CHAPTER IV

Effect of Sodium Fluoride on Nucleic Acids and Protein Profiles in the Tissues of Growing Chick, *Gallus gallus domesticus*.

The type and quantity of pollutants generated by man's activities are a function of population growth, urbanization, industrial and commercial development, changing technologies and consumption of goods.

Among various pollutants, fluoride has been listed fairly high in a series of high priority air pollutants (Shaikh, 1985). Fluoride emitting (airborne) and effluent releasing (waterborne) industries exist in the outskirts of Baroda and the chief recipient of the effluents is the Mahi estuary. Marine and estuarine animal species seem to have the capacity for fluoride accumulation (Groth,1975; Shaikh, 1980). Significant skeletal storage of fluoride has been reported in fishes (Groth, 1975; Minocha,1980). Once fluoride enters the food web it gets distributed in the ecosystem. Fluoride accumulates at easily measurable levels in skeletal tissues and it also gets into soft tissues. The distribution of fluoride in the body has been shown to conform to some extent to the pattern of distribution of calcium. Due to the high affinity of fluoride for apatite, calcified tissues acquire by far the highest concentration of fluoride of all tissues (Call *et al.*, 1965).

The accumulation of fluoride in the animal system cause obvious derangement in the metabolic machinery which is interlinked with the growth and function of the cell and tissues. *In vitro* studies conducted by Berry and Trillwood (1963) have proved that fluoride inhibits cell growth and function. Addition of 30 ppm fluoride to organ culture of rapidly growing bone is known to inhibit mitotic index and suppresses DNA synthesis (Proffit and Ackerman, 1964). Capacity of fluoride ions to inhibit protein synthesis has also been suggested (Helgeland, 1976). However, even though the cytotoxic effect of fluoride on cell proliferation and DNA synthesis has been established in cell and tissue cultures, there is apparent lack of evidence on similar effect of fluoride in animal system. Therefore, the present experiment was aimed

at understanding the effect of chronic fluoride poisoning on nucleic acids and protein profiles in the liver and small intestine of growing chicks of domestic fowl *Gallus gallus domesticus*.

MATERIAL AND METHODS

Female Rhode Island Red chicks of a day old were purchased from Government Hatchery. Feeding and management were as previously described (Chapter 3). Six chicks selected at random from both control and experimental groups were killed by decapitation at 1, 5, 10, 20 and 30 day of experiment. For initial biochemical values six birds were sacrificed on the day of procurement. Left liver lobe and a piece of small intestine, immediately following duodenum were removed quickly after death. Known quantity of tissues were homogenized in chilled 10% perchloric acid and distilled water for nucleic acids and protein assays respectively.

Analytical Methods

Nucleic acids were extracted by employing the method of Schneider (1957).

Deoxyribonucleic acid (DNA) was estimated using the improved diphenylamine method of Gilles and Myers (1965).

Ribonucleic acid (RNA) was assayed according to Mejbaum (1939).

Total protein was estimated according to the method of Lowry *et al.* (1951).

Statistics

The data in text and figures are presented as mean ± SEM of six observations and their statistical significance were evaluated using Student's 't' test.

RESULTS

Deoxyribonucleic acid

Liver: The mean DNA content in the liver of control chicks registered a chronological increase. However, the DNA level in the liver of experimental birds increased only upto day 10 of fluoride administration. Thereafter the level of DNA showed signs of decline (Table I). Compared to control chicks significant ($p < 0.001$) reduction in the DNA content was obvious in fluoride poisoned birds on days 20 and 30 of experimental regimen (Figure 1).

Small Intestine: From table 2 it is apparent that the average DNA content in the small intestine of control chicks follow the pattern akin to that of liver. Fluoride adversely affected the DNA content in the small intestine by day 20 of experiment. Further intoxication led to more reduction in DNA content (Figure 2).

Ribonucleic acid

Liver: The level of RNA in the liver of control chicks fluctuated at different stages of experimental regimen. A parallel change in the RNA content was quite obvious in the liver of experimental birds (Table I). However, compared to controls the mean RNA content in the liver of fluoride treated birds registered a significant ($p < 0.05$) fall by day 20 of experiment. More significant ($p < 0.001$) reduction in RNA content was observed in the liver of fluoride treated birds on day 30 of experiment (Figure 3).

Small Intestine: The average RNA content in the small intestine of control birds remained almost constant throughout the experimental regimen, except on day 10 and 30 where increase in RNA content was quite pronounced. As compared to control chicks the experimen

TABLE I : Fluoride induced alterations in the cellular proliferative and synthetic activities in the chick liver.

Parameters studied	Duration of Treatment (Days)										
	0	1		5		10		20		30	
		Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp	Con.	Exp
DNA (mg/g/tissue)	1.17±0.06@	1.19± 0.07	1.17± 0.06	1.67± 0.12	1.62± 0.11	1.68± 0.09	1.68± 0.09	1.86± 0.05	**** 1 43± 0 05↓	1.97± 0.05	**** 1.45± 0.04↓
RNA (mg/g Tissue)	0.53±0.05	0.54± 0.07	0.53± 0.08	0.45± 0.06	0 47± 0.07	0 82± 0.08	0.84± 0.06	0.68± 0.05	* 0.51± 0.04↓	1.32± 0.04	**** 0.96± 0.04↓
Protein (mg/100 mg tissue)	14.88±0.83	15.01± 0.99	14.98± 0.67	18 56± 1 31	17.25± 1 47	17.67± 0.75	17.39± 1.34	21.98± 0.83	*** 17 12± 0.71 ↓	22.11± 0.94	**** 15.23± 1.16 ↓
DNA/RNA	2.14±0.15	2.17± 0.14	2.19± 0.16	3.45± 0.21	3.64± 0.27	2.07± 0.15	2.09± 0.17	2.74± 0.18	2.76± 0.20	1.49± 0.17	1.51± 0.14
RNA/Protein	0.0039± 0.0002	0.0039± 0.0002	0.0036± 0.0003	0.0024± 0.0002	0.0025± 0.0002	0.0046± 0.0003	0.0046± 0.0003	0.0031± 0.0002	0.0032± 0.0003	0.0059± 0.0002	* 0.0066± 0.0002↓

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

TABLE II : Fluoride induced alterations in the cellular proliferative and synthetic activities in the chick small intestine.

Parameters studied	Duration of Treatment (Days)										
	0	1		5		10		20		30	
		Con.	Exp.	Con.	Exp.	Con.	Exp.	Con.	Exp	Con	Exp
DNA (mg/g tissue)	1.77±0.07[@]	1.76± 0.08	1.78± 0.09	2 25± 0.10	2 13± 0 11	2 77± 0 11	2.76± 0.13	2.82± 0.08	**** 2.33± 0.06↓	2.88± 0.10	*** 2.22± 0.15↓
RNA (mg/g Tissue)	0.57±0.03	0.57± 0.02	0.57± 0.03	0 40± 0.02	0.39± 0.03	1.01± 0 04	1.03± 0.05	0.56± 0.02	**** 0.41± 0 02↓	0.76± 0.03	**** 0.54± 0.03↓
Protein (mg/100 mg tissue)	11.22±0.91	11.20± 1.23	11.32± 1.13	12.71± 0 87	12.31± 1.34	15 22± 0.98	14.84± 1.34	14.37± 1.03	* 11.17± 0.92 ↓	15 71± 1.22	*** 09.74± 0.93 ↓
DNA/RNA	3.12±0 24	3.28± 0.26	3.10± 0.25	5 62± 0.21	5.39± 0.32	2 71± 0.19	2.68± 0.20	5.07± 0.25	5.05± 0.34	3.75± 0.19	3.76± 0.24
RNA/Protein	0.0052± 0.0003	0.0051± 0.0002	0.0052± 0.0002	0.0032± 0.0002	0.0032± 0 0003	0 0067± 0.0002	0.0069± 0.0003	0.0037± 0.0001	0 0037± 0.0002	0.0049± 0.0001	*** 0.0056± 0.0002↑

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

Fig. 1. DNA Content in Liver

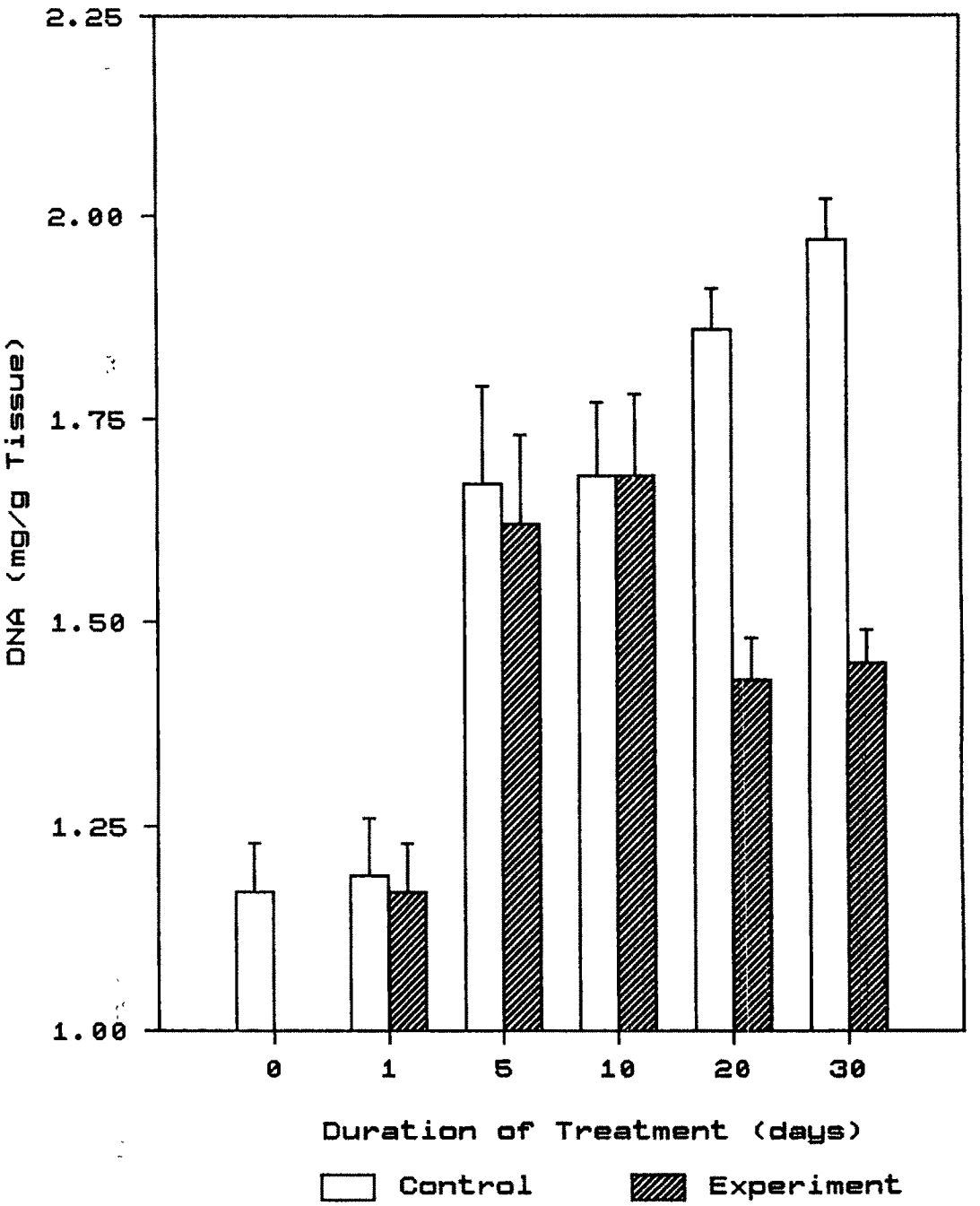


Fig. 2. DNA Content in Intestine

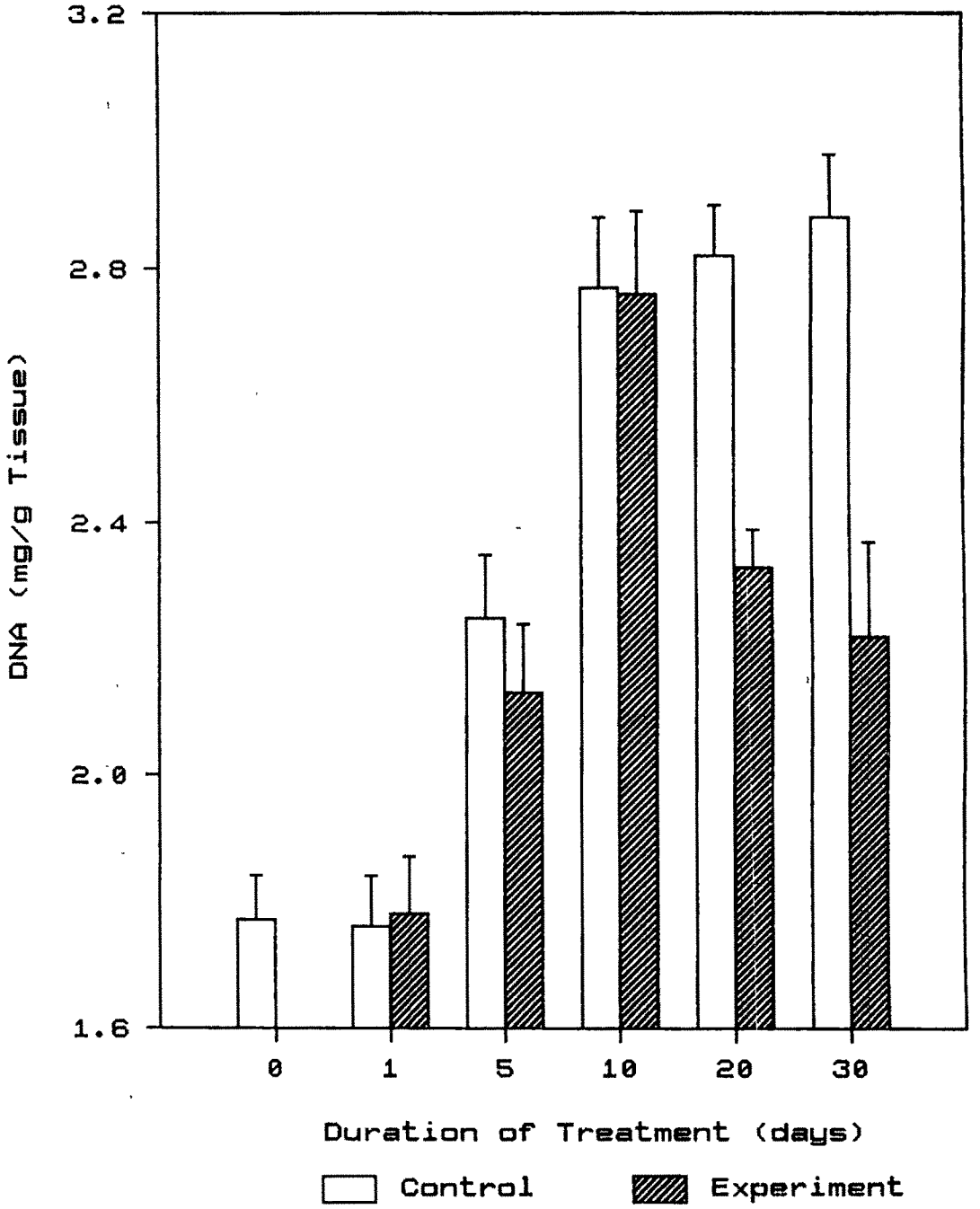


Fig. 3. RNA Content in Liver

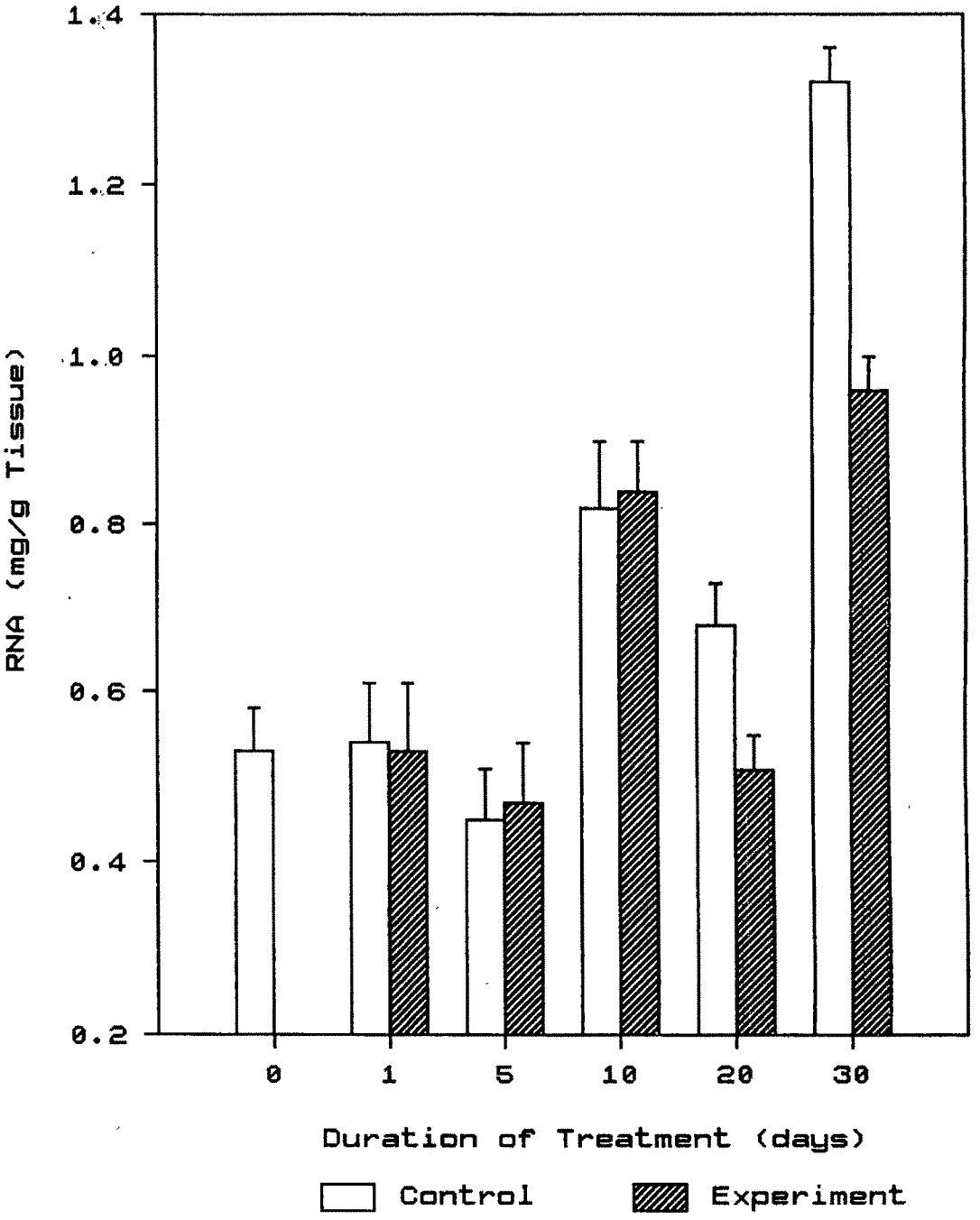


Fig. 4. RNA Content in Intestine

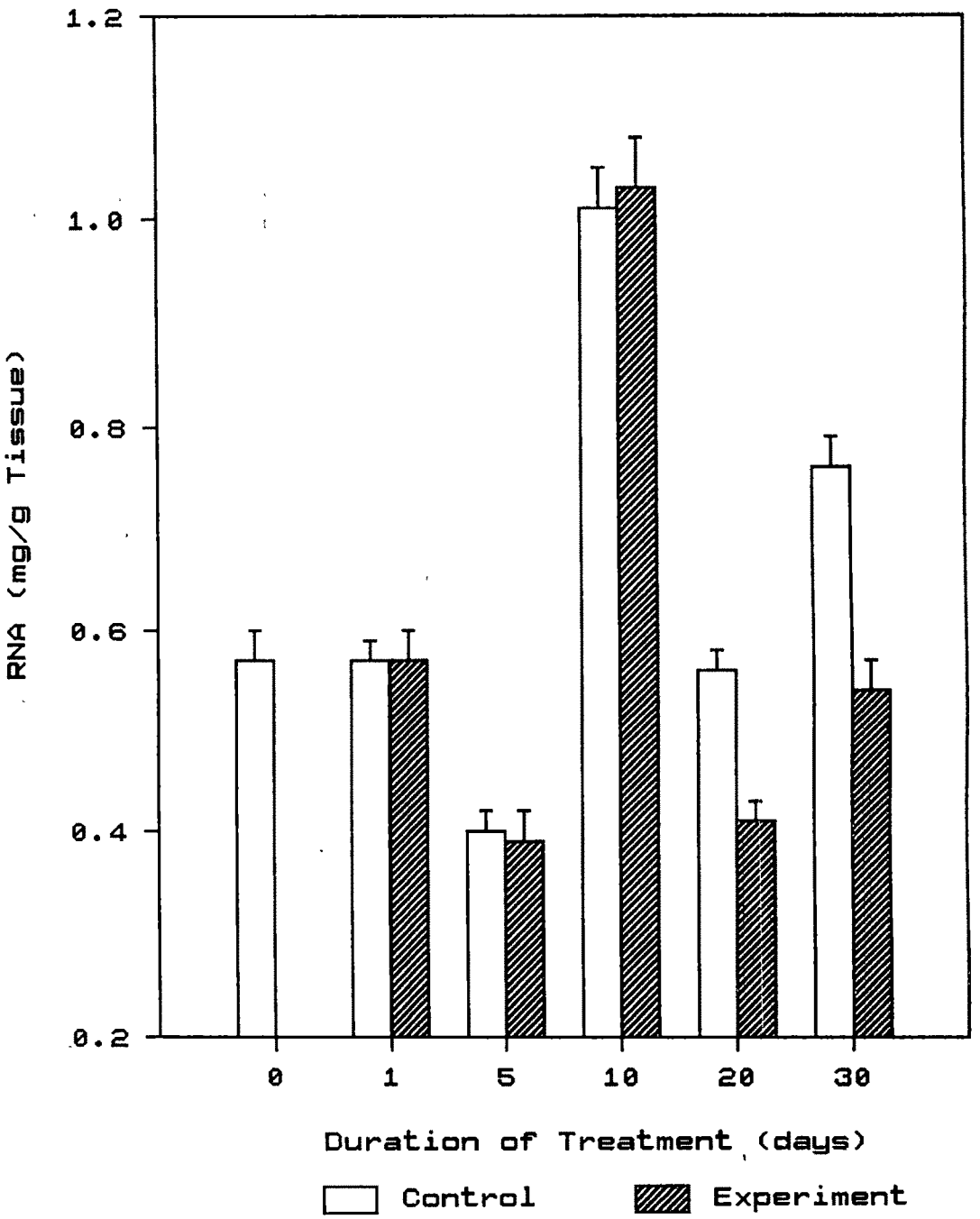


Fig. 5. Protein Content in Liver

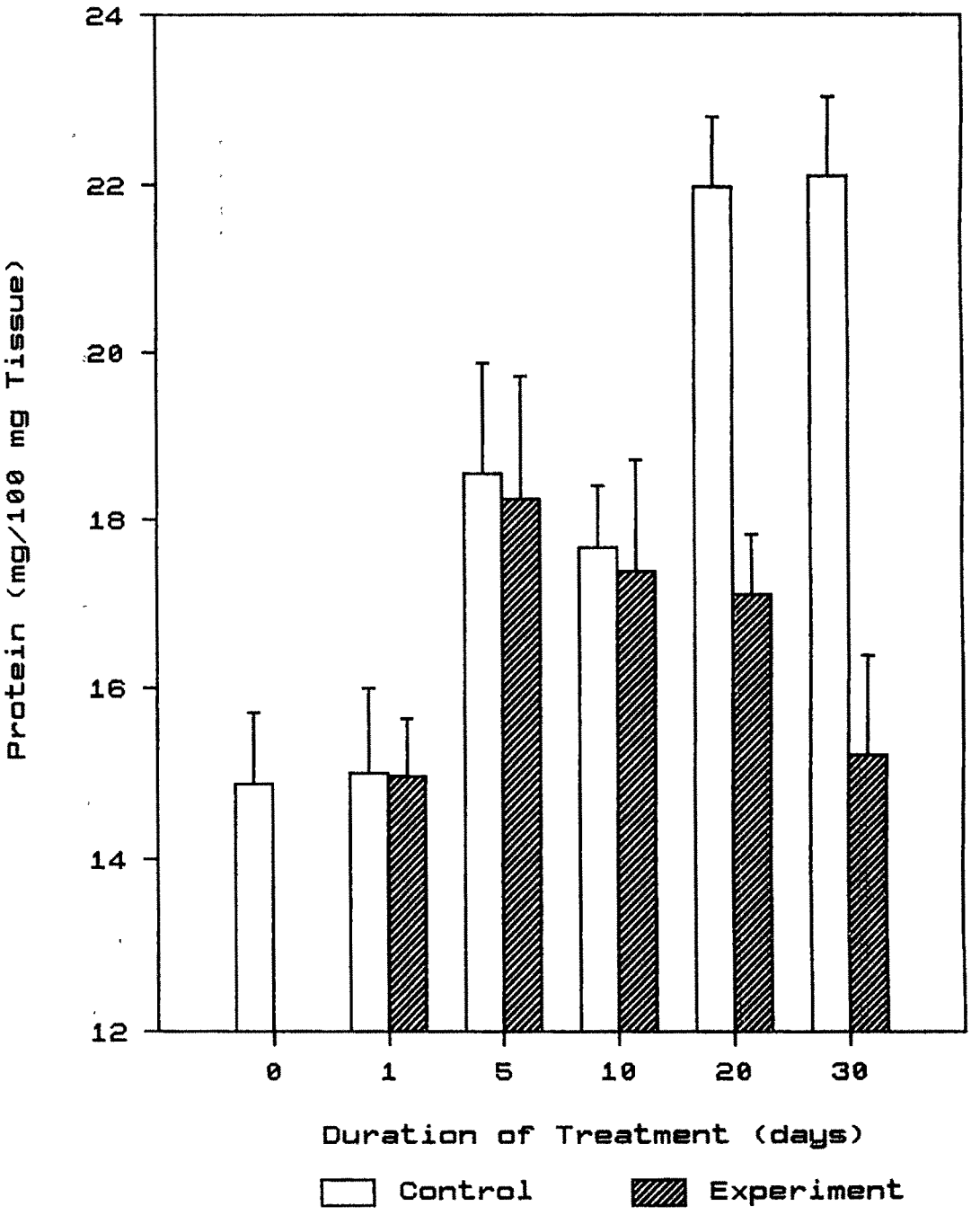


Fig. 6. Protein Content in Intestine

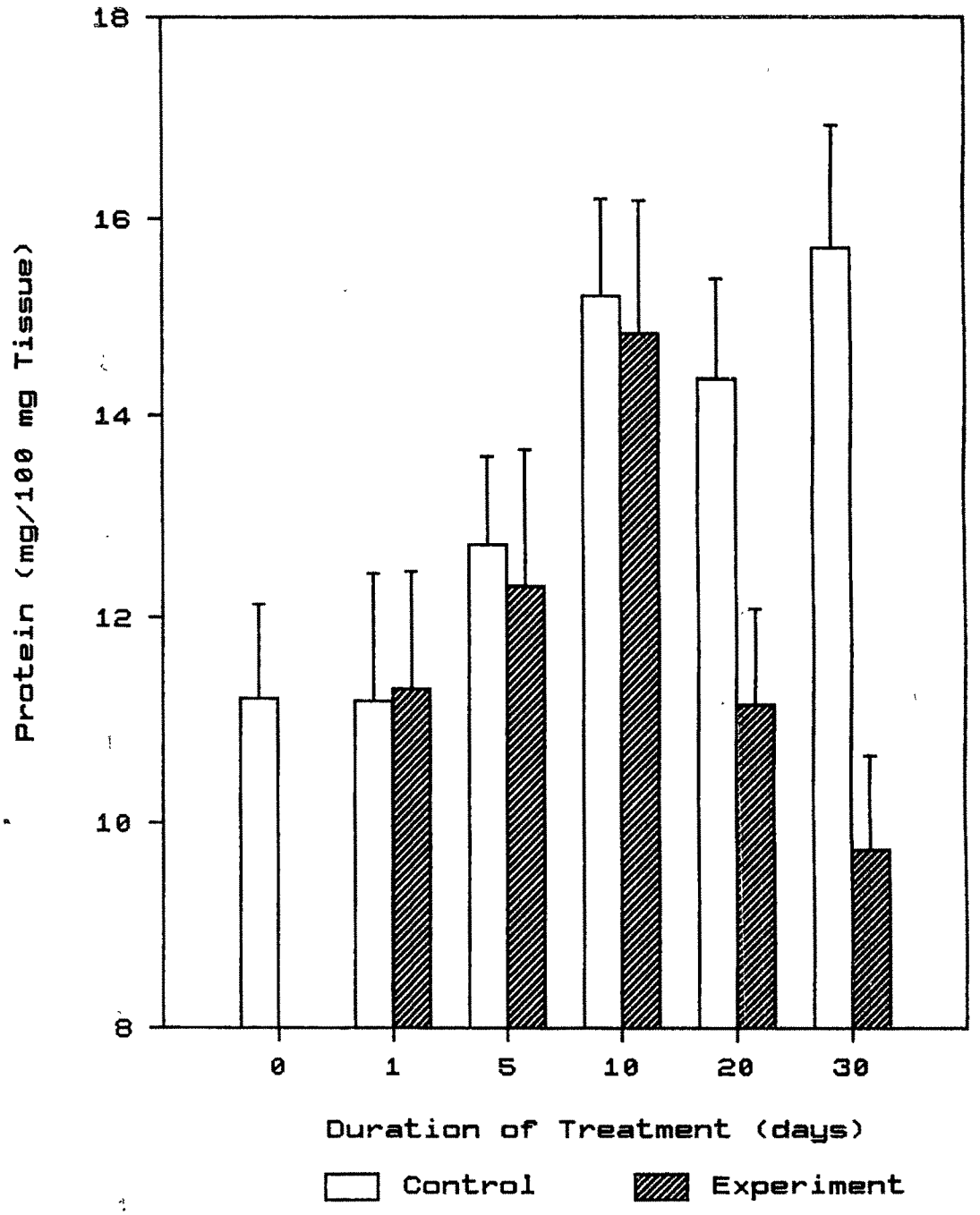


Fig. 7. DNA to RNA Ratio in Liver

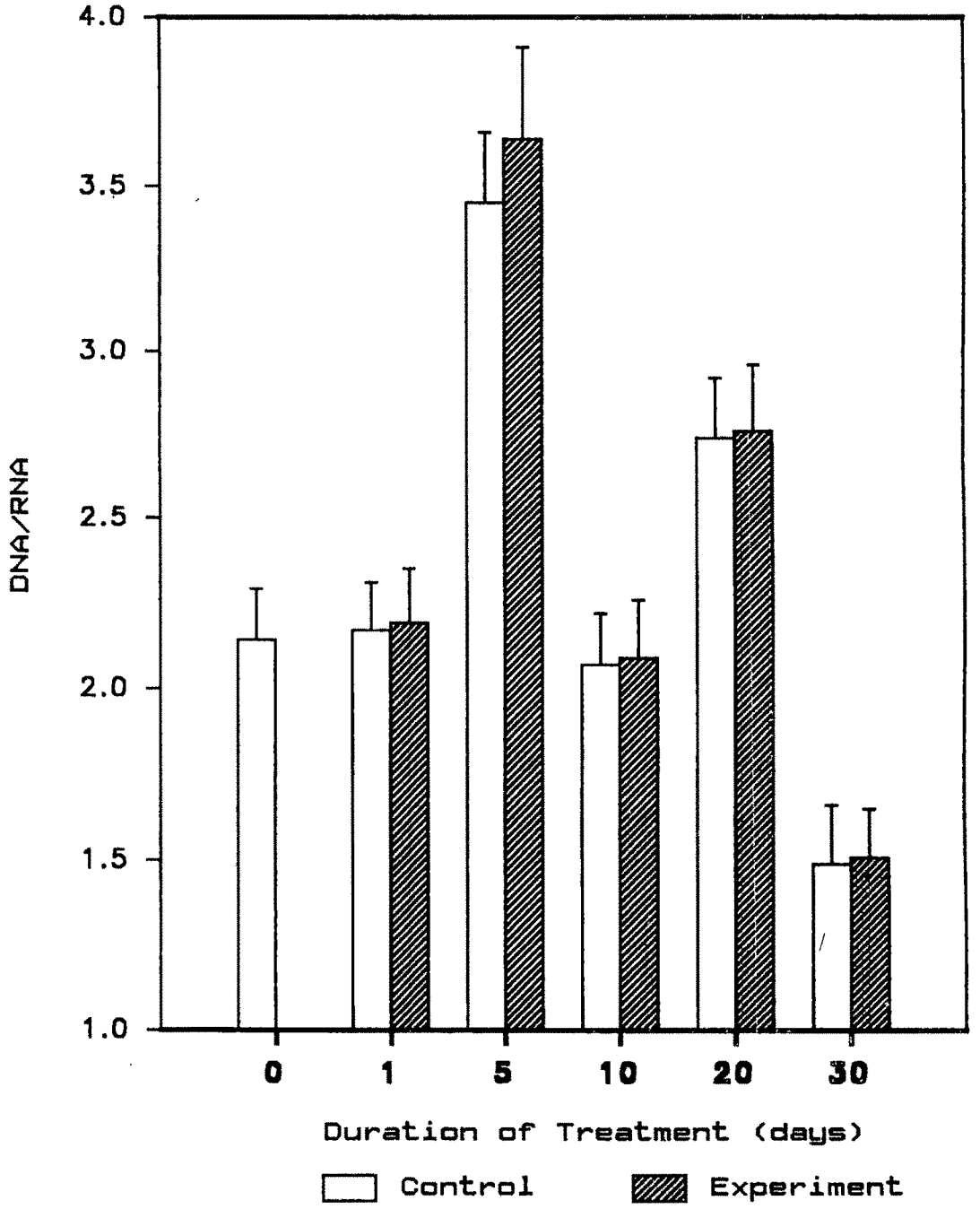


Fig. 8. DNA to RNA Ratio in Small Intestine

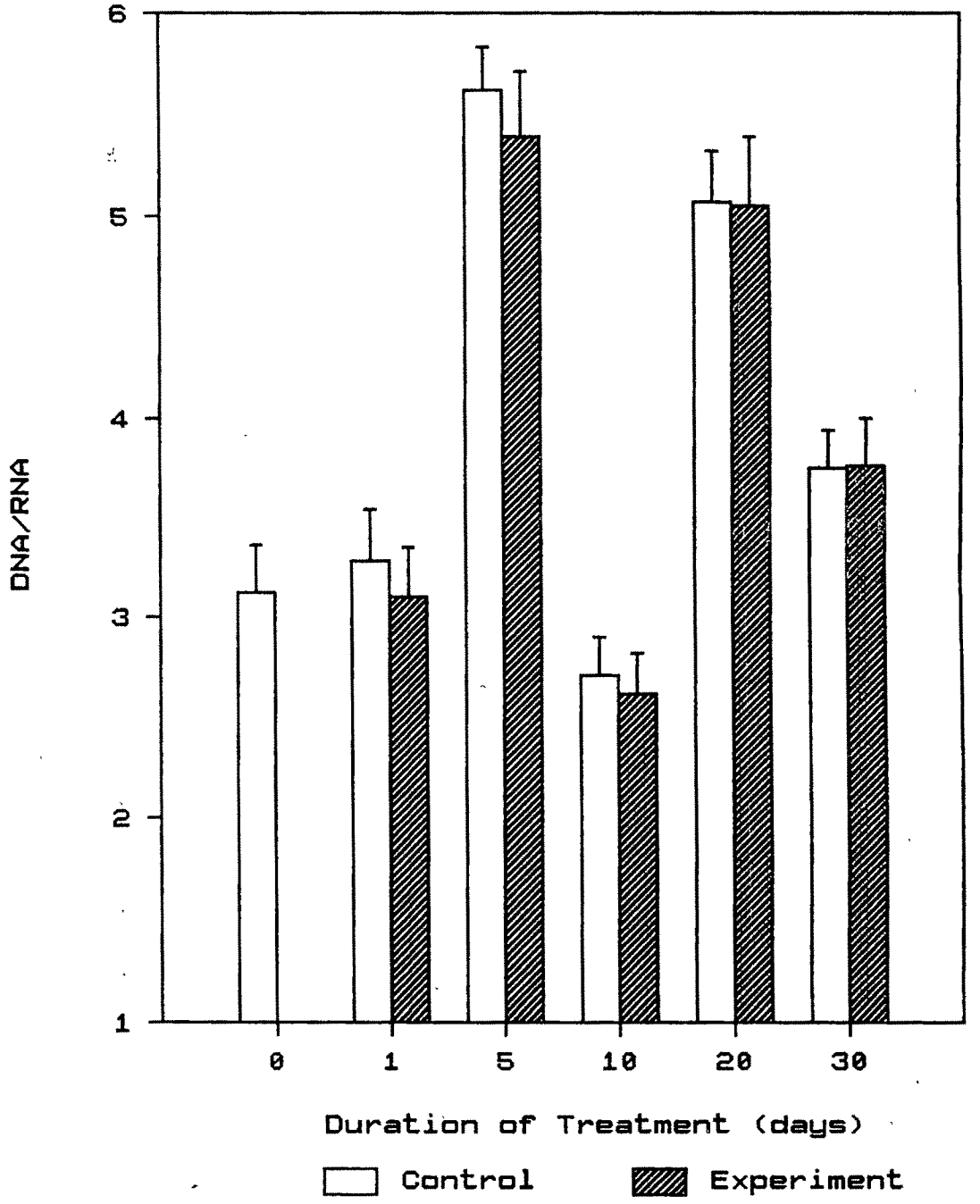


Fig. 9. RNA to Protein Ratio in Liver

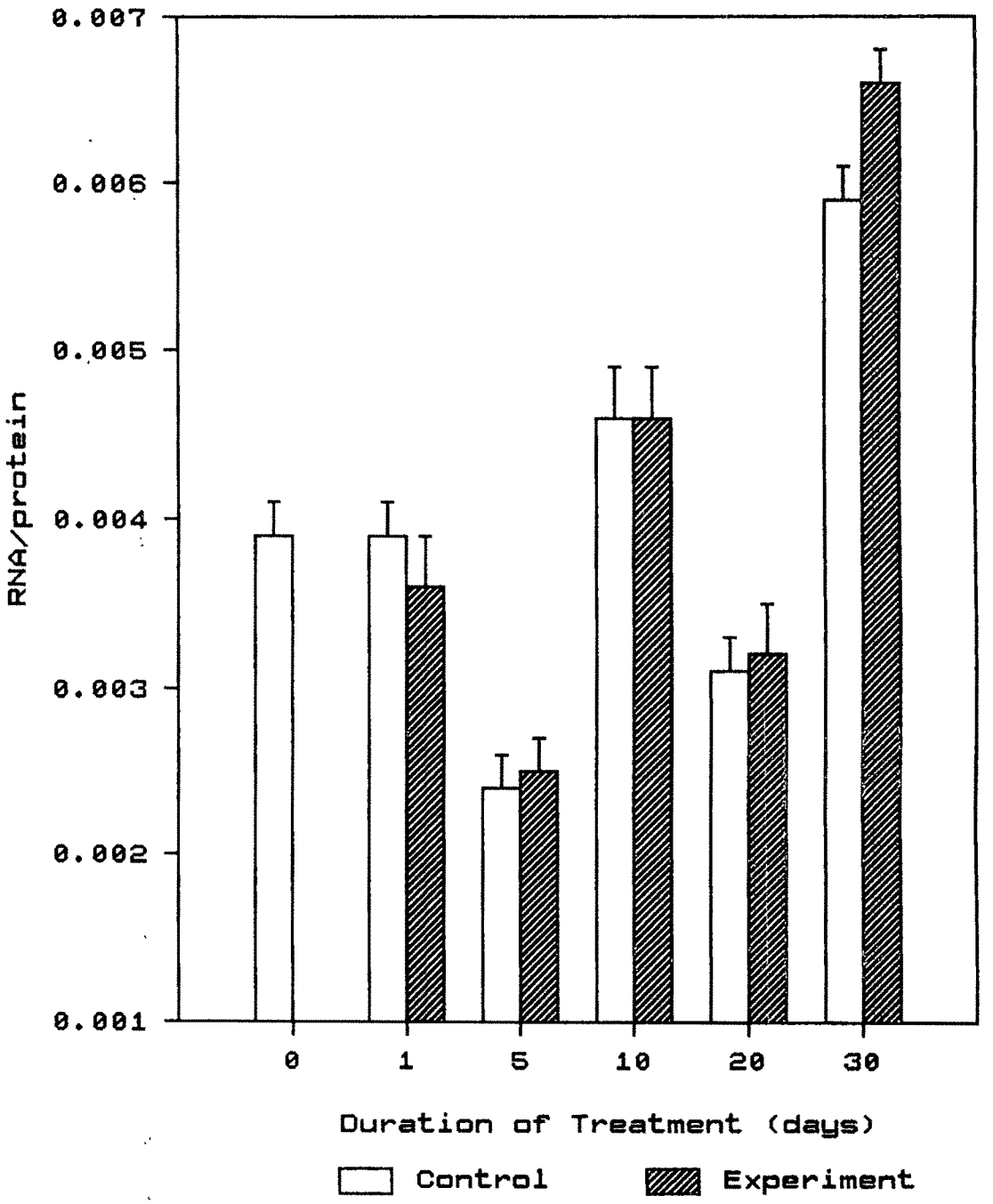
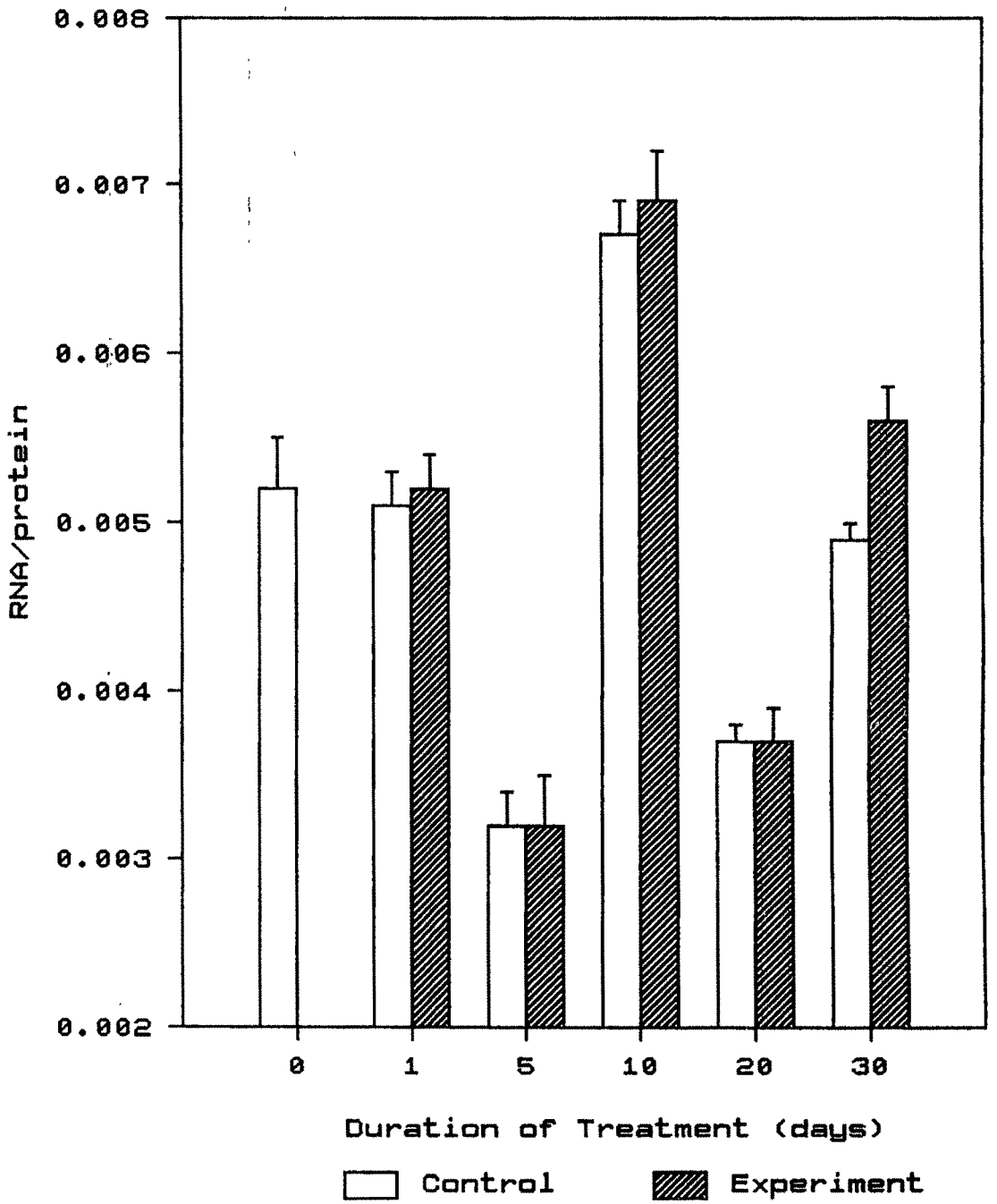


Fig. 10. RNA to Protein Ratio in Intestine



tal birds showed significant ($p < 0.001$) reduction in RNA content on day 20 and 30 of experiment (Figure 4).

Protein

Liver: It is clear from table I that the mean protein content in the liver of control chicks increased with the age of bird. Fluoride intoxication however, reduced the protein content in the liver by day 20 of treatment schedule. The difference in the level of protein between control and experimental birds was more pronounced in the last phase of fluoride treatment (Figure 5).

Small Intestine: Fluoride seems to have adversely affected the protein profile in the small intestine by day 20 of intoxication (Table II). Further reduction in protein content was observed in chick which received subacute fluoride for 30 days (Figure 6).

DISCUSSION

Elsewhere in the present study (Chapter 7), it was observed that chronic fluoride poisoning leads to cholinergic dysfunction. The vagal influence on DNA synthesis has been well established. Shimazu (1983) observed hampered DNA synthesis in regenerating liver following subdiaphragmatic vagotomy. Cholinergic denervation (vagotomy) is known to delay and suppress hepatic DNA synthesis in rats (Tanaka *et al.*, 1987). Hence it is possible that fluoride induced parasympathetic neuropathy might be one of the reasons for reduced DNA turnover.

Moreover, fluoride is also known to inhibit DNA synthesis directly. Berry and Trillwood (1963) have recorded that sodium fluoride at higher concentrations decreases the rate of cell division in cell line cultures. Other reports with similar conclusions were later confirmed by Albright (1964), Hongslo *et al.*(1974), Holland (1979) and Imai *et al.* (1983). Sodium fluoride

also inhibits DNA synthesis in isolated nuclei (Proffit and Ackerman, 1964) and in cultured lymphocytes (Jain and Susheela, 1987). Imai *et al.* (1983) argued that fluoride induced inhibition of proteins, which are essential for the replication of DNA could be the reason for reduced DNA turnover.

Chicks which received subacute sodium fluoride also showed lower level of total proteins than control, thus indicating depletion and derangement of synthetic machinery. It has been found that fluoride has the capacity to bind plasma protein and intracellular protein (WHO, 1970). Several *in vitro* studies have confirmed that fluoride inhibits protein synthesis in Landshutz ascites (Hogan, 1969). Reduction in protein content due to inhibitory action of fluoride on protein synthesis has been reported in tissue pieces (*in vitro*) *eg* dental pulp tissues (Helgeland, 1976), islets of Langerhans from rat (Lin *et al.*, 1976) and bone (Proffit and Ackerman, 1963). Kathpalia and Susheela (1978) reported that rabbits given sublethal amount of fluoride doses showed reduction in protein content of various tissues. Shaikh (1985) has observed lowered protein content in liver and muscle of fish exposed to fluoridated water. Marks *et al.* (1963) found that fluoride could inhibit the protein synthesis in reticulocytes. Fluoride has the ability to inhibit protein synthesis even in cell-free systems (Lin *et al.*, 1966; Hoerz and McCarty, 1971).

However, it is well established that the inhibition of protein synthesis could take place either through defective transcription or through deranged translatory process. In the present study, even though the level of RNA was reduced by day 20 of fluoride administration, DNA/RNA ratio remained unaltered throughout the experimental regimen (Figure 7and 8). This indicates that the transcription has not been affected by sodium fluoride. However, the hike in RNA/protein ratio on day 30 of experiment (Figure 9 and 10), points to possible derangement in the translatory process. Marks *et al.* (1965) have shown that the sodium fluoride induced inhibition of protein synthesis in reticulocytes is associated with a pro-

gressive dissociation of the polyribosome. Hence, it is possible to surmise that the inhibition of protein synthesis by fluoride is probably due to the inhibition of new peptide chain and the dissociation of ribosome.