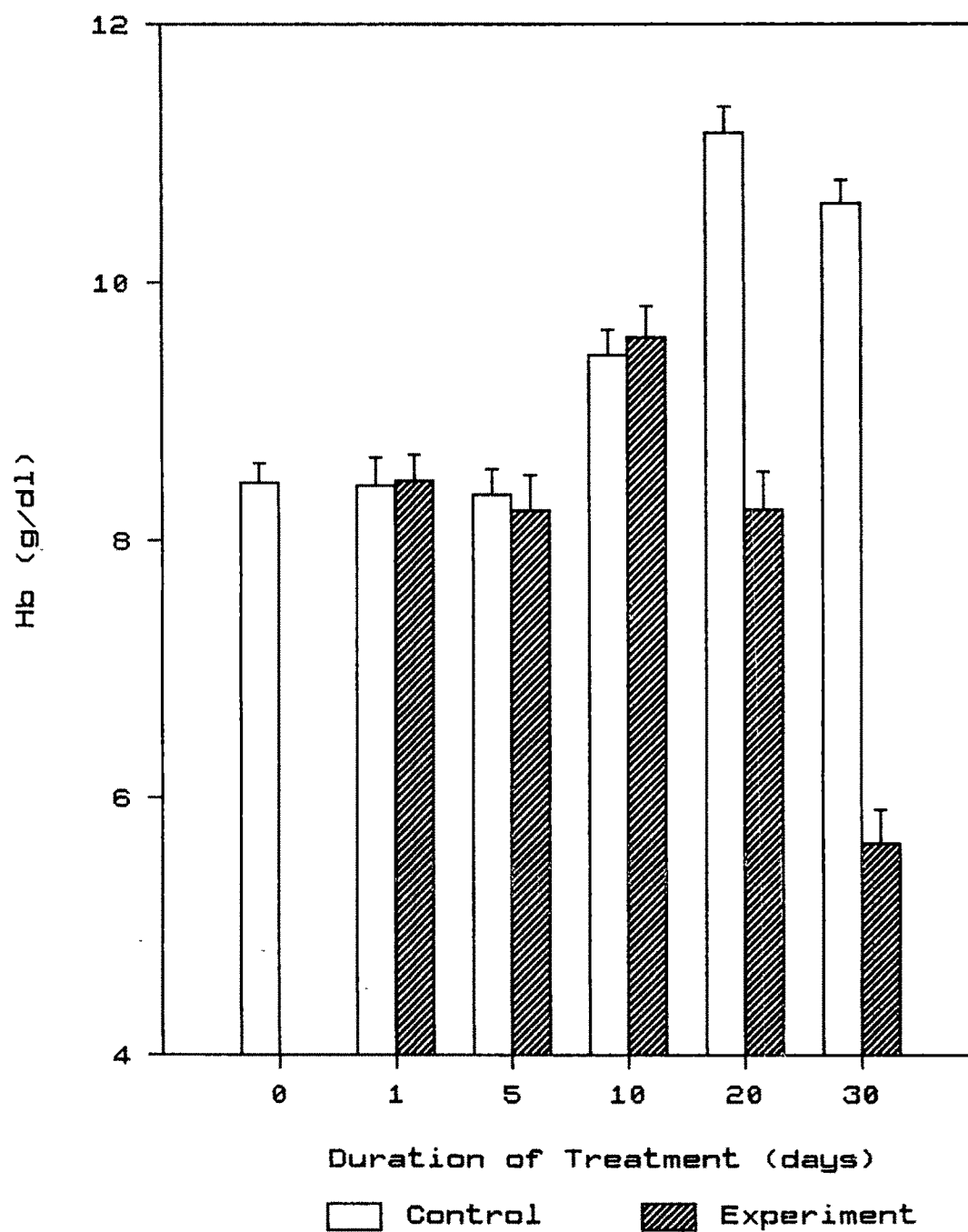


Fig. 5. Mean Corpuscular Volume

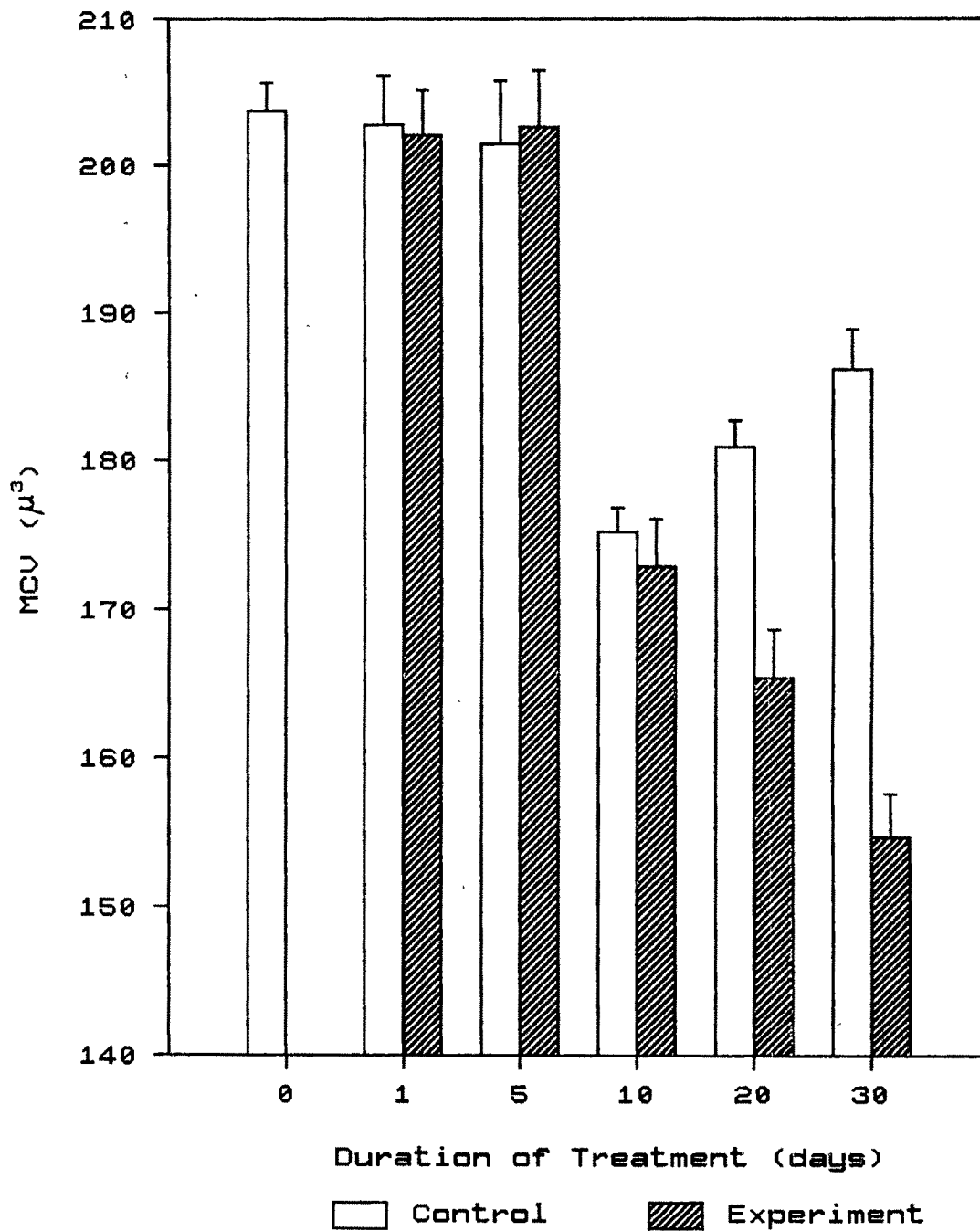


Fig. 6. Mean Corpuscular Haemoglobin

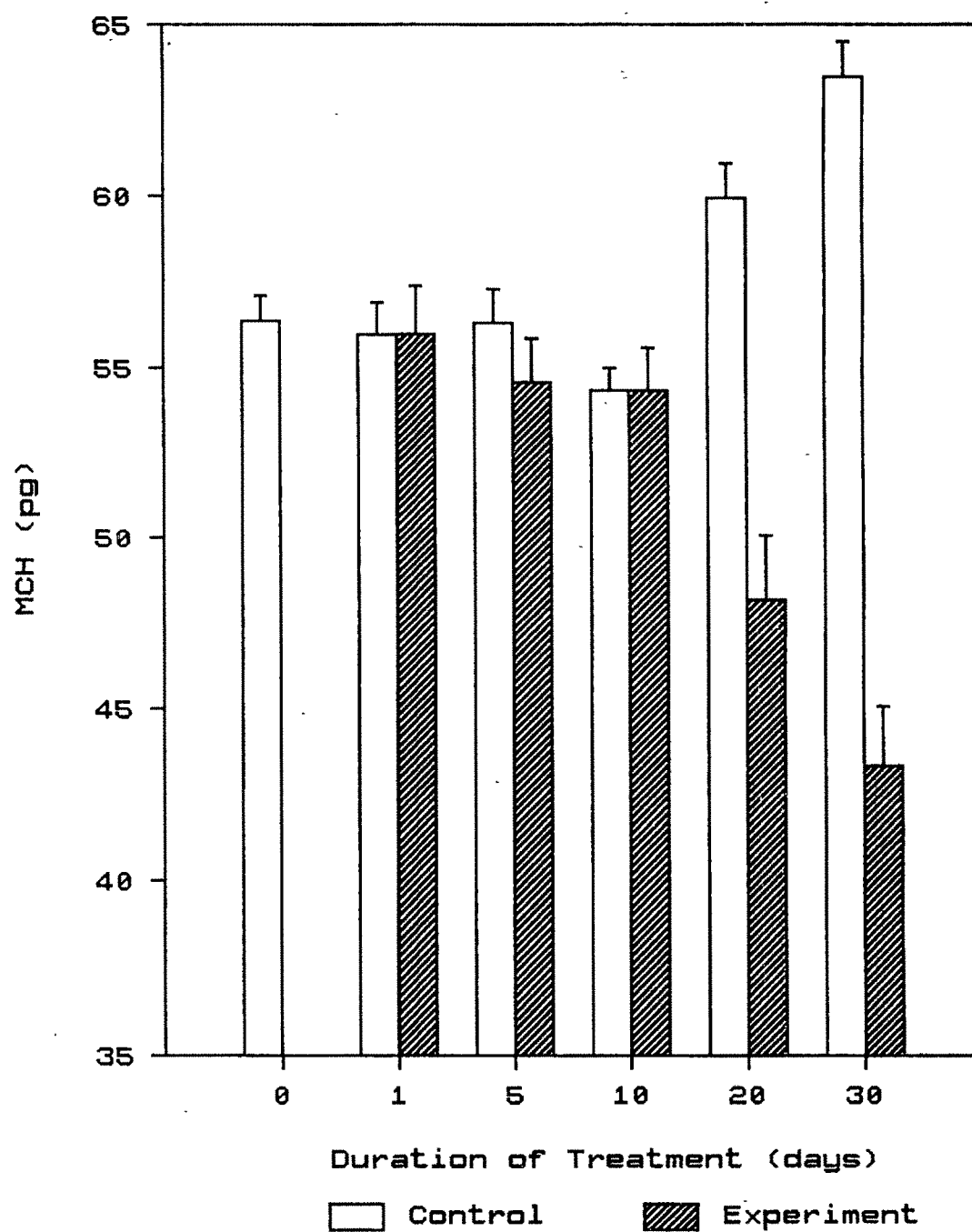
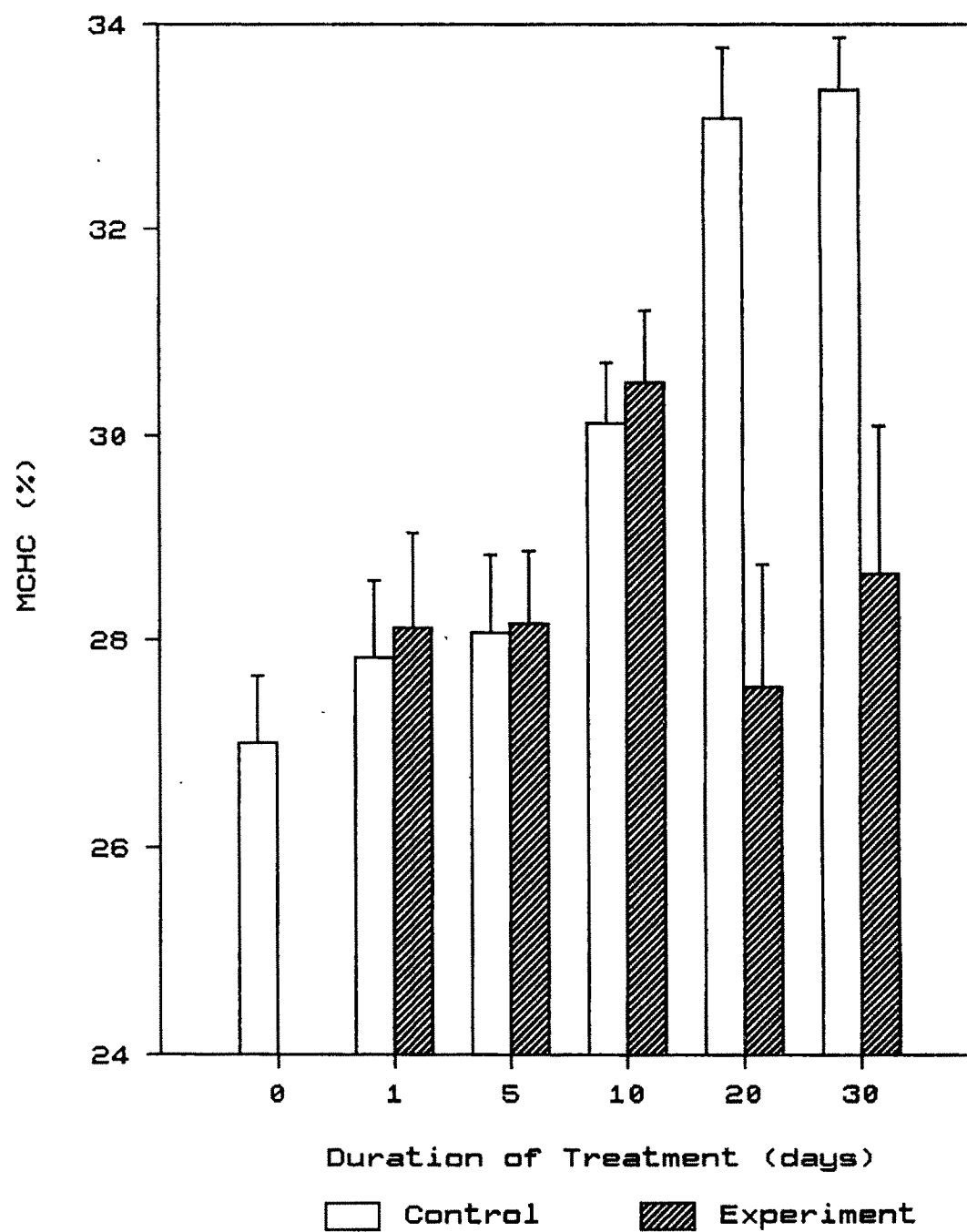


Fig. 7. Mean Corpuscular Haemoglobin Concentration



treated birds had fallen further by 30 % towards the end of the experiment (Figure 2).

Administration of sublethal dose of fluoride to developing chick had not altered the Fe and Hb values initially. Nevertheless, both Fe and Hb values in experimental birds declined considerably by day 20 (Table I). The magnitude of fluoride intoxication is intensified with the duration of administration. This is evidenced by the lowest values of Fe and Hb observed among birds treated with fluoride for 30 days (Figures 3 and 4).

As seen in table 1 the MCV and MCH values in control birds gradually declined till day 10 of experiment. Thereafter, an increase in both MCV and MCH values was quite apparent. However, in experimental birds MCV and MCH values declined steadily till the termination of experiment (Figures 5 and 6).

The mean MCHC values in growing chicks during the various periods of study are represented in figure 7. The MCHC value in control chicks had increased progressively till the end of experimental regimen. However, the MCHC in fluoride treated birds followed the pattern only upto day 10. An obvious drop of 17 and 14 % of MCHC value was observed among experimental birds on days 20 and 30 of treatment as compared to respective controls.

DISCUSSION

Present investigations on erythrocyte population, iron and haemoglobin contents of the blood of developing chicks have shown definite degenerative changes in response to fluoride poisoning. The decrease in RBC count during the last two phases of fluoride intoxication is well paralleled by the concomitant decrease in haemoglobin content. Kahl *et al.* (1973) reported reduced RBC count in rat subjected to low doses of fluoride. A drop in erythrocyte number was recorded in camels from fluoride intoxicated area (Karram and Ibrahim, 1992).

Chronic fluoride administration has been shown to cause erythropenia in rabbit (Susheela and Jain, 1983). While reasoning the possible cause of erythropenia in fluoride treated rabbits, Susheela and Jain (1983) had suggested that reduced levels of adrenal cortical steroids, other than aldosterone, might have induced erythropenia. Hypertrophy and hypofunction of adrenal gland in fluoride ingested subject have been documented (Rao and Susheela, 1979). A similar situation in fluoride treated chicks cannot be ruled out. Moreover, the fluoride induced antimitotic activity (Chapter 4) might have also contributed its share in altering erythrocyte population.

Anaemia and decrease in iron content have been reported by several workers in mammals and birds following fluoride exposure. Roshan (1959) reported anaemia in rabbits exposed to low concentrations of fluoride. Soldatovic and Nadeljkovic-Tomic (1971) recorded decrease in the haemoglobin and iron contents in rabbits that were orally administered with 1-20 mg F/kg b.w. daily for thirty days. Severe symptoms of anaemia in mice were seen when they were given 50 ppm fluoride in drinking water for 19 days (Messer *et al.*, 1973). Inhalation of fluoride (hydrofluoric acid gas) by doves resulted in lesions in lungs, hyper-anaemia and weight loss (Ronzani, 1909). Hirao (1972) noted anaemia in experimental animals following fluoride ingestion which was also reflected in weight loss. These findings are in agreement with the present study on the developing chicks subjected to fluoride. The haemoglobin decrease may be attributed to fluoride inhibition of globin synthesis (Tom *et al.*, 1971) in the affected RBCs. Inadequate nutrition observed in the present study (Chapter 3) could also lead to anaemia. Similar observation made by Suttie (1968) gives additional support for the present findings.