

## CHAPTER 9

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**Influence of transient (90 days) hypo./hypercorticalism in RIR pullets on histomorphology and hormones of adrenal, thyroid and ovary and growth kinetics of liver and lymphoid organs.**

### **Introduction**

In the post-hatch avian growth and development, the various organs and, the body as a whole, undergo both physical growth and physiological maturation, to attain the characteristic adult size, histoarchitectural features and functional competence. The role of endocrine secretions in regulating growth and functional maturity in the post-hatch immature phases cannot be overlooked. The growth retardatory effects manifested by hypophysectomy in cockreals (King, 1969), and by thyroidectomy in ducks and fowls (see Assenmescher, 1973), are evidences to this end. There are also reports suggesting the influence of adrenal steroids on growth and development of fowls with both hypercorticalism and hypocorticalism being shown to inhibit weight increase in the post-hatch periods (Blivaiss, 1947; Winchester and Davis, 1952; Howard and Constable, 1958; Baum and Meyer, 1960; Nagra *et al*, 1963; Nagra and Meyer 1963; Nagra *et al.*, 1965; Raheja *et al.*, 1971; King and King, 1973; Kallicharan and Hall, 1974; Carasia, 1987; Bartov, 1982; Kuhn *et al.*, 1984; Akiba *et al.*, 1992;

Hayashi *et al.*, 1994). Both antagonistic and parallel adrenal-gonad relationships have been documented for adult birds ( Riddle *et al.*, 1924; Legait and Legait, 1959; Fromme-Bouman, 1962; Patel *et al.*, 1986; Ramachandran and Patel, 1986; Ramachandran *et al.*, 1987; Ramachandran and Patel, 1988; Ayyar *et al.*, 1992). However, the influence of either hypo. or hypercorticalism in cockreals and pullets on growth and development of gonads and attainment of sexual maturity have never been studied. Besides, an effective functional relationship between corticosteroids and thyroid hormones has been shown to be the feature both in immature and adult stages (Kuhn *et al.*, 1984) and, both these hormones had been shown to affect gonadal functions and influence the reproductive axis (Ayyar *et al.*, 1992; Patel *et al.*, 1985; Ramachandran and Patel, 1986; Patel *et al.*, 1993; Singh, 1993). It is apparent from these, that chronic mild HPR or HPO in the rearing stages would have subtle or dramatic effects on growth and maturation of various organs, histomorphological features of ovary and oviduct, as well as the serum profiles of various hormones.

Studies on temporal alterations in these respects in the immature stages, from hatch till sexual maturity, could prove relevant in assessing features like, time of attainment of sexual maturity and egg laying performance. Previous studies have revealed some qualitative and quantitative effects in this respect under induced chronic mild HPR or HPO in pullets upto 90 days of age (Dandekar, 1998; chapters 3, 4, 6 and 7). It is likely that, the above observed changes in the adult stage could be a consequence of the changes induced by HPR or HPO on histomorphology of the endocrine and reproductive organs and, the alterations in the serum profiles of other hormones in the immature stages. The present study is attendant to this line of thinking and attempts to assess the influence of

mild HPR or HPO in pullets upto 90 days of age, on growth kinetics and histomorphology of adrenal, thyroid ovary and oviduct as well as the serum profiles of corticosterone,  $T_3$ ,  $T_4$ , and progesterone during the experimental period and, possibly relate these changes with the previously reported effects on attainment of sexual maturity, laying performance and egg composition.

## Material and methods

As detailed in chapter 1

## Results

### *Body and organ weights :*

The weights of adrenal, thyroid and ovary and of the body as a whole (fig1,2a,b), showed a steady increment from 0-90 days, with a peak growth rate between 60 and 90 days in the control chicks. The HPO chicks showed greater growth rate and heavier body weights at 30 and 60 days but, at 90 days, their body weight was significantly lower due to a significantly reduced growth rate between 60 and 90 days (Table 1). The adrenal, thyroid and ovary of HPO chicks depicted significantly greater growth rates throughout, and hence, their weights were higher than those of the control chicks. Whereas the adrenal and thyroid of HPO chicks showed peak growth rates between 30 and 60 days, the ovary showed peak growth rate between 60 and 90 days. The oviduct of HPO chicks showed similar weight as that of control chicks at 90 days though with a higher growth rate and weight at 60 days. The body weight of HPR chicks

was similar to that of control chicks at 90 days, though it was significantly less at 60 days. The peak growth rate in HPR chicks occurred between 60 and 90 days with lesser rate between 0-30 and 30-60 days. The weight of adrenal was significantly lower at 30 days and greater at 60 days but identical to controls at 90 days, due to differential growth rates. The thyroid of HPR chicks showed consistently better growth rates and higher weights throughout. The ovary of HPR chicks showed consistently increasing growth rates compared to controls, with significantly higher weight at 60 and 90 days. The oviduct of HPR chicks weighed slightly heavier at 90 days due to more pronounced growth rate between 0-30 and 60-90 days. The growth kinetic ratio was higher for thyroid, adrenal and ovary in the case of HPO chicks due to significantly greater ratios throughout. In the case of HPR chicks, growth kinetic ratio was significantly more only in the case of ovary mainly due to significantly higher ratio between 0-30 and 30-60 days (Table 1,3). The growth kinetic ratio was higher for thyroid, adrenal and ovary in case of HPO chicks due to significantly greater ratios throughout. In the case of HPR chicks, growth kinetic ratio was significantly more only in the case of ovary, mainly due to significantly higher ratios between 0-30 and 30-60 days (table 3).

The absolute and relative weights of liver and lymphoid organs of HPR and HPO pullets showed a significant increment at 90 days (table 2) (fig. 2c,d). The growth rates and growth kinetic ratios of liver and lymphoid organs also showed an increment at 90 days (table 4).

#### *Hormonal changes :*

The CORT and  $T_3$  concentrations showed a similar trend of decrease from 30-90 days in both control and HPR chicks, with maximum decrease at 60 days. Whereas the concentration of CORT was higher in

HPR chicks, the concentration of  $T_3$  was lower in HPR chicks. Whereas the serum  $T_4$  concentration showed almost a constant level from 30-90 days in control chicks, the same showed an increase in HPR chicks. The relative concentration of  $T_4$  appeared to be higher at 30 and 90 days and lower at 60 days in HPR chicks compared to control chicks. The serum CORT concentration in HPO chicks was lower than control chicks at all ages and showed a similar trend of significant decrease at 60 days. Though the serum progesterone concentration showed a similar trend of decrease at 60 days followed by a slight increase at 90 days in both control and HPR chicks, the relative levels at all ages was significantly lower in HPR chicks. The serum progesterone concentration in HPO chicks showed a continuous decrease from 30-90 days, unlike the control chicks which showed a decrease at 60 days and increase at 90 days. In general, the relative levels of serum progesterone were significantly lower in HPO chicks (Table 5a) (fig. 3). The absolute and relative weights of liver in HPR and HPO chicks showed an increment at 30, 60 and 90 days, whereas the lymphoid organs (thymus, bursa and spleen) showed differential changes at 30 and 60 days followed by increased weights at 90 days. The overall growth rates and growth indices of liver and lymphoid organs were higher in HPR pullets throughout the period of study (Table 2,4).

#### *Histological observations :*

*Thyroid :* The thyroid of NLD chicks showed medium sized follicles with cuboidal epithelium and varying contents of colloid at 30 days. At 60 days, the epithelial cell height was reduced and the follicles showed increased colloid content. By 90 days, the follicles were enlarged with rich colloid content and reduced cell height. The follicles of HPR chicks at 30 days was characterised by small to medium sized follicles lined with cuboidal epithelium and depleted colloid content. However, at 60 and 90

days, the epithelial cell height gradually got reduced with progressive retention and increase in colloid content. The thyroid of HPo chicks also showed prominent follicles with depleted colloid content and cuboidal epithelium at 30 days. Cell height was reduced and only fewer follicles depicted colloid depletion at 60 days. At 90 days, the follicles were medium to large sized with moderate colloid depletion (Plate 1).

*Adrenal* : The adrenal of 30 day old NLD chicks, showed prominent active cortical cords with relatively less but active medullary cords. At 60 days, the cortical cords appeared very prominent but less active with condensed nucleus. There were signs of medullary activation. By 90 days, the cortical cords were well formed with active looking cells and depicting secretory exhaustion. The cortical cords of HPR chicks were hypertrophied with lesser proportion of medullary tissue at 30 days. At 60 days, both cortical and medullary cords were prominent with hypertrophied cortical cells and, greater secretory exhaustion being seen in the medullary cells. At 90 days, the cortical cords were prominent with hypertrophied epithelium and the cells represented an admixture of active and inactive states. The cortical cords of HPO chicks were prominent and hypertrophied throughout. There was relatively more secretory exhaustion of cortical cells at 60 days than at 30 and 90 days while, at 90 days, even the medullary cells showed active state (Plate 2).

*Ovary* : At 30 days, the ovary of NLD chicks showed many primordial and primary follicles. These follicles underwent progressive enlargement and growth through 60-90 days. At 60 days, the stromal tissue was hypertrophied with signs of differentiation into interstitial glands. The granulosa was prominent and thecal condensation had started by 90 days. The follicles were enlarged with well formed theca and yolk

Table 1. Changes in Body weight (in gms) and absolute and relative weights of thyroid, adrenal, ovary and oviduct (in mg) in HPR and HPO chicks under NLD

	One day old chicks	30 days		60 days		90 days	
		absolute wt.	relative wt	absolute wt.	relative wt.	absolute wt.	relative wt.
Body weight	26.63 ±3.43	117.23±16.23	--	312.85±18.22	--	600.0±16.32	--
	HPR	103.84±12.85	--	250.44±22.27	--	596.0±29.55	--
	HPO	156.0±11.54	--	380.6±23.0	--	533.3±12.47	--
Thyroid weight	4.33 ±.40	12.0±.81	10.23±1.13	19.66±1.20	6.28±.55	33.00±1.80	5.5±.83
	HPR	15.30±.93*	14.85±.94*	22.00±1.11	8.64±.50**	38.00±1.51*	6.40±.57
	HPO	14.00±.91	9.33±1.44	29.36±1.97***	7.72±.005	43.5±1.55*	8.15±.87*
Adrenal weight	12.75 ±3.92	21.50±1.64	18.33±2.08	30.60±1.24	9.78±.80	68.00±2.44	11.33±.87
	HPR	17.30±1.12*	16.79±1.53	43.16±1.49***	16.96±.61***	68.6±1.96	11.62±.68
	HPO	31.50±1.05***	21.0±2.51	52.5±1.91***	13.81±2.06*	85.50±2.06***	16.03±.67***
Ovary weight	22.66 ±4.26	34.63±4.03	28.99±3.0	65.20±2.93	20.84±1.58	116.3±9.31	19.38±.92
	HPR	43.66±4.44	42.38±3.62*	90.71±1.29***	35.65±1.96***	163.33±8.8***	27.17±1.59**
	HPO	42.00±4.91	28.00±2.30	111.66±3.03***	29.38±2.56**	232.3±11.12**	43.56±1.06***
Oviduct weight	0.89 ±0.07	21.40±2.52	18.24±1.59	57.33±3.19	18.32±.63	112.30±1.88	18.72±1.19
	HPR	28.87±2.23*	27.80±1.69**	55.00±3.01	21.61±.65*	122.7±1.76***	20.78±1.83
	HPO	22.5±2.09	15.00±1.48	58.50±2.92	15.39±1.08*	108.66±2.01	20.37±1.04

Values : Mean ± SE, n=12, \* P<.05, \*\*P<.005, \*\*\*P<.0005.

Table 2. Changes in absolute and relative weights of liver and lymphoid organs (in gms) of HPR and HPO chicks under NLD.

(Weight in gms)	one day old	30 days		60 days		90 days	
		absolute wt.	relative wt	absolute wt.	relative wt.	absolute wt.	relative wt.
Liver weight	3.75 ±0.27	C	3.48 ±.15	29.70 ±.303	6.44 ±.199	20.6 ±.129	11.41 ±.218
		HPR	3.47 ±.145	33.45 ±.165	8.93 ±.21***	35.10 ±.079***	14.73 ±.098***
		HPO	4.94 ±.47*	32.97 ±.139	7.4 ±.17	19.4 ±.132	24.98 ±.189
Thymus weight	0.193 ±0.049	C	0.392 ±.036	2.48 ±.013	1.282 ±.012	4.09 ±.008	2.12 ±.218
		HPR	0.270 ±.023*	2.62 ±.023*	1.19 ±.009	4.70 ±.010	3.34 ±.178***
		HPO	0.150 ±.034***	1.00 ±.007***	0.83 ±.011***	2.18 ±.013***	3.07 ±.211***
Bursa weight	0.083 ±0.017	C	0.148 ±.007	1.26 ±.010	0.45 ±.010	1.43 ±.019	1.56 ±.289
		HPR	0.220 ±.012***	2.16 ±.013***	0.97 ±.011***	3.84 ±.013***	3.36 ±.232***
		HPO	0.140 ±.005	0.95 ±.010	0.73 ±.020***	1.92 ±.006	2.21 ±.217*
Spleen weight	0.150 ±0.085	C	0.137 ±.019	1.16 ±.017	0.528 ±.005	1.68 ±.014	0.72 ±.021
		HPR	0.120 ±.016	1.18 ±.005	0.400 ±.012	1.57 ±.008	1.06 ±.023***
		HPO	0.156 ±.021	1.03 ±.064	0.681 ±.013	1.72 ±.025	0.96 ±.011***

Values : Mean ± SE, n=12, \* P&lt;.05, \*\* P&lt;.005, \*\*\* P&lt;.0005.



Table 3. Monthly growth rates and growth indices of body (gms) and organs (mg) of HPR and HPO chicks under NLD.

		30 days		60 days		90 days		Overall	
		Growth rate	Growth index	Growth rate	Growth index	Growth rate	Growth index	Growth rate	Growth index
Body weight	C	3.02	--	6.51	--	9.57	--	6.37	--
	HPR	2.57	--	5.02	--	11.18	--	6.25	--
	HPO	4.11	--	7.68	--	5.09	--	5.63	--
Thyroid	C	0.255	0.084	0.255	0.039	0.444	0.046	0.318	0.049
	HPR	0.366	0.142	0.222	0.044	0.534	0.047	0.374	0.059
	HPO	0.322	0.078	0.512	0.066	0.471	0.092	0.435	0.077
Adrenal	C	0.219	0.096	0.303	0.046	1.24	0.129	0.613	0.096
	HPR	0.151	0.058	0.862	0.171	0.848	0.075	0.620	0.099
	HPO	0.625	0.152	0.700	0.091	1.10	0.216	0.808	0.143
Ovary	C	0.399	0.132	1.01	0.155	1.70	0.177	1.04	0.163
	HPR	0.700	0.272	1.56	0.310	2.32	0.207	1.52	0.243
	HPO	0.644	0.156	2.32	0.302	4.02	0.789	2.33	0.413
Oviduct	C	0.588	0.194	1.19	0.182	1.83	0.191	1.20	0.188
	HPR	0.837	0.325	0.947	0.188	2.25	0.201	1.32	0.211
	HPO	0.625	0.152	1.20	0.156	1.67	0.328	1.16	0.206

Values : Mean  $\pm$  SE, n=12, \* P<.05, \*\*P<.005, \*\*\*P<.0005.

Table 4. Monthly growth rates and growth indices of liver and lymphoid organs (gms) of HPR and HPO chicks under NLD.

		30 days		60 days		90 days		Overall	
		Growth rate	Growth index	Growth rate	Growth index	Growth rate	Growth index	Growth rate	Growth index
Liver	C	0.086	0.028	0.098	0.015	0.165	0.017	0.116	0.018
	HPR	0.086	0.033	0.182	0.036	0.193	0.017	0.153	0.024
	HPO	0.135	0.038	0.082	0.010	0.197	0.038	0.138	0.024
Thymus	C	0.0033	0.001	0.033	0.005	0.027	0.002	0.021	0.003
	HPR	0.0025	0.0009	0.030	0.005	0.071	0.006	0.034	0.005
	HPO	0.0014	0.0003	0.022	0.0028	0.074	0.014	0.031	0.005
Bursa	C	0.0021	0.0006	0.010	0.001	0.037	0.003	0.016	0.002
	HPR	0.0045	0.001	0.025	0.004	0.079	0.007	0.036	0.005
	HPO	0.0019	0.0004	0.019	0.0024	0.049	0.0096	0.023	0.004
Spleen	C	0.0004	0.0001	0.013	0.001	0.0064	0.0006	0.0063	0.0009
	HPR	0.001	0.0003	0.0093	0.001	0.022	0.001	0.010	0.001
	HPO	0.0002	0.00004	0.0175	0.0022	0.0093	0.0018	0.0090	0.001

Values : Mean  $\pm$  SE, n=12, \* P<.05, \*\*P<.005, \*\*\*P<.0005.

Table 5a. Changes in serum corticosterone, T<sub>3</sub>, T<sub>4</sub> and progesterone in HPR and HPO chicks under NLD.

		30 days	60 days	90 days
<b>Corticosterone</b>	<b>C</b>	2.48 ±.09	2.23 ±.06	2.02 ±.17
	<b>HPR</b>	3.67 ±.09***	3.16 ±.11***	2.80 ±.18**
	<b>HPO</b>	2.30 ±.66	1.33 ±.28*	1.71 ±.15
<b>T<sub>3</sub></b>	<b>C</b>	0.690 ±.05	0.528 ±.02	0.593 ±.08
	<b>HPR</b>	0.678 ±.09	0.491 ±.02	0.532 ±.007
	<b>HPO</b>	0.571 ±.03*	0.309 ±.07***	0.551 ±.023
<b>T<sub>4</sub></b>	<b>C</b>	3.016 ±.10	3.03 ±.15	3.18 ±.31
	<b>HPR</b>	3.79 ±.22**	2.53 ±.19*	4.16 ±.28*
	<b>HPO</b>	2.88 ±.18	1.23 ±.11***	2.80 ±.33
<b>Progesterone</b>	<b>C</b>	0.511 ±.07	0.120 ±.05	0.266 ±.04
	<b>HPR</b>	0.340 ±.02*	0.069 ±.06	0.075 ±.08*
	<b>HPO</b>	0.144 ±.07***	0.120 ±.006	0.170 ±.05

Values : Mean ± SE, n=12, \* P<.05, \*\*P<.005, \*\*\*P<.0005.

Table 5b. Changes in corresponding ratios of serum hormones of HPR and HPO chicks under NLD.

		30days	60 days	90 days
$T_3 : T_4$	C	0.228	0.174	0.186
	HPR	0.179	0.194	0.127
	HPO	0.198	0.251	0.196
$T_3 : \text{CORT}$	C	0.278	0.236	0.286
	HPR	0.184	0.155	0.190
	HPO	0.248	0.232	0.768
$T_4 : \text{CORT}$	C	1.216	1.35	1.53
	HPR	1.03	0.800	1.48
	HPO	1.25	0.924	3.90
$T_3 : \text{Body wt.}$	C	0.0058	0.0016	0.0009
	HPR	0.0065	0.0019	0.0009
	HPO	0.0038	0.0008	0.0010
$T_4 : \text{Body wt.}$	C	0.025	0.0092	0.0053
	HPR	0.0364	0.0099	0.0070
	HPO	0.019	0.0032	0.0052
$T_3 : \text{Thyroid wt.}$	C	0.057	0.020	0.0179
	HPR	0.044	0.022	0.0140
	HPO	0.400	0.0081	0.0126
$T_4 : \text{Thyroid wt.}$	C	0.251	0.154	0.0963
	HPR	0.247	0.115	0.109
	HPO	0.205	0.032	0.064
$\text{CORT} : \text{Body wt.}$	C	0.021	0.0071	0.0034
	HPR	0.035	0.012	0.0047
	HPO	0.015	0.0034	0.0013
$\text{CORT} : \text{Adrenal wt.}$	C	0.155	0.072	0.030
	HPR	0.212	0.073	0.040
	HPO	0.073	0.022	0.0083

Values : Mean  $\pm$  SE, n=12, \* P<.05, \*\*P<.005, \*\*\*P<.0005.

Table 6. Histometric data of the ovarian follicles in ovaries of HPR and HPO pullets under NLD

			S <sub>1</sub> (<30µm)	S <sub>2</sub> (31-90µm)	S <sub>3</sub> (91-120µm)	B1 (121-240µm)	B2 (241-440µm)	S (6-200µm)	B (200-300µm)	L (>300µm)	Total
30 days	C	POF	23 (38%)	29 (48%)	3 (5%)	5 (8%)	--	58	2	--	60
		AF	2 (9%)	--	--	--	--	--	--	--	2 (3%)
	HPR	POF	14 (29%)	17 (35%)	6 (12%)	11 (22%)	--	41	7	--	60
		AF	--	4 (23%)	--	--	--	--	--	--	2 (3%)
	HPO	POF	9 (14%)	34 (53%)	14 (22%)	7 (10%)	--	61	3	--	64
		AF	--	2 (6%)	--	--	--	--	--	--	2 (3%)
60 days	C	POF	21 (29%)	35 (49%)	7 (10%)	6 (8%)	3 (4%)	66	6	--	72
		AF	8 (38%)	3 (8%)	1 (14%)	1 (16%)	--	--	--	--	12 (16%)
	HPR	POF	4 (6%)	43 (63%)	8 (12%)	13 (19%)	--	56	12	--	68
		AF	1 (25%)	2 (5%)	1 (12%)	1 (8%)	--	--	--	--	--
	HPO	POF	--	51 (70%)	11 (15%)	8 (11%)	3 (4%)	65	7	1	73
		AF	--	10 (20%)	2 (18%)	1 (12%)	1 (33%)	--	--	--	14 (19%)

Values : Mean

continued.....

			S <sub>1</sub> (<30µm)	S <sub>2</sub> (31-90µm)	S <sub>3</sub> (91-120µm)	B1 (121-240µm)	B2 (241-440µm)	S (6-200µm)	B (200-300µm)	L (>300µm)	Total
90 days	C	POF	--	25 (47%)	5 (9%)	17 (32%)	6 (11%)	35	14	4	53
		AF	--	11 (44%)	2 (40%)	3 (18%)	--	--	--	--	16 (31%)
	HPR	POF	4 (8%)	3 (6%)	10 (21%)	22 (47%)	8 (17%)	24	18	5	47
		AF	--	--	6 (60%)	6 (27%)	--	--	--	--	12 (25%)
	HPO	POF	3 (6%)	21 (40%)	12 (23%)	8 (15%)	8 (15%)	46	6	1	52
		AF	1 (33%)	2 (10%)	1 (8%)	1 (12%)	--	--	--	--	5 (10%)

Values : Mean

Fig. 1. Body weight and body growth rates of HPR and HPO chicks under NLD.

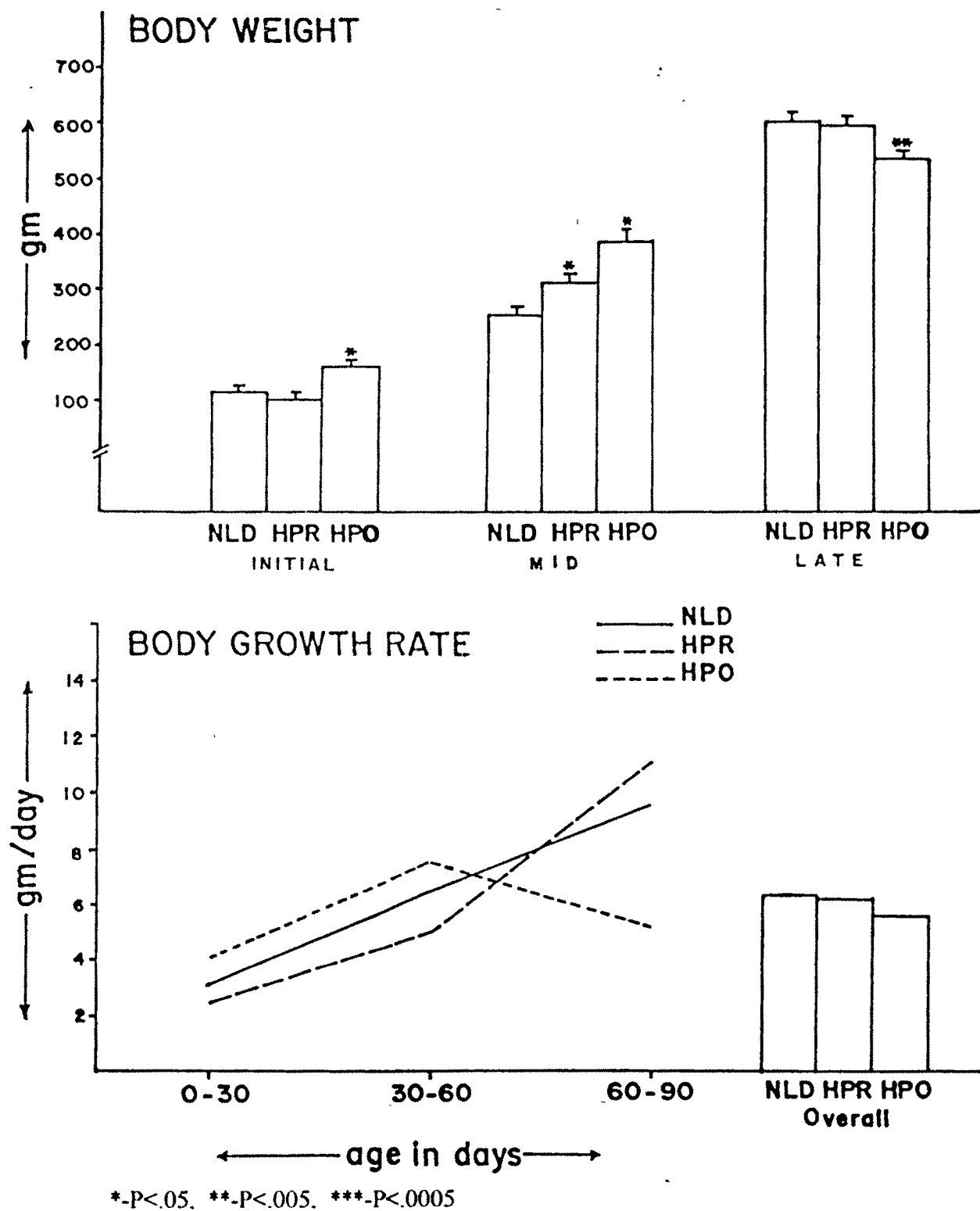


Fig. 2a. Absolute and relative weights of thyroid and adrenal (in mg) of HPR and HPO chicks under NLD.

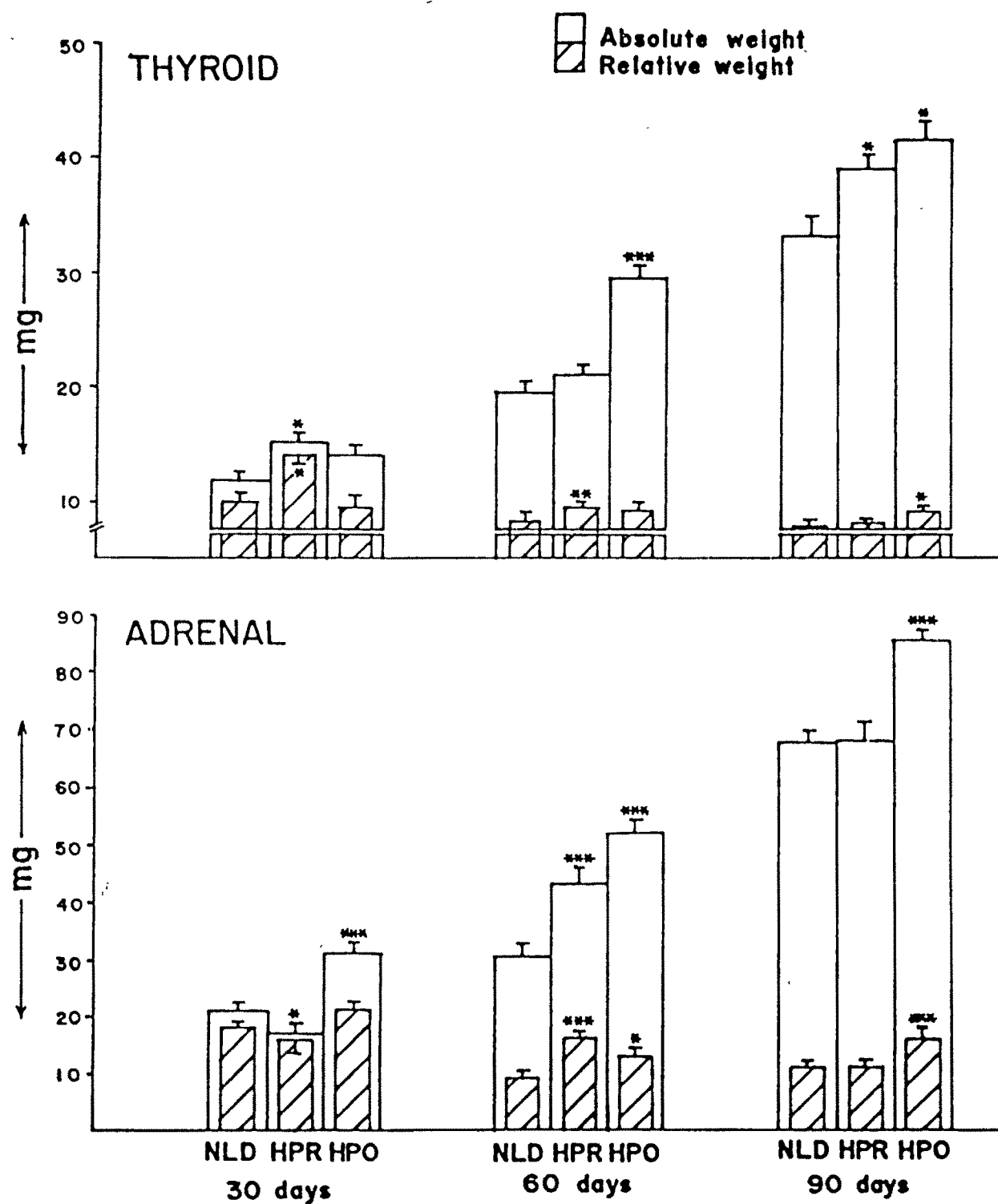




Fig. 2b. Absolute and relative weights of ovary and oviduct (in mg) of HPR and HPO chicks under NLD.

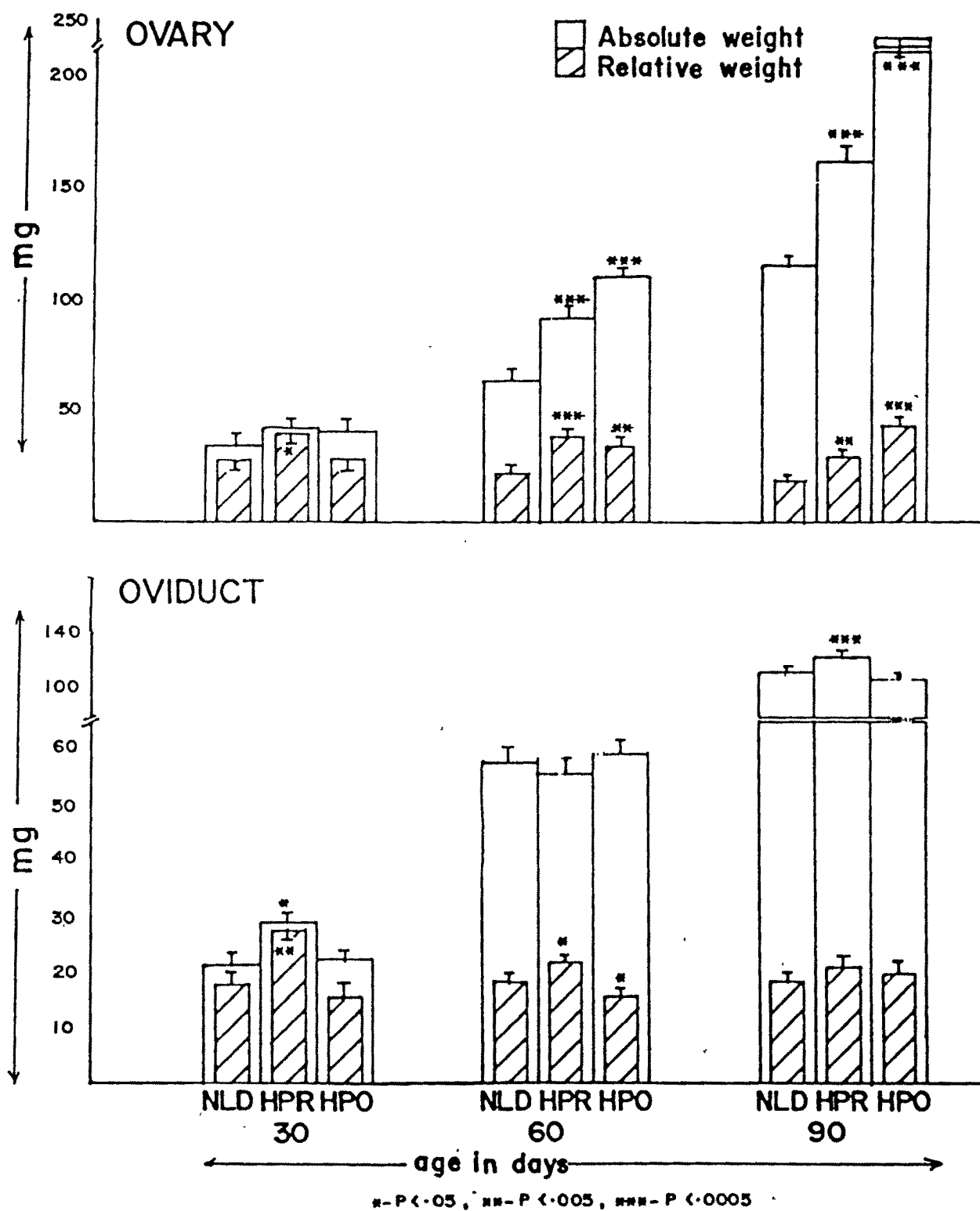
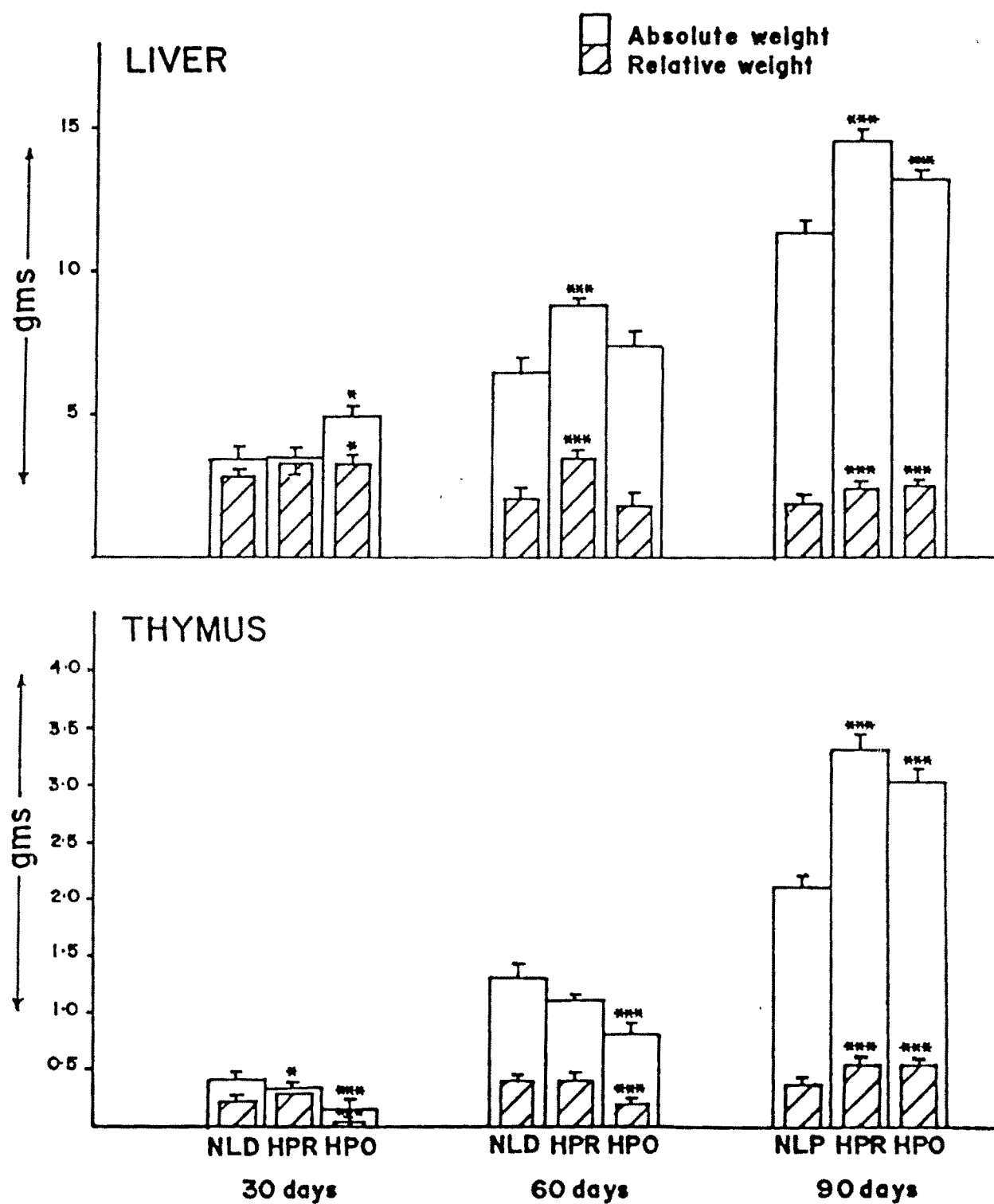


Fig. 2c. Absolute and relative weights of liver and thymus (in gms) of HPR and HPO chicks under NLD.



\*-P < 0.05, \*\*-P < 0.005, \*\*\*-P < 0.0005

Fig. 2d. Absolute and relative weights of bursa and spleen (in gms) of HPR and HPO chicks under NLD.

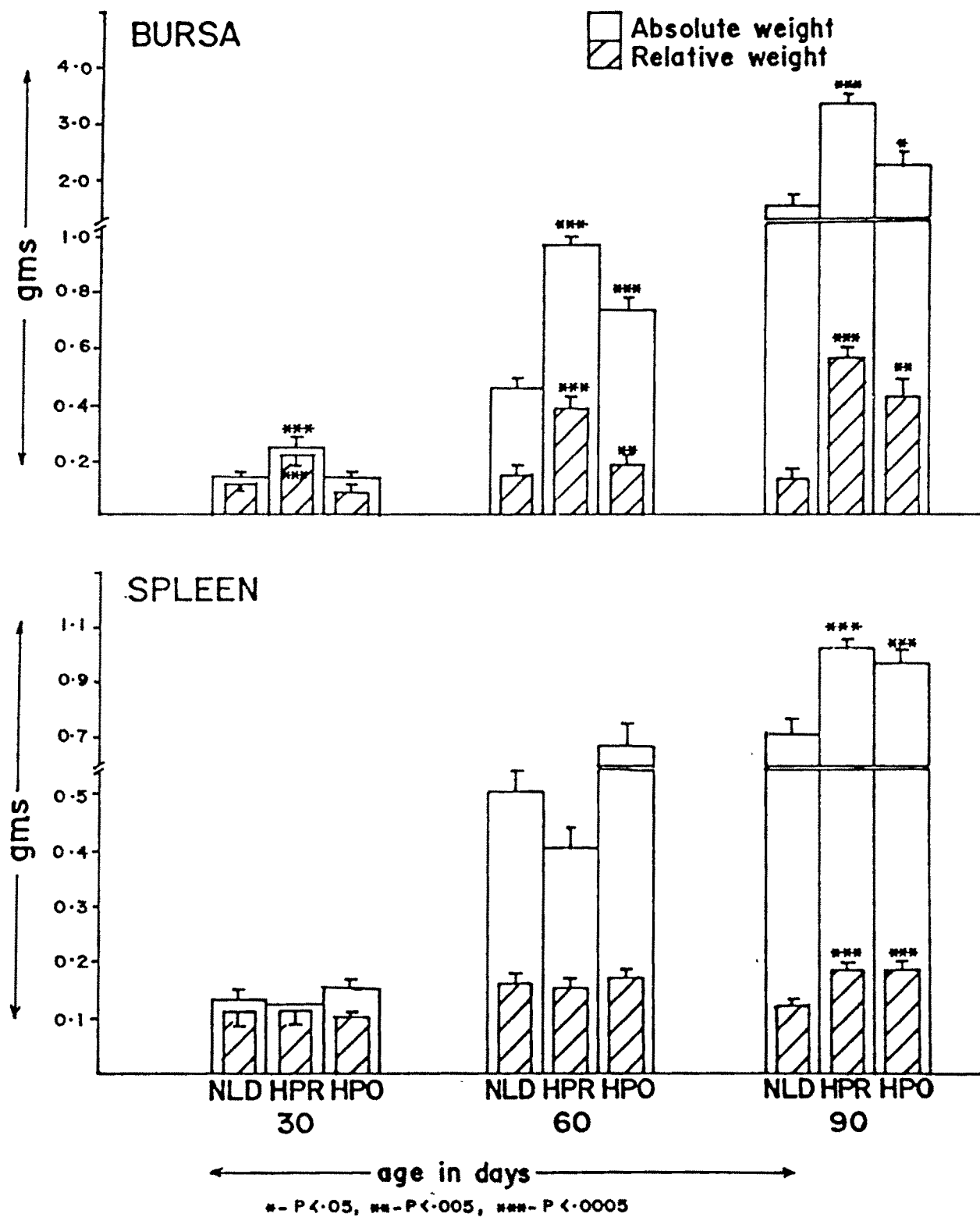
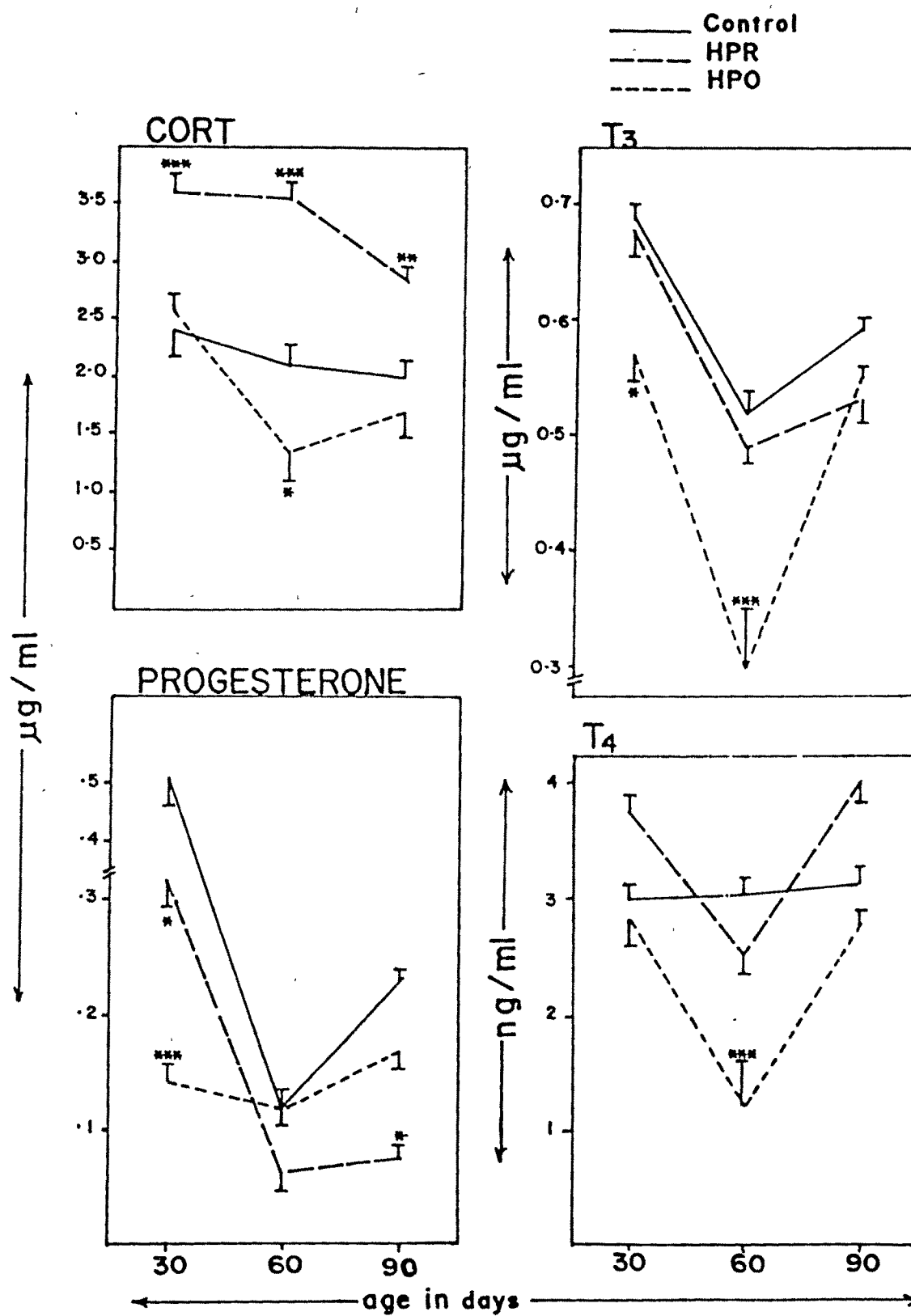


Fig 3. Serum hormone levels of HPR and HPO chicks under NLD, at 30, 60 and 90 days.



### **Plate 1 (Figs. 1-9)**

Photomicrographs of thyroid of HPR, control and HPO chicks (320 X).

Fig. 1. Thyroid of 30 day old HPR chick showing follicles with varying degrees of colloid content and a low follicular epithelium.

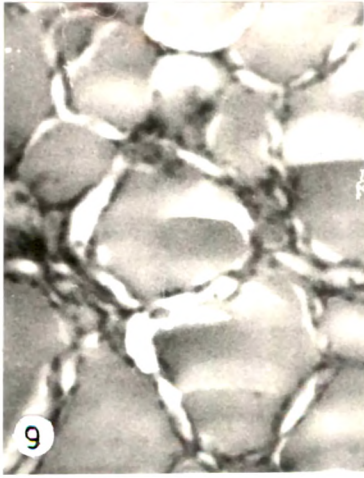
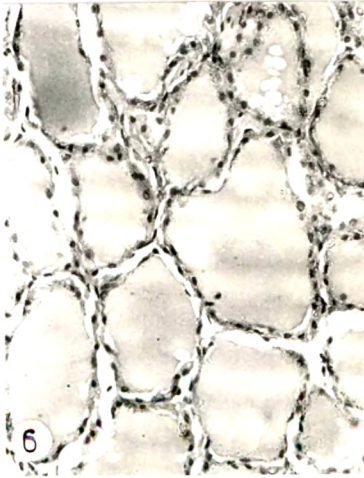
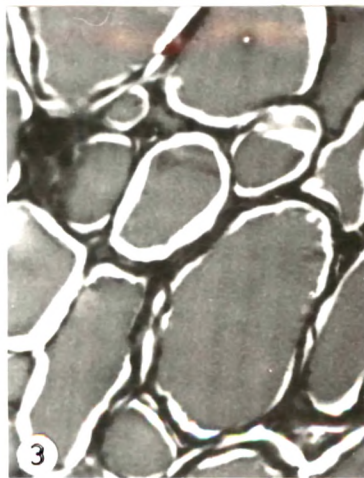
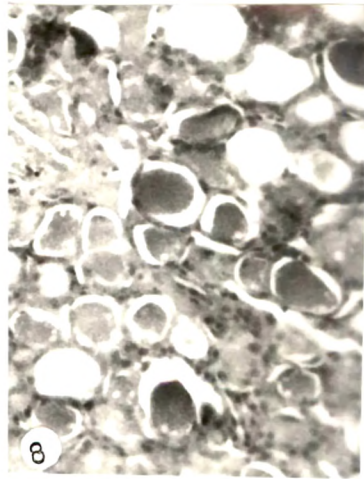
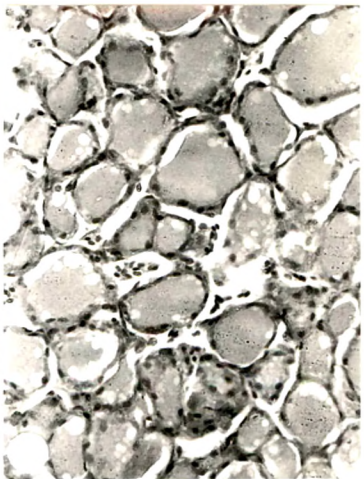
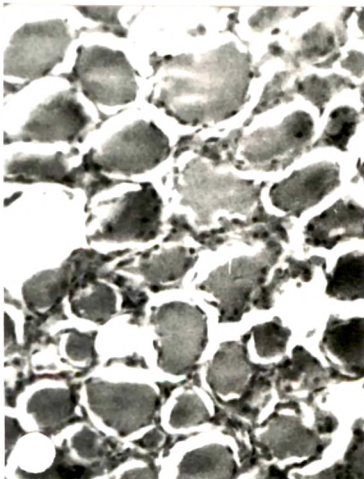
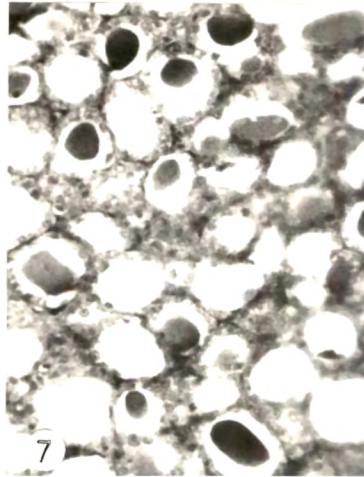
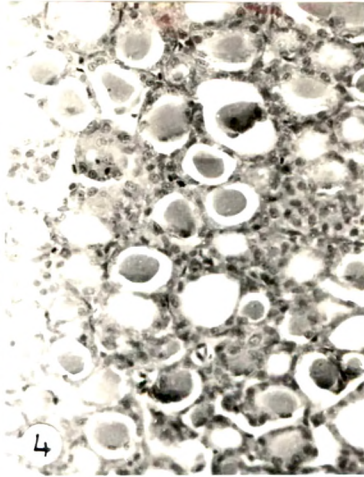
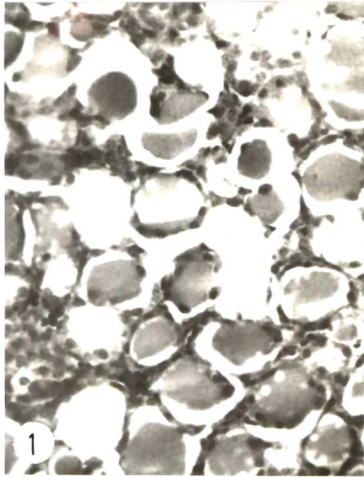
Figs. 2-3. Thyroid of 60 and 90 day old HPR chick showing colloid filled follicles. Follicular epithelium cuboidal at 60 days (fig. 2) or flat at 90 days (fig. 3).

Figs. 4-6. Thyroid of 30, 60 and 90 day old control chick showing cuboidal to low follicular epithelium. Some of the follicles colloid filled, whereas some show mild depletion of colloid.

Fig. 7. Thyroid of 30 day old HPO chick showing active cuboidal epithelium and overall colloidal depletion.

Fig. 8. Thyroid of 60 day old HPO chick. Note the colloid filled follicles with flat epithelium.

Fig. 9. Thyroid of 90 day of HPO chick showing follicles with mild colloid depletion.



**Plate 2 (Figs. 10-18)**

Photomicrographs of adrenal of HPR, control and HPO birds (320 x).

Fig. 10. Adrenal of 30 day HPR chick. Note the prominent cortical cords and nuclei.

Fig. 11. Adrenal of 60 day HPR chick showing less active cortex.

Fig. 12. Adrenal of 90 day HPR chick showing mild hypertrophy and more or less an inactive state.

Fig. 13. Adrenal of 30 day control chick showing prominent and active medulla.

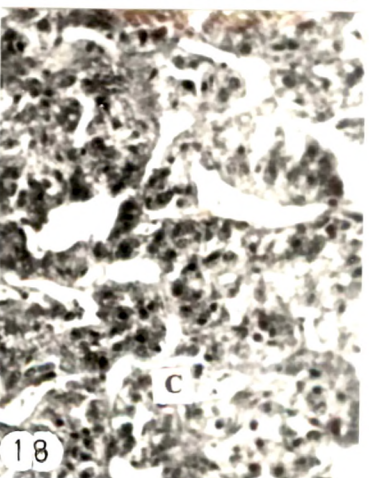
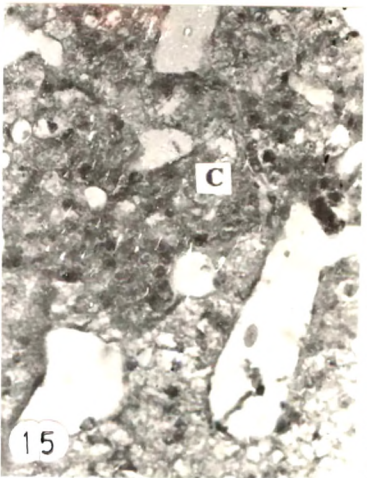
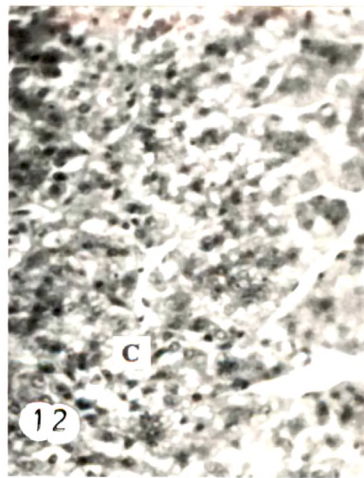
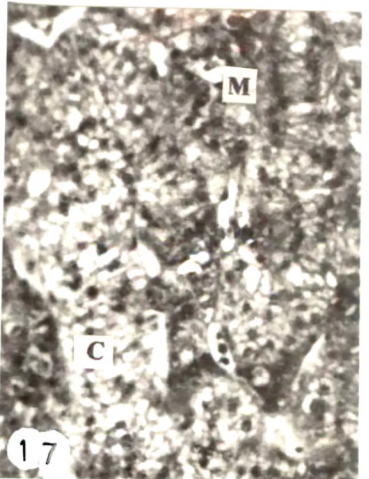
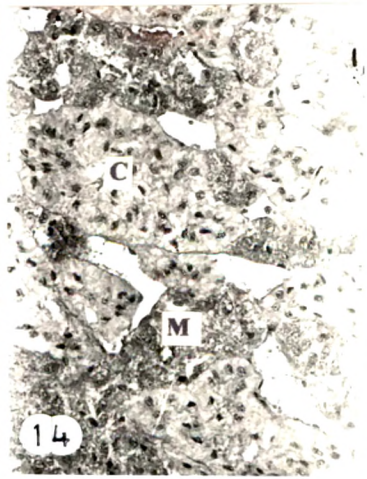
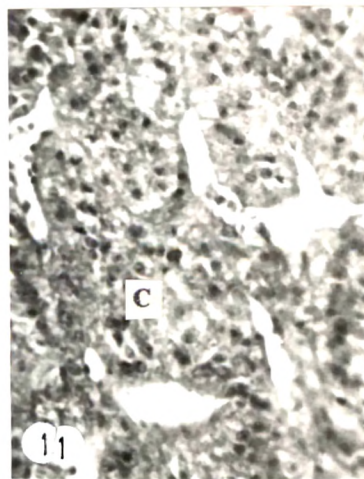
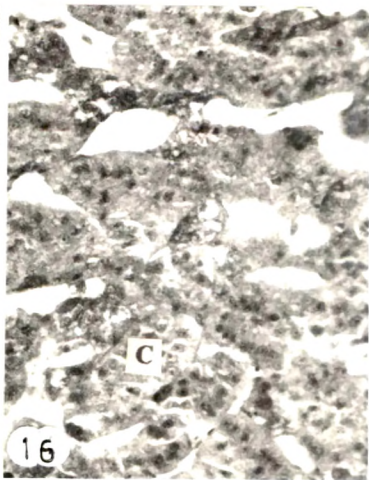
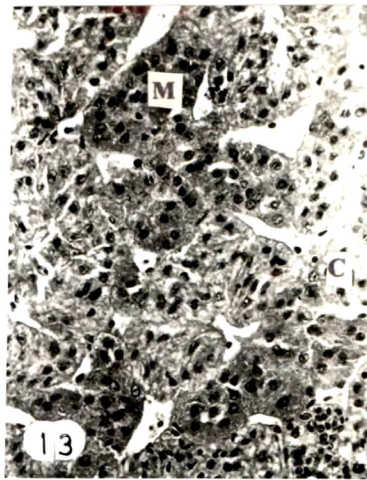
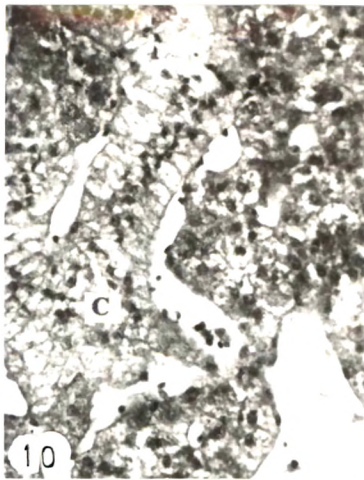
Fig. 14. Adrenal of 60 day control chick. Note the condensation of nuclear elements in cortex. Medullary secretion indicated.

Fig. 15. Adrenal of 90 day control chick. Cortical cords well formed with active cells and showing secretory exhaustion. Medullary cell activity prominent.

Figs. 16-17. Adrenal of 30 and 60 day HPO chick showing active cortex and medulla.

Fig. 18. Adrenal of 90 d HPO chick showing secretory exhaustion in cortical cells and active state by nuclear appearance.







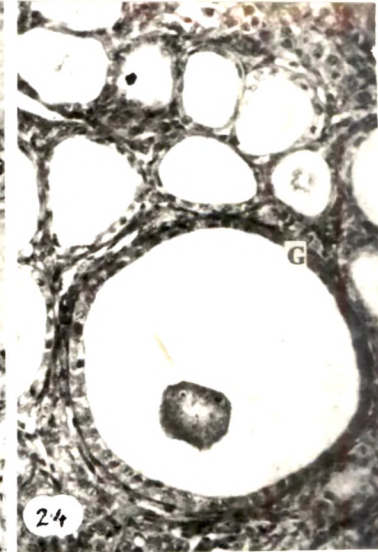
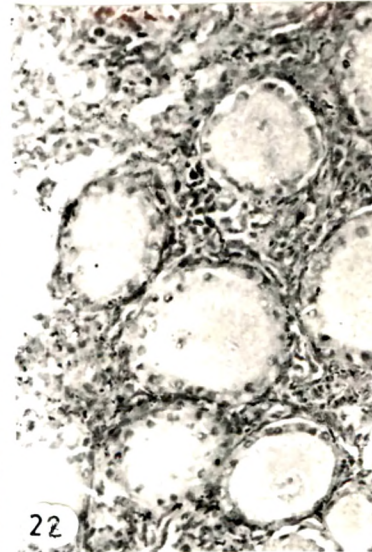
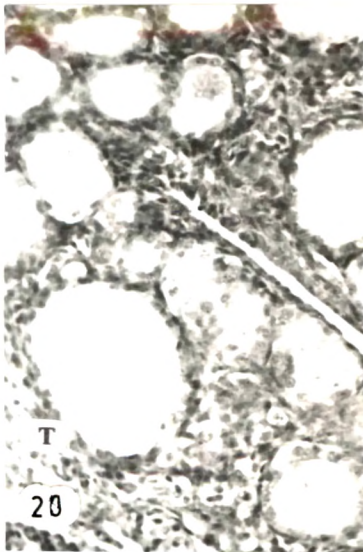
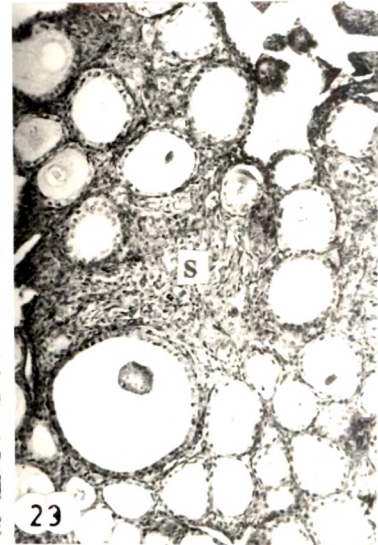
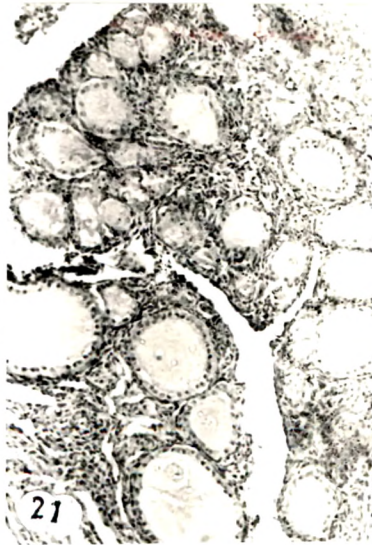
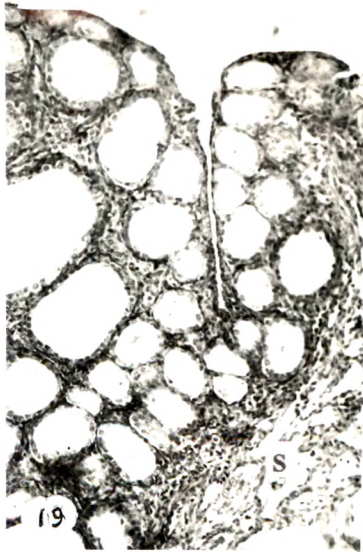
**Plate 3 (Figs. 19-24)**

Photomicrographs of 30 day old ovary of HPR , control and HPO chicks (160 and 320 x).

Figs. 19-20. Ovary of 30 day old HPR chick showing many medium sized follicles. Note the condensed theca and hypertrophied stroma.

Figs. 21-22. Ovary of 30 day old control chick showing many primary and primordial follicles.

Figs. 23-24. Ovary of 30 day HPO chick showing enlarged primary and primordial follicles with active granulosa cells and a compact stromal tissue.



**Plate 4 (Figs. 25-30)**

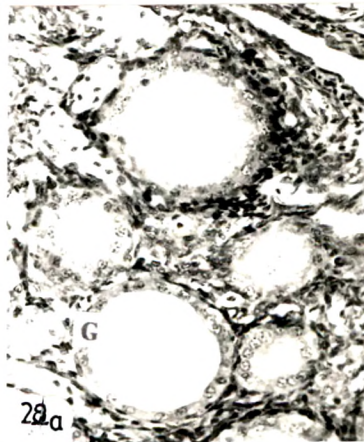
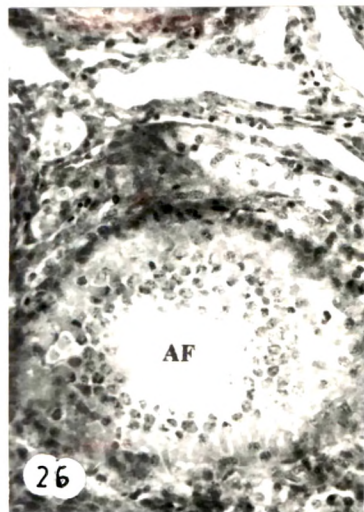
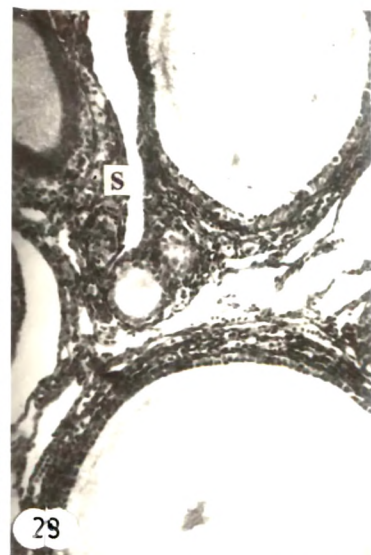
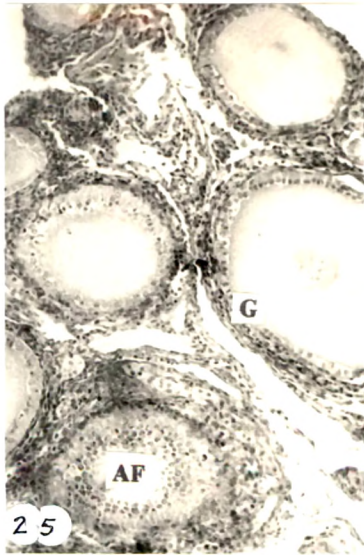
Photomicrographs of 60 day old ovary of HPR, control and HPO chicks (160 and 320 x).

Figs. 25-26. Ovary of HPR chick showing many large follicles with prominent granulosa. Note the follicular atresia.

Figs. 27-28 (a-b). Ovary of control chick showing small medium (fig. 27,28a) and large (fig. 28b) follicles with prominent granulosa. Note the hypertrophied stroma and condensed theca.

Figs. 29-30. Ovary of HPO chick showing larger sized follicles with prominent granulosa surrounded by loose stroma. Theca thin and fibrous.





**Plate 5 (Fig. 80, 160 and 320 x)**

Photomicrographs of 90 day old ovary of HPR, control and HPO chicks (80, 160 and 320 x).

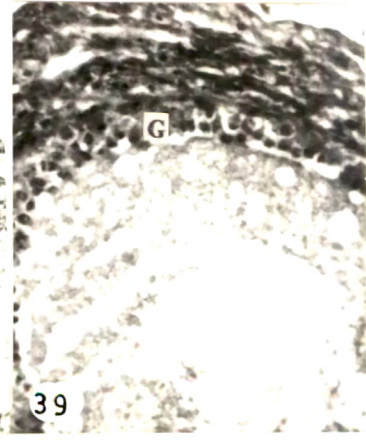
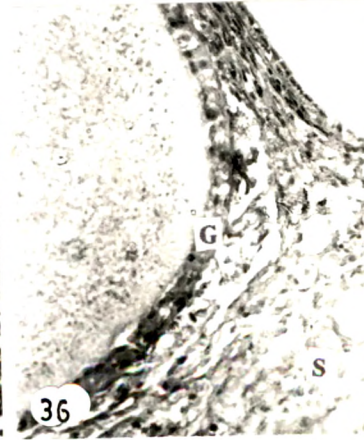
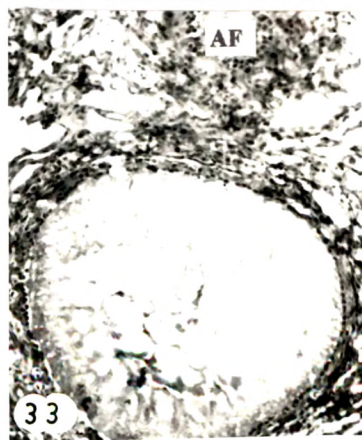
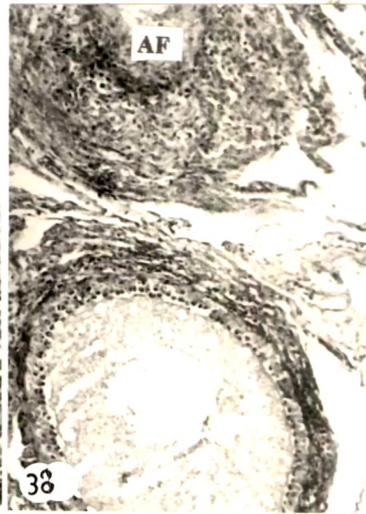
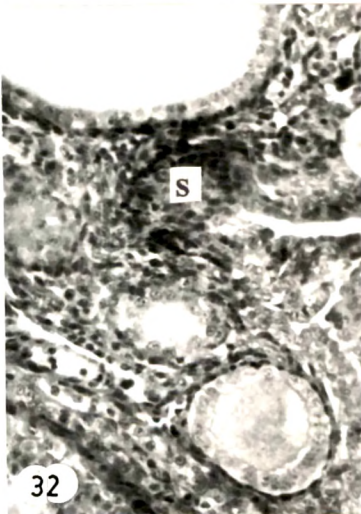
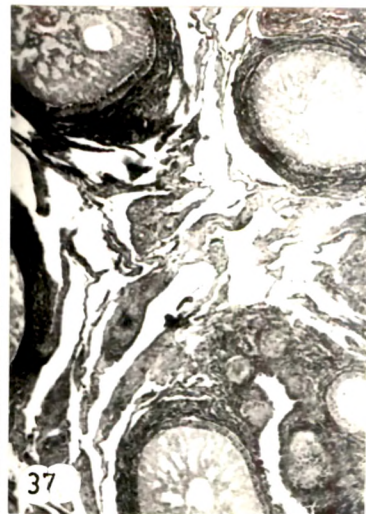
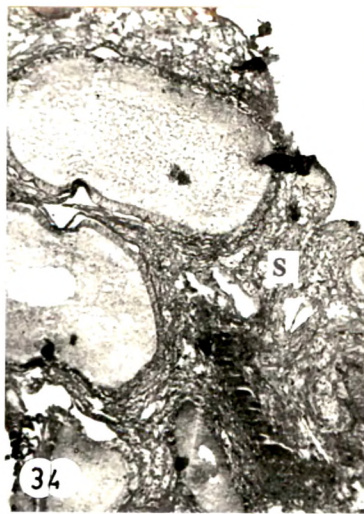
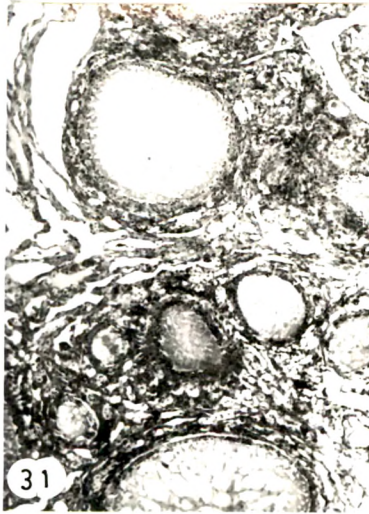
Figs. 31-33. Ovary of HPR chick showing primary and primordial follicles and a dense and compact stroma.

Figs. 34-36. Ovary of control chick showing overall less number of follicles. Theca thin and fibrous, stromal tissue appears loose.

Figs. 37-39. Ovary of HPO chick showing medium to large sized follicles, but overall population less. Atretic changes evident.







granules. The interstitial glands were well developed. The ovary of both the HPR and HPO chicks showed a similar histoarchitecture as that of control chicks except for hyperplastic granulosa and theca with loose stromal tissue at 90 days in HPR chicks and thin and fibrous theca with loose stromal tissue at 60 days (Plate 3,4,5).

The histometrics of the ovarian follicles show a temporal progression from 6-30  $\mu\text{m}$  to 240-440  $\mu\text{m}$  sized follicles from 30-90 days in control chicks. The histology of ovary of HPR and HPO chicks showed a similar follicular hierarchy. However, the ovary of HPR chicks showed a greater rate of follicular transition from small to big and big to large follicles compared to controls. The percentage of follicles undergoing atresia in ovary of HPR chicks was significantly more at 30 days, less at 60 days and similar at 90 days as compared to the control. The population of follicles of < 400  $\mu\text{m}$  size was less in HPR ovary throughout. In contrast, the ovary of HPO chicks had similar rate of transition from small to big follicles at 30-60 days but significantly less at 90 days compared to control. Moreover, the transition of big to large follicles was nil. The percentage of follicular atresia in ovary of HPO chicks was similar to that of control at 30 and 60 days but significantly less at 90 days. The number of follicles less than 200  $\mu\text{m}$  size were similar to control at 30 and 60 days and significantly more at 90 days (Table 6).

## DISCUSSION

The data on body and organ weights and the growth indices of organs clearly reveal subtle differential effects of corticosterone or

metapyrone implantation. Though there are no dramatic differences in serum CORT levels, subtle hypercorticalism and hypocorticalism are evident by the recorded relatively higher levels of the hormone in corticosterone implanted chicks and, lower levels in metapyrone implanted chicks (table 5a). Most of the investigations to date intended to study the impact of HPR / HPO have employed acute administration of corticosterone and metapyrone or dexamethasone (Freeman *et al.*, 1979; Joseph and Ramachandran, 1992,1993; Joseph *et al.*, 1995)). There is only one study which had employed and, documented the appropriateness of implantation of corticosterone as a more meaningful experimental model (Davison *et al.*, 1985). These workers demonstrated significant increase in CORT levels subsequent to implantation. However, the dosage employed was incomparably higher ( 40mg/kg body weight V/s 1mg corticosterone) and the duration of study much shorter (15days V/s 90days) as compared to the present study. Whereas there was no significant difference in the body weight of HPR pullets, the body weight of HPO pullets was lesser at 90 days. However, the absolute and relative weights of various organs were higher in both HPR and HPO chicks; but a careful scrutiny of the growth rate and growth index reveals differential effects at 30, 60 and 90 days. Whereas the body growth rate was slightly lower during the first two months with peak rate during the second month and significantly greater during third month in HPR chicks, the body growth rate was significantly higher during the first two months with a significantly depressed marginal growth rate during 3<sup>rd</sup> month in HPO chicks (Fig. 2a). Though data on body weight and relative weights of organ do not project clear cut differences between HPR and HPO, data in terms of body growth rate and growth index of organs afford more meaningful comparison. Such a comparison reveals increased body growth rate during the 3<sup>rd</sup> month in HPR chicks with reduced rates during first two months and, depressed



growth rate during 3rd month with increased growth rates during first two months in HPO chicks. In contrast, the growth index of various organs like liver, oviduct and lymphoid organs show a reverse set of changes in the form of lower growth index during the 3rd month, with higher growth indices during first the two months in HPR chicks and, higher growth index during the third month as contrasted with lower growth indices during the first two months in HPO chicks. Overall, the relative weight and growth index of liver and lymphoid organs are relatively higher in HPR pullets, and lower in HPO chicks, which clearly indicate favourable influence of corticosterone levels within an optimum range, on growth of liver and lymphoid organs in the early phase of post-natal development. This is in keeping with the reports of some other workers, as well as the previous observations in this respect in this study in relation to rearing photoperiods (see chapter 8). The increased relative weight and growth index of liver and lymphoid organs in HPO chicks during the 3rd month, seem to be essentially due to relatively reduced body weight increase between the 2<sup>nd</sup> - 3<sup>rd</sup> months in these chicks.

Decrement in serum levels of T<sub>3</sub>, T<sub>4</sub> and progesterone with age is more-or-less manifested in all the three groups of chicks with relatively low levels of all the hormones in HPR chicks. Though an influence of corticosterone on thyroid hormone levels and also on the peripheral conversion of T<sub>4</sub> to T<sub>3</sub> have been clearly established in both birds and mammals (Singh *et al.*, 1967; Braveman *et al.*, 1970; Sterling, 1970; Chopra, 1977; Asteir and Newcomer, 1978; Decuypere *et al.*, 1983; Rudas and Pethes, 1984; Williamson and Davison, 1987), the present data on thyroid hormone level in HPR or HPO chicks do not reveal any such influence. This may be clearly due to the fact that the changes in CORT levels are only subtle and not as markedly altered to influence thyroid hormone levels. However, the favourable influence of corticosterone is

more clearly illustrated by the hormone:body weight ratios, which are higher with respect to CORT,  $T_4$  and  $T_3$  in HPR chicks and lower in HPO chicks (table 5b). The influence of HPO is also clearly manifested by the significantly higher relative weights of adrenal throughout, presumably due to an altered feedback effect.

Both HPR and HPO seem to have a favourable influence on the reproductive axes as the weights and growth indices of ovary and oviduct were significantly greater in these chicks. Though the similar favourable response appears enigmatic, it may be speculated that, while the influence of HPO may represent an activated HHG axis, that of HPR may represent an increased sensitivity of the ovarian tissue. The histological appearance and the histometric data tend to suggest an increase in somatic component to be the main contributing factors in increasing the ovarian weight in both HPR and HPO, rather than an actual increment in the germinal component. The histometric data show more-or-less similar hierarchial progression of follicles in terms of size in control and HPR pullets. Though the rate of transition from small to big follicles was slightly higher during the 1<sup>st</sup> and 2<sup>nd</sup> months in HPR pullets, the rate of transition from small to big and big to large in the 3<sup>rd</sup> month was almost identical to controls during the 3<sup>rd</sup> month. Even the pool of follicles of size less than 200 $\mu$ m showed a similar gradual decrease during 1<sup>st</sup> to 3<sup>rd</sup> month in control and HPR chicks. However, in the HPO chicks, the transition of follicles of higher size hierarchy was significantly retarded, which is not only reflected by the relatively lesser number of big and large follicles but, also by the almost static pool size of follicles of <200 $\mu$ m between the 2<sup>nd</sup> and 3<sup>rd</sup> months, indicating almost no progression in follicular growth. The serum progesterone levels were significantly lower in both HPR and HPO chicks with an almost constant level in HPR and slightly reduced level during 2<sup>nd</sup> and 3<sup>rd</sup> months in HPO

chicks. It is difficult to relate these changes in serum progesterone level with either HPR or HPO status and also with the observed changes in the ovarian tissue. It is likely that the turnover of progesterone and its relative rate of conversion to androgen and oestrogen might be different in HPR and HPO chicks. Moreover, the intraovarian level of progesterone and androgen and oestrogen, as well as, the sensitivity to these hormones may also be differentially affected due to HPR or HPO. Though these aspects may not be greatly affected/alterd under HPR, it may be more relevantly affected under HPO. Such an assumption is compatible with the previously reported laying capacity of HPO and HPR birds (Dandekar, 1998) wherein HPO chicks depicted reduced egg laying, while the HPR did not show any significant difference. Apparently, the retardation in folliculogenesis coupled with increased follicular atresia, may be favourably related with the HPO induced reduced lay in the adult stage. However, the possible subtle alterations in the intra-ovarian mechanisms need to be elucidated to make more meaningful valid explanation. But in general, it is evident from the present observations and previous reports, that the early HPO during the rearing stage of pullets has some negative influence on ovarian functions and egg laying capacity of such pullets. Further studies on these line may be fruitful in establishing the relationship between adrenal steroids and ovarian functions in the domestic fowl.