

CHAPTER III

Effect of Fluoride on Body Growth and the Development of Digestive Organs
in the Postnatal Chicks of Domestic Fowl, *Gallus gallus domesticus*.

Fast growing industries are a boon to any affluent society. However, the attendant problem of pollution often acquires unmanageable proportions, which are critical for the very lives whose betterment they were intended.

Due to its natural occurrence in earth's crust, as well as its widespread use in industries, in cosmetics and in pharmaceuticals, fluoride is considered as one of the major pollutants in ecotoxicological studies (Groth, 1975). Fluoride gains entry into the circulation mainly through absorption from gastric and duodenal regions of the intestine (Stookey *et al.*, 1964b). Not surprisingly, therefore, the stomach and intestinal disorders are common among patients with osteofluorosis (Czerwinski and Lankosz, 1977). In acute fluoride toxicity, one of the most striking changes in humans are the alterations found in gastrointestinal mucosa (Waldbott, 1963). Acute hemorrhagic gastroenteritis with patches of hyperaemia and oedema dominate the events (Roholm, 1936). An epidemiological survey conducted in an endemic fluorotic area in North India, revealed severe gastrointestinal problems among 47% of the population surveyed (Susheela and Das, 1988). Hence, it becomes apparent that fluoride, when present as a pollutant causes severe damage to digestive organs.

Damage to digestive system at the growing stage of an organism will in turn affect the growth and development of the organism in question. This is especially true in the case of poultry, where at the early period of growth, the transition from embryonic absorption of yolk to utilization of food is accompanied by many changes in the developmental process including changes in patterns of growth of the organs of supply (eg intestine, pancreas, liver) and the organs of demand (eg muscle, fat) (Nitsan *et al.*, 1991). At the early period of ex-ovo development, intense growth of organs of supply is the characteristic feature. However, in the later period of growth, the emphasis on development changes from the organs of supply to that of the organs of demand (Katanbaf *et al.*, 1988). A pollutant like fluoride, which is widely used in poultry industry as a pesticide and has been known to adversely affect the

gastrointestinal tract might possibly curb the rate of development of organs of supply at the crucial period of early development (postnatal). Such a change often leads to stunted growth and severe loss in terms of economy. To examine such a possibility the present work was designed to study the body growth as well as the growth of digestive organs *viz.*, small intestine, pancreas and liver, in fluoride intoxicated RIR chicks.

MATERIAL AND METHODS

Day old female Rhode Island Red chicks were purchased from a Government Hatchery Baroda. They were divided into two groups of thirty each by random selection and kept in metal cages of 150 x 100 x 75 cm with 14/10 light /dark cycle. The chicks were fed on a commercial starter diet throughout the experimental period. Water was available *ad libitum* for both the groups. Birds in the first group (experimental) were given orally 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 given at early morning hours. After fluoride treatment food and water were withdrawn for 1 h. Chicks in the other group were treated similarly with distilled water and were considered as controls.

On days 1, 5, 10, 20 and 30 following the commencement of experiment, six birds from each group were weighed and killed by decapitation. The small intestine (after removal of chyme), gizzard (whole and empty), pancreas and liver were removed and weighed. To obtain the initial weight of organs, six birds were sacrificed on the day of purchase. Allometric growth was calculated according to the equation $(O_n/O_h)/(BW_n/BW_h)$, where O is the organ weight; n is the day of experiment; h is the initial weight and BW is the body weight (Fisher, 1984).

Statistics

Each value is expressed as mean \pm standard error of mean (SEM) and the difference of means was analysed by Student's 't' test with 95 percent confidence limit.

RESULTS

Body weight

The average weight of the control chicks registered a gradual increase till day 10 of experiment. The body weight, however, increased rapidly between 10 day (44 g) and 20 day (87 g). This tempo in growth was maintained in the final stage of experiment (Table I). Fluoride administration hindered the body growth by day 10 of experiment. By day 20, the difference in size of the body between control and experimental birds became more conspicuous (Plate Ia). At the end of the experiment the weight of the fluoride poisoned chicks was almost half as that of the control birds (Plate Ib; Figure 1).

Food content in gizzard

As the body grows, a parallel increase in the amount of food content was observed in the gizzard of control birds (Figure 2). However, fluoride administration abated the food intake by day 10 of experiment. Compared to controls, a decrease of 45 and 71 percent of food content was observed in the gizzard of experimental birds on day 20 and 30 respectively.

Absolute weight and Allometric growth of digestive organs

Small intestine: The initial weight of small intestine in control chick doubled by day 5 of experiment. This is followed by a gradual increase till day 10. Between day 10 and 20 the intestine weight gain was two fold. However, the pace of growth again slowed down towards

the end of the experiment (Figure 3). Fluoride imparted its adverse effect on small intestine by day 10. Thereafter compared to control birds the weight of small intestine in experimental birds remained at a significantly ($p < 0.001$) low level.

Maximum allometric growth of the small intestine was observed in the control chicks on day 5 and day 20 of experiment (Table II). However, compared to control birds hampered allometric growth was observed in the fluoride intoxicated chicks on days 10, 20 and 30 (Figure 4).

Pancreas: In control chicks steep increase in the weight of pancreas was noticed at each stage of experiment, except on day 1 and 10, where the increase in weight was slow and gradual (Figure 5). Nevertheless, compared to control chicks an apparent reduction in the weight of pancreas was observed in the experimental birds by day 10 of fluoride administration. More definite decrease in the weight of pancreas was recorded in the fluoride poisoned birds on day 20 and 30.

The allometric growth of pancreas in control birds was approximately double than that of the body growth for the entire duration of experiment except, on day 1 (Figure 6). However, fluoride administration reduced the allometric growth on day 20 and 30 of experiment.

Liver: From figure 7, it is obvious that in control birds the average weight of liver increased gradually till day 20 of experiment. However, between day 20 and 30 the weight of liver increased by 92 percent. Compared to control chicks significant reduction in liver weight was observed in experimental birds on day 10, 20 and 30 of fluoride administration.

Allometric growth of liver was considerably less than that for the pancreas and small intestine, with minimum value on day 30 of experiment (Figure 8). Fluoride administration retarded the allometric growth of liver by day 20. Further intoxication resulted in more significant ($p < 0.01$) reduction in allometric growth (Table II).

TABLE I : Body Weight, food content in gizzard, absolute weights of small intestine (minus chyme), pancreas and liver of female chicks at different phases of experiment.

Parameters studied	Duration of Treatment (Days)									
	0	1	5	10	20	30	Exp	Con.	Exp	Con.
Body weight (gm)	31.00±0.90 ^a	31.20±1.10	34.70±1.10	34.01±1.10	44.00±2.10	87.00±4.50	54.80±5.50 ↓	172.0±10.40	95.20±12.00 ↓	
Food content in gizzard (gm)	0.155±0.02	0.669±0.04	1.158±0.03	1.163±0.04	1.495±0.07	2.481±0.08	1.369±0.12 ↓	4.168±0.14	1.214±0.10 ↓	
Weight of small intestine (gm)	0.802±0.03	0.815±0.04	1.634±0.05	1.613±0.07	1.916±0.06	4.239±0.08	1.989±0.05 ↓	5.614±0.14	2.677±0.11 ↓	
Weight of pancreas (gm)	0.066±0.01	0.067±0.01	0.147±0.02	0.141±0.02	0.191±0.01	0.467±0.05	0.275±0.04 ↓	0.786±0.05	0.331±0.04 ↓	
Weight of liver (gm)	1.021±0.08	1.052±0.04	1.217±0.08	1.213±0.09	1.449±0.04	2.497±0.09	1.464±0.14 ↓	4.802±0.18	2.095±0.16 ↓	

@ Values are expressed as mean ± SEM of six experiments. * p < 0.05; ** p < 0.02; *** p < 0.01; **** p < 0.001

TABLE II : Allometric growth of small intestine, pancreas and liver of female RIR chicks at specific phases of experiment.

Name of the Organ	Duration of Treatment (Days)											
	1			5			10			20		
	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp.
Small Intestine	1.017 ± 0.03	1.021 ± 0.04	1.804 ± 0.04	1.816 ± 0.06	1.684 ± 0.05	1.477 ± 0.07 ↓	1.883 ± 0.04	1.393 ± 0.06 ↓	1.261 ± 0.04	1.088 ± 0.06 ↓		
Pancreas	1.015 ± 0.04	1.000 ± 0.05	1.961 ± 0.05	1.973 ± 0.05	2.039 ± 0.02	1.963 ± 0.03	2.522 ± 0.02	2.348 ± 0.05 ↓	2.147 ± 0.02	1.636 ± 0.02 ↓		
Liver	1.029 ± 0.05	1.034 ± 0.06	1.056 ± 0.05	1.050 ± 0.07	1.021 ± 0.07	1.039 ± 0.06	0.871 ± 0.02	0.808 ± 0.02 ↓	0.848 ± 0.03	0.669 ± 0.03 ↓		

@ Values are expressed as mean ± SEM of six experiments. * p < 0.05; *** p < 0.01; **** p < 0.001

Plate Ia. The control (C) and experimental (E) birds after 20 days of treatment.

Plate Ib. The control (C) and experimental (E) birds after 30 days of treatment.



Fig. 1. Body Weight

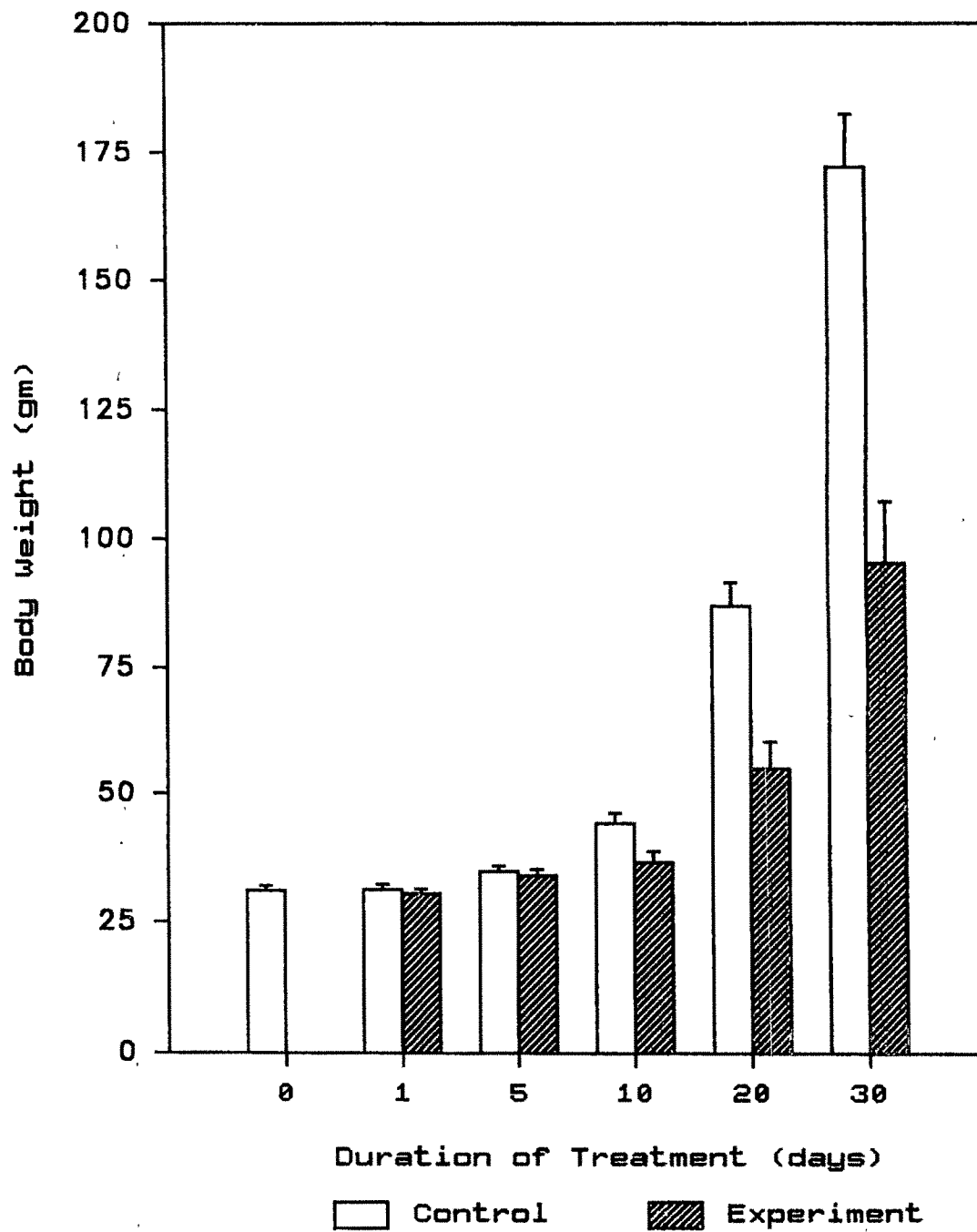


Fig. 2. Food Content in Gizzard

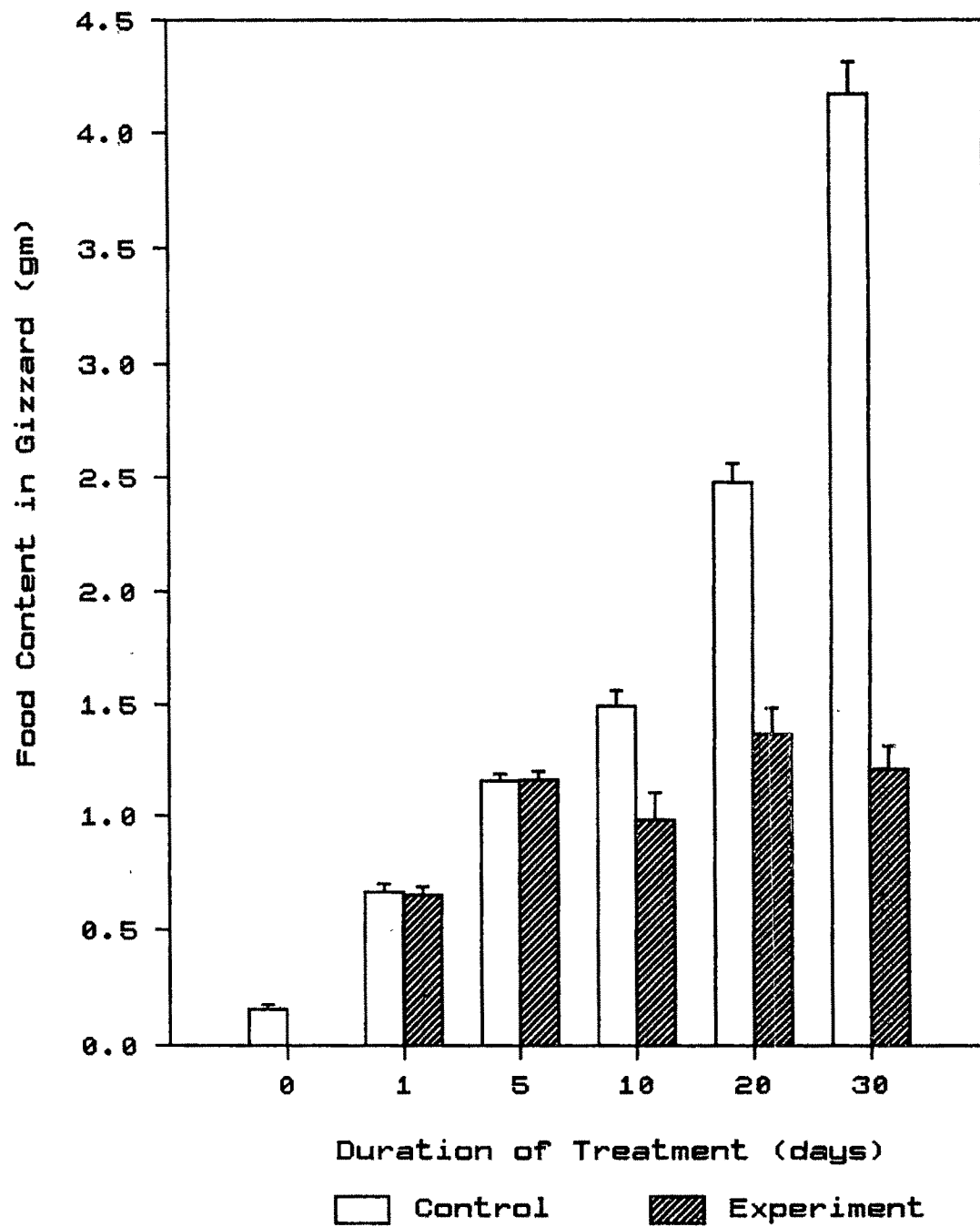


Fig. 3. Weight of Small Intestine

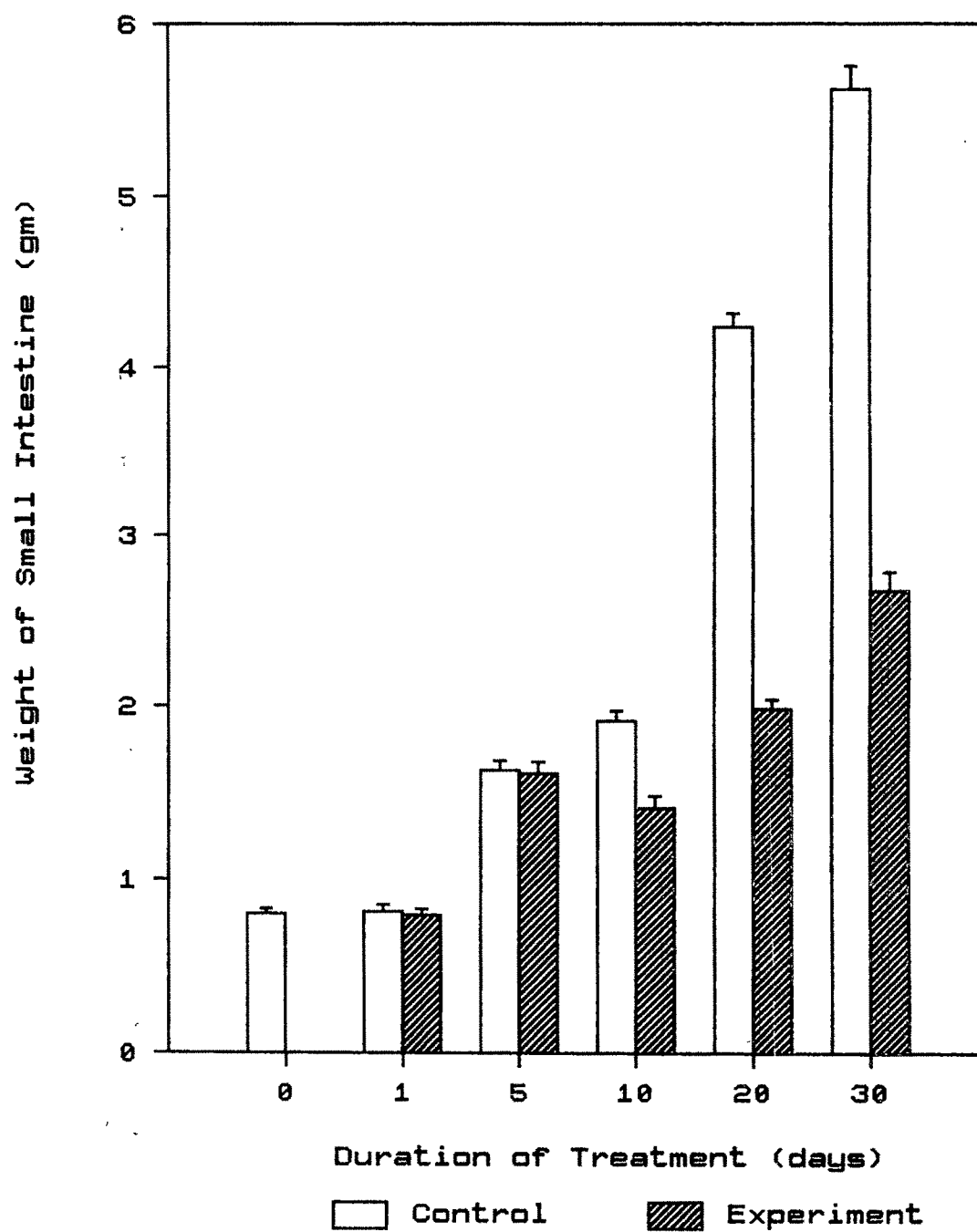


Fig. 4. Allometric Growth of Small Intestine

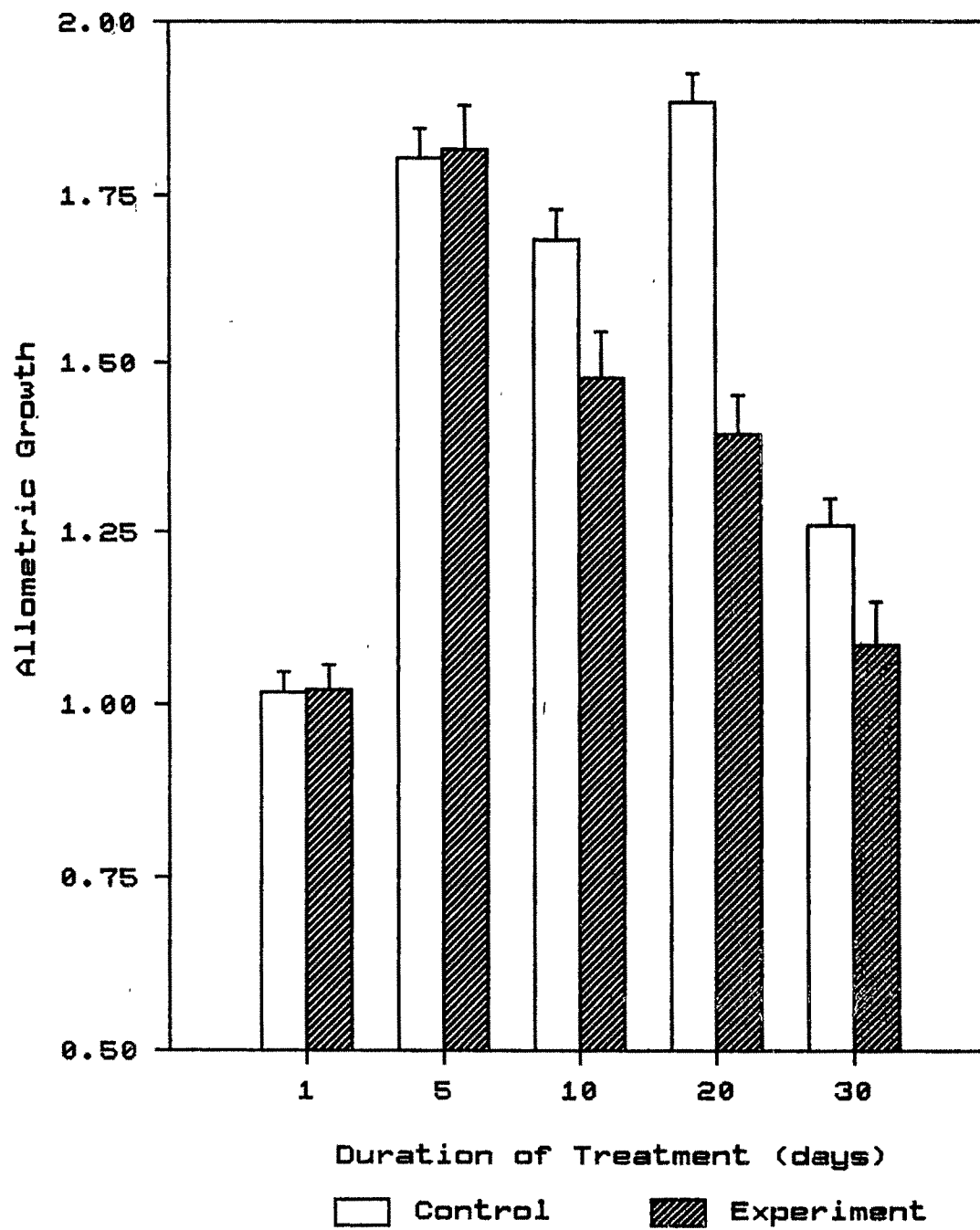


Fig. 5. Weight of Pancreas

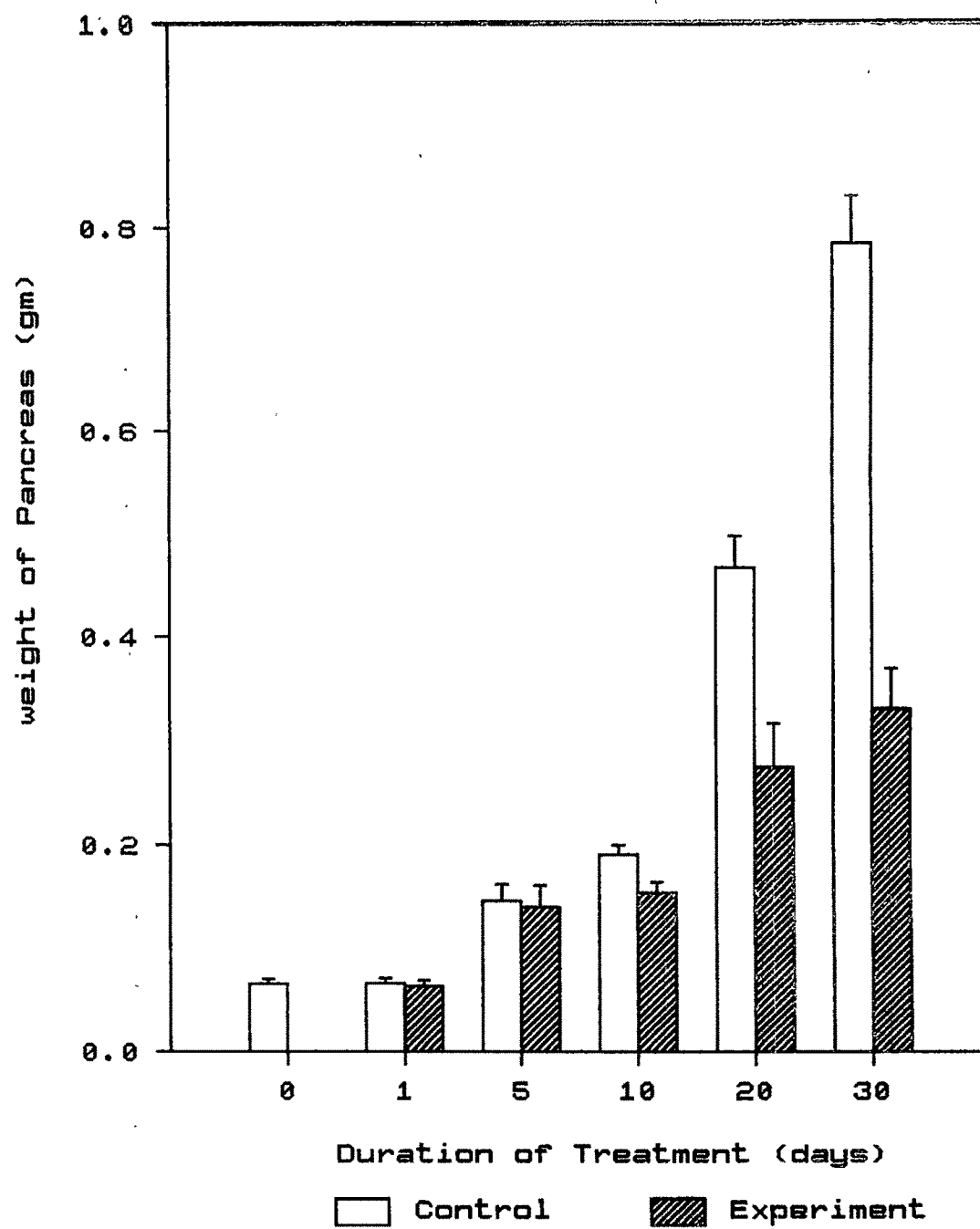


Fig. 6. Allometric Growth of Pancreas

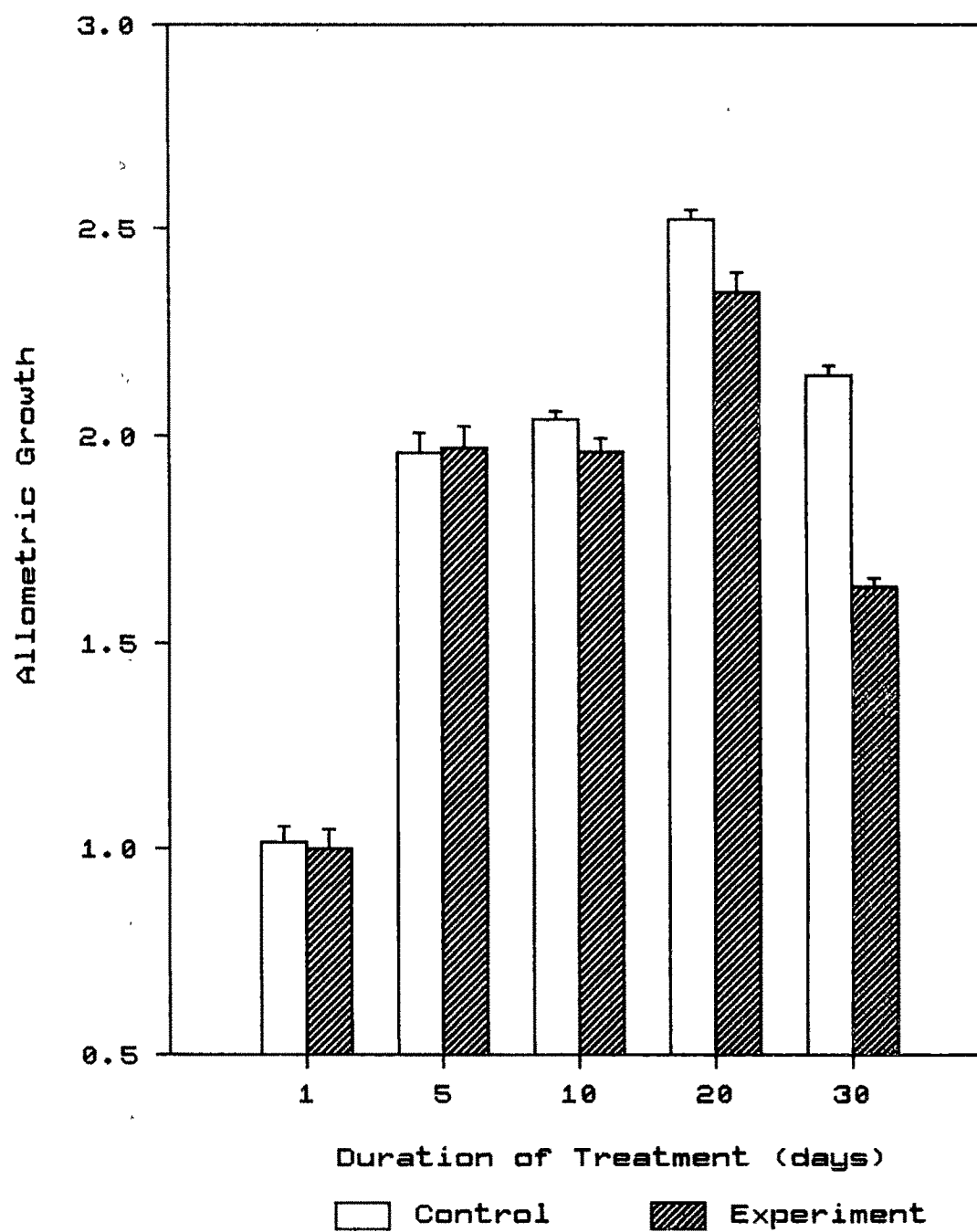


Fig. 7. Weight of Liver

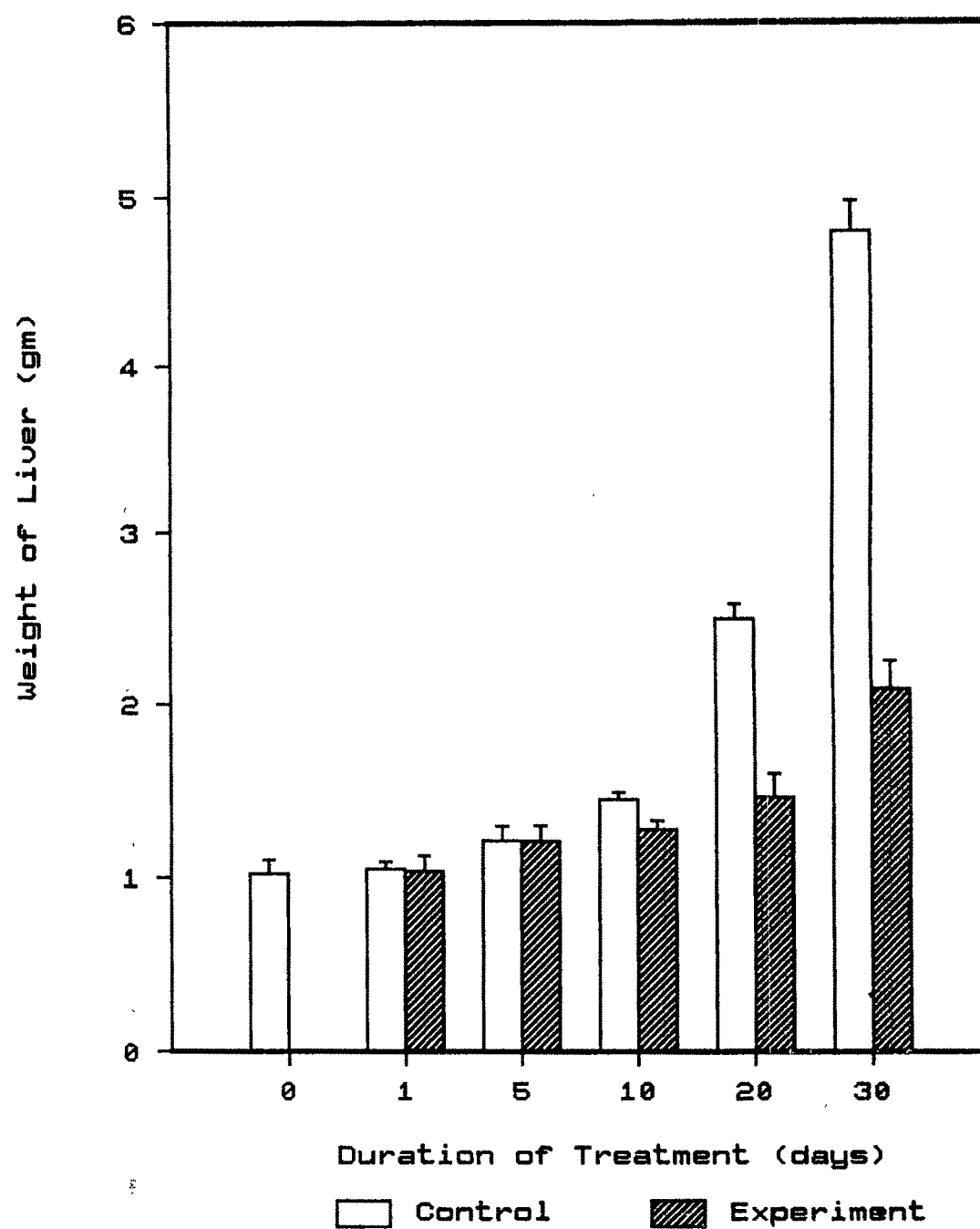
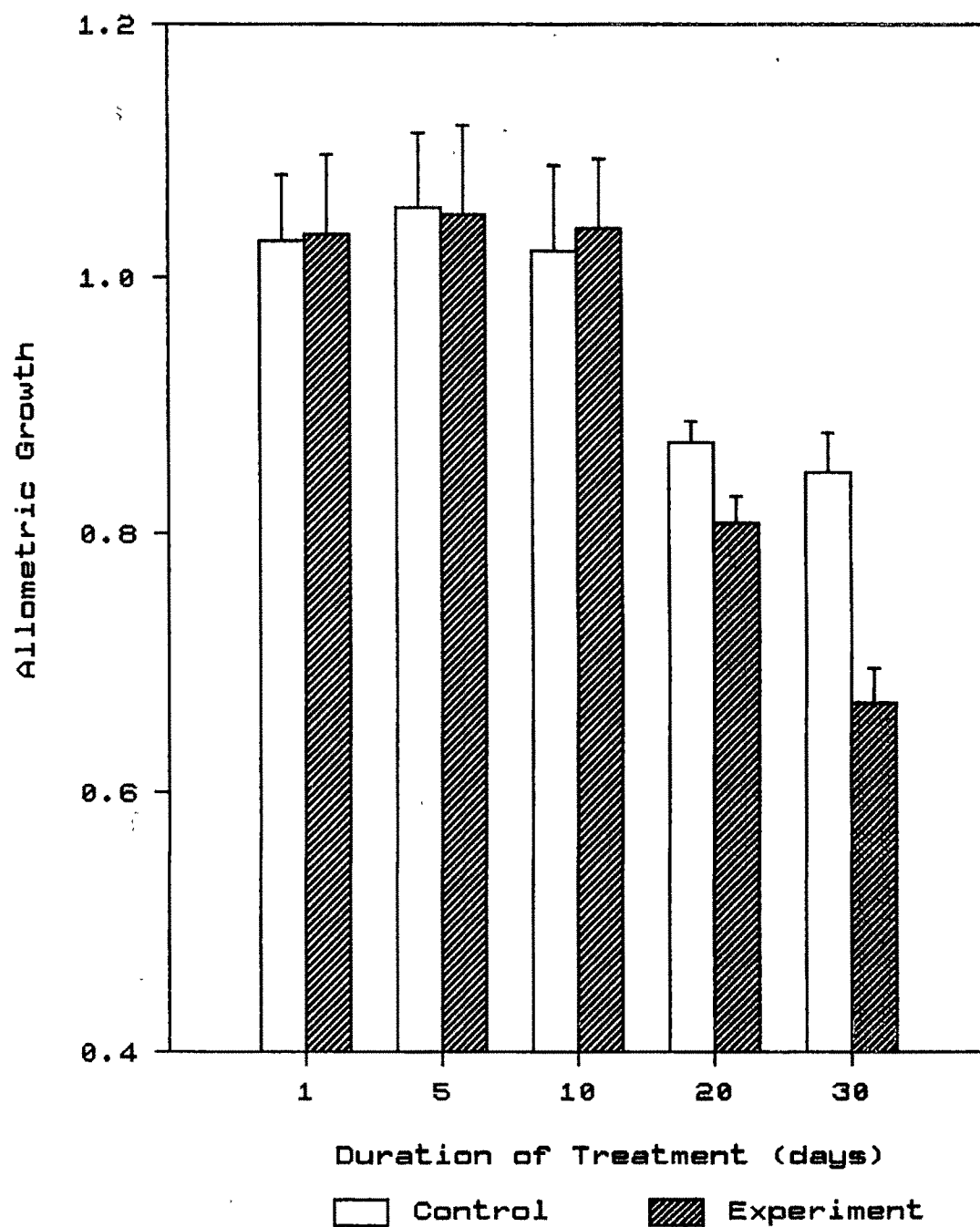


Fig. 8 . Allometric Growth of Liver



DISCUSSION

Chicks subjected to sublethal dose of sodium fluoride exhibited obvious reduction in growth of the body. Growth retardatory effect of fluoride has been well documented. Hampered regenerative growth of caudal fin was recorded in fish exposed to fluoridated water (Shaikh, 1985). Oral administration of sodium fluoride was found to curtail tail growth in autotomized house lizard (Suresh and Hiradhar, 1990a). Pankhurst *et al.* (1980) have observed significant reduction in growth of *Artemia* exposed to fluoride levels as low as 5 ppm fluoride. Moore (1971) recorded 52% reduction in the final average size of blue crab exposed to 20 ppm fluoride. Deficient growth accompanied by skeletal and dental lesions were noticed in sheep and cattle exposed to high concentration of fluoride (Velu, 1932). Reduction in body weight was also noticed in rats subjected to subacute dose of sodium fluoride (Pillai *et al.*, 1989b).

Retarded body growth observed in the experimental birds might be a reflection of direct toxicity of fluoride. Several *in vitro* studies have proved that fluoride inhibits mitotic activity in cell and organ cultures (Berry and Trillwood, 1963; Proffit and Ackerman, 1964). It was also noticed that fluoride inhibits cellular proliferative activity in chick (Chapter 4). Hence, it is possible that fluoride induced inhibition of mitotic activity might be one of the reasons for stunted growth observed in the experimental birds. Moreover, it is well known that an organism needs energy for its growth and development. The chief source of energy is carbohydrates. Therefore, it is apparent that any alteration in the carbohydrate metabolism leads to defective growth. From chapter 6 it is clear that fluoride severely altered the energy yielding metabolic activities in postnatal chicks. This could additionally hamper the progress of body growth in fluoride treated birds.

Yet another reason for defective growth observed in the experimental birds might be because of decreased food intake due to possible anorexia brought about by fluoride. In the present study it was observed that the amount of food in the gizzard of fluoride treated birds was relatively less than that of the control birds. This indicates lowered food intake by fluoride intoxicated chicks. This finding is in agreement with that of Suttie (1968), who also observed anorexia and inadequate nutrition in animals subjected to sublethal dose of fluoride.

The chicks at the early days of post-hatched development depend mainly on vitelline residue for energy requirement (Murakami *et al.*, 1988; Nitsan *et al.*, 1991). Later the bird gets adapted to exogenous food. This is associated with dramatic increase in gastrointestinal tract (Murakami *et al.*, 1988). Hence, the drop in allometric growth of the organs of supply viz., small intestine, pancreas and liver, observed in the fluoride treated birds certainly curtails the progress of body growth. Moreover, along with the increase in weight of the digestive organs, it is essential to the secretory activity of the pancreas to achieve maximal growth at the early age (Corring and Bourdon, 1977). Fluoride is known to inhibit cellular synthetic activity (Chapter 4). Hence, it is also possible that the exogenous fluoride might inhibit secretory activity of pancreas. Lack of pancreatic enzymic hydrolysis in the intestinal lumen could decrease the apparent digestibility of the dietary compounds and in turn reduce body growth.