CHAPTER 10

Effect of step-down photoperiod and transient hyper. or hypocorticalism on serum hormone profile and histo-morphology of some organs during post-hatch development.

Introduction :

The chicks of precocial species, like the domestic fowl, undergo a rapid phase of development involving organ growth and maturation of the reproductive axis, culminating in attainment of sexual maturity and adult body size. The transition from pullets to hens, is characterized by dramatic histoarchitectural and functional alterations of the ovary and oviduct. Dynamic alterations in endocrine milieu can be envisaged to play a major role in growth kinetics and functional maturation of the reproductive system. This has been emphasized by the reported growth retardatory influence of both hypophysectomy and thyroidectomy in chicks of duck and fowl (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). Similarly, experimental manipulations resulting in decreased or increased corticosterone levels have also been shown to retard body weight gain (Dandekar, 1998). In adult birds, both thyroid hormones and adrenocorticosteroids have been shown to influence the reproductive axis though, the relationship between these hormones and the functions of gonads could be either parallel or inverse (Riddle *et al.*, 1924; Legait and Legait, 1959; Fromme-Bouman, 1962; Patel *et al.*, 1986; Thapliyal and Pandha, 1967 a & b; Jallages and Assenmacher, 1973, 1974; Oishi and Konishi, 1978; Patel *et al.,* 1985; Ramachandran and Patel 1986; Ramachandran *et al.*, 1987; Ramachandran and Patel, 1988; Ayyar *et al.*, 1992). Previous study from this laboratory on adult feral and domestic pigeons had attested the concept of a parallel adrenal-gonad and inverse thyroid-gonad relationships in the former species and, inverse adrenal-gonad and parallel thyroid-gonad relationship in the latter (Patel, 1993; Singh, 1993). In keeping with these concepts, another study on one month and two months old white leghorn chicks revealed increased size and hastened functional maturation of testis under hypocorticalism and retardation of growth and functional maturation under hypercorticalism (Joseph and Ramachandran, 1993). Recent study involving induction of transient mild hypo/hypercorticalism from 0-90 days in RIR pullets, showed subtle effects on features of egg laying during the first cycle of lay and composition of eggs (Dandekar, 1998; chapters 3, 4, 6 and 7). The alterations induced by hypo/hypercorticalism on growth kinetics and histomorphology of adrenal, thyroid, ovary and oviduct and serum level of T_3, T_4, corticosterone and progesterone during the experimental period, were also studied to relate with the observed influences on egg laying (see chapter 9).

A common practice that had been perfected over the years to improve egg laying performance in domestic fowl is the rearing of pullets in a controlled artificial photoperiod (Dunn *et al.*, 1990; Lewis *et al.*, 1996 a & b; Sandowal and Gernat, 1996; Etches, 1996). In the previous study, exposure of chicks to long photoperiod (18:6) from 0-90 days of age followed by shifting to NLD (12:12), a step-down photic schedule, had revealed some negating influence on egg laying in RIR hens (see chapter

2). The effect of LP on growth kinetics and histomorphological alterations of adrenal, thyroid, ovary and oviduct, and serum hormone levels of T_3, T_4, corticosterone and progesterone were also assessed in pullets during the experimental period to draw some correlation (see chapter 8). As both, a long photoperiod as well as hyper / hypocorticalism during the rearing stages, were shown to affect various facets of egg laying of RIR hens, a subsequent study tried to evaluate the influence of LP and HPR/HPO on laying performance which resulted in differential effects on attainment of sexual maturity and yield of eggs (see chapters 2 & 4). The present study in this context evaluates the changes in growth kinetics and histometrics of adrenal, thyroid, ovary and oviduct as well as serum corticosterone, T_3, T_4 and progesterone in pullets subjected to a combination of experimental schedule of LP and HPR/HPO to draw possible correlation if any with attainment of sexual maturity and egg laying.

Materials and Methods :

As detailed in chapter 1

Results :

Body and organ weights :

The body weight of HPR and HPO chicks was almost identical to that of LP control chicks at 90 days. However, at 30 days, the weight of both HPR and HPO chicks tended to be higher than that of the control chicks, significantly in the latter. At 60 days, the weight of both these experimental groups of chicks was lesser than that of controls (table 1). Whereas a peak growth rate in LP control chicks occurred between 30 and 60 days, the same occurred for HPR and HPO chicks between 60 and 90 days (fig. 1). The weights of adrenal, thyroid and ovary were significantly greater in HPR chicks at 90 days, while the weight of oviduct was significantly less. In the case of HPO chicks, the weights of adrenal, ovary and oviduct were significantly greater at 90 days compared to those of the control chicks (Table 1)(fig. 2a,b). These differences were reflected in the overall growth kinetics of these organs (Table 3). Whereas the growth kinetic ratio of thyroid of control chicks showed a progressive increase from 0-90days, that of HPR chicks was steady from 0-60 days with a maximal ratio between 60 and 90 days. The HPO chicks showed a maximum growth kinetic ratio between 60-90 days and minimum between 30-60 days. The growth kinetic ratio of adrenal in LP control chicks was more or less constant throughout, though slightly higher between 0-30 days and slightly lower between 30-60 days. The adrenal of HPR chicks showed a progressively increased growth kinetic ratio from 30-90 days, while that of HPO chicks showed a steady high growth kinetic ratio throughout. The growth kinetic ratio of oviduct of control chicks was maximal between 30-60 days and minimal between 60-90days. Whereas the ratio of ovary in HPR chicks showed a gradual increase, the increase was maximally significant between 60 and 90 days. The ovary of HPO showed the greatest ratio between 30 and 60 days. The growth kinetic ratio for oviduct was maximum for control chicks between 30-60 days while, it was so between 0-30 days in HPR chicks and between 60-90 days in HPO chicks. The absolute weights of liver, thymus, bursa and spleen were significantly increased in HPO chicks as compared to LP control chicks, while in HPR chicks, significant increment was observed in weights of spleen and bursa only. Similar observations were made for the relative weights of these organs in HPO chicks (table 2)(fig. 2c,d). Overall growth ratios and growth indices of liver, thymus and bursa were higher in HPR and HPO chicks, whereas the same for spleen showed reciprocal changes (table 4).

Hormonal profiles :

In general, serum corticosterone levels showed a progressive decrease from 30-90 days in all the three groups of chicks. In general, serum T_3 and T_4 levels showed progressive decrease from 30 to 90 days in control chicks. Though serum T_3 and T_4 levels were significantly lower than the levels of control chicks at 30 days the levels increased thereafter to levels comparable to those of 60 and 90 day controls. In the case of HPO chicks, the serum T_3 level was significantly lower at 90 days as compared to control chicks while, there was no significant difference in the case of serum T_4 at any of the stages. The serum progesterone level showed fluctuations in control chicks, with maximum level at 30 and 90 days and minimum level at 60 days. The serum progesterone level in HPR chicks showed a similar pattern but was significantly lower compared to the control levels at 30, 60 and 90 days. In the case of HPO chicks, the serum progesterone levels tended to remain constant at 30 and 60 days with no significant difference compared to the control levels, but at 90 days, it increased to a significantly higher level compared to the control level (table 5a).

Histological observations :

Thyroid : The thyroid of control chicks showed colloid filled follicles with small cuboidal epithelium at 30 days. At 60 days, the follicles were small to medium sized with rich colloid content and low cuboidal epithelium and a few of the follicles depicted colloid depletion. Even at 90 days, the follicles were small to medium sized with rich colloid content, though many more follicles were empty compared to the thyroid of 60 days old chicks. The thyroid of HPR chicks showed follicles of various sizes with, most of them colloid filled and with low cuboidal epithelium. At 60 days, the follicles were lined by cuboidal epithelium with evidence of colloid depletion. At 90 days the follicles appeared colloid filled and lined by a flat epithelium. By contrast, the thyroid of HPO chicks at 30 days showed follicles lined by active cuboidal epithelium and with varying degrees of colloid depletion. At 60 days, the follicles appeared colloid filled and lined by flat epithelium. At 90 days also, the follicles were lined by flat epithelium and generally colloid filled, though some of them depicted colloid loss (plate 1).

Adrenal : Both the cortical and medullary cords were prominent and active in the control chicks from 30 days. There was progressive hypertrophy and secretory exhaustion as marked by vacoulization through 60-90 days. The 30 day old adrenal of HPR chicks showed prominent cortical cells and signs of secretory exhaustion. The medullary cells appeared prominent but with no signs of secretory exhaustion. In contrast, the adrenal of 30 day old HPO chick showed relatively inactive cortical cords though, the cells appeared prominent and enlarged. The medullary cords showed differential activity. By 60 days, the cortical cords of both HPR and HPO chicks seemed well formed but with a greater degree of secretory exhaustion in HPR. The medullary cells appeared more active in HPO than in HPR chicks. At 90 days, the adrenal of HPR chicks showed prominent hypertrophied cortical cords with greater degree of secretory exhaustion as marked by vacoulated cells. Medullary cells also appeared prominent and showed greater secretory activity marked by vacoulization. In contrast, the adrenal of 90 day old HPO chicks showed relatively inactive cortical cords with only few cells showing histological signs of secretion. However, the medullary cords were prominent and hypertrophied and, the cells appeared highly active with signs of secretory exhaustion (plate 2).

Ovary : The ovary of 30 day old control chicks showed precocious enlargement of follicles with prominent hypertrophied granulosa and thecal condensation and signs of deposition of yolk. By 60 days, both the granulosa and theca were hypertrophied and active with further enlargement of follicles. By 90 days, progressive hirerarchial development of follicles was evident with the granulosa and theca appearing less active. The ovary of 30 day old HPR and HPO chicks showed prominent hypertrophied granulosa and stromal differentiation in interstitial glands. The ovary of HPR chicks seem to contain apparently more number of follicles while, in the ovary of HPO chicks, enlargement of follicles and disproportionate yolk deposition were evident. At 60 days, whereas the ovary of HPR chicks showed enlargement of follicles and well developed stromal tissue, that of HPO chicks showed follicles of various sizes with hypertrophied granulosa and yolk deposition. Thecal differentiation was evident but, the cells did not appear very active. Interstitial gland differentiation was prominent. The 90 day old ovary of HPR chicks showed many small and medium sized follicles but, no large follicles. Granulosa and theca were well differentiated but they appeared active only in some follicles. Granulosa cell hyperplasia was evident and stromal tissue was loosely organised. In contrast, the ovary of 90 day old HPO chicks showed many medium to large sized follicles with very few small follicles. Granulosa and theca were prominent around large follicles and yolk deposition was evident in these. Stromal tissue was hypertrophied but loosely organised (plate 3,4,5).

The histometrics of ovarian follicles showed a temporal progression from 6-30µm to 240-440µm sized follicles from 30 to 90 days in all the three groups of chicks. The total number of follicles was slightly more in the ovary of control chicks but the progression of follicular development into higher hierarchial sizes appeared slower in the HPR and HPO chicks, more conspicuously in the latter, compared to the control chicks at 60 days. By 90 days, the ovary of both HPR and HPO chicks seemed to have lesser number of follicles but with greater progression into large sized follicles. The percentage of atretic follicles was least in the ovary of HPR

Table 1. Changes in body weight (in gms) and absolute and relative weights of thyroid, adrenal, ovary and oviduct (in mg) of HPR and HPO pullets under LP.

	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	C	120.0 ±10.8	--	317.5±5.65	--	640±8.96	--
		HPR	130.0 ±14.1	--	386.6±4.71	--	610±21.2	--
		HPO	150.0 ±7.07	--	360±4.14	--	627.5±16.1	--
Thyroid weight	4.33 ±.40	C	9.00 ±.28	7.50 ±.56	22.66 ±1.32	5.86 ±.32	41.5 ±1.33	6.80 ±.13
		HPR	8.66 ±.37	6.66 ±.46	20.00 ±1.76	6.29 ±.20	48.7±1.3***	7.86 ±.30**
		HPO	9.00 ±.57	6.00 ±.34*	19.66 ±1.12*	5.46 ±.43	43.3 ±1.29	6.90 ±.19
Adrenal weight	12.75 ±3.92	C	20.66 ±1.76	17.21 ±.64	39.0 ± 2.66	10.08 ±.16	55.66 ±3.02	9.12 ±.62
		HPR	16.33 ±1.09*	12.5 ±.70***	41.0 ±3.80	12.91 ±.87	71 ±2.97**	11.45 ±.47*
		HPO	23.00 ±2.07	15.33 ±.41	48.0 ±2.28*	13.3 ±.28***	74 ±3.32***	11.79 ±.19*
Ovary weight	22.66 ±4.26	C	35.66 ±2.25	29.71 ±2.3	164.0 ±4.98	42.41 ±2.96	188.66 ±5.12	30.92 ±.52
		HPR	43.0 ±3.11*	33.07 ±2.8	111±3.75***	34.96 ±1.15*	222.5 ±5.53***	35.88 ±3.82
		HPO	47.66 ±3.27*	31.77 ±2.21	153.6 ±4.86	42.68 ±.76	219.33 ±6.1**	34.95 ±1.89
Oviduct weight	0.89 ±0.07	C	16.00 ±.96	13.33 ±.41	71.0 ±2.38	18.36 ±.28	95.33 ±3.75	15.62 ±1.08
		HPR	20.00 ±1.02*	15.38 ±.49**	57.0 ±2.25**	17.95 ±1.55	77.06 ±2.77**	12.42 ±2.27
		HPO	17.00 ±.81	11.33 ±1.51	50.6 ±2.5***	14.07±.5***	117.33 ±4.02**	18.69 ±1.88

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

Table 2. Changes in weights of Liver and lymphoid organs (in gms) of HPR and HPO pullets under LP.

(weight in gms)	One day old chicks		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	4.03 ±.24	3.36 ±.24	8.43 ±.29	2.18 ±.061	10.16 ±2.79	1.66 ±.086
		HPR	4.34 ±.17	3.33 ±.096	7.12 ±.54*	2.24 ±.093	11.17 ±2.91	1.80 ±.054
		HPO	4.84 ±.25*	3.22 ±.050	7.93 ±.59	2.20 ±.013	13.08 ±2.68	2.18 ±.034***
Thymus weight	0.193 ±0.049	C	0.258 ±.023	0.21 ±.010	1.62 ±.050	0.42 ±.040	1.67 ± .09	0.27 ± .006
		HPR	0.268 ±.34	0.206 ±0.22	0.86 ±.016***	0.27 ±.004***	1.76 ±.12	0.284 ±.026
		HPO	0.295 ±.026	0.196 ±0.18***	1.48 ±.081	0.41 ±.026	2.43 ±.31*	0.388 ±.021***
Bursa weight	0.083 ±0.017	C	0.095 ±.008	0.079 ±.008	1.50 ±.008	0.388 ±.031	1.65 ±.13	0.271 ±.030
		HPR	0.214 ±.008***	0.164 ±.005***	0.933 ±.016***	0.29 ±.028***	1.48 ±.17	0.239 ±.016
		HPO	0.216 ±.016***	0.144 ±.009***	0.793 ±.011***	0.22 ±.008***	2.56 ±.21***	0.408 ±.010***
Spleen weight	0.150 ±0.085	C	0.085 ±.005	0.070 ±.003	0.589 ±.014	0.152 ±.014	0.747 ±.037	0.122 ±.008
		HPR	0.140 ±.026*	0.107 ±.010***	0.511 ±.014	0.160 ±.007	0.722 ±.020	0.116 ±.007
		HPO	0.216 ±.012***	0.144 ±.004***	0.465 ±.026***	0.12 ±.005***	1.31 ± .098***	0.208 ±.011***

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

Table 3. Monthly growth rate and Growth indices of Body (gms) and thyroid, adrenal, ovary and oviduct (mg) of HPR and HPO pullets under LP.

		0-30 days		30-60 days		60-90 days		Overall	
		growth rate	growth index	growth rate	growth index	growth rate	growth index	growth rate	growth index
Body weight	C	3.12	--	8.88	--	7.44	--	6.48	--
	HPR	3.44	--	6.24	--	10.09	--	6.59	--
	HPO	4.11	--	7.00	--	8.90	--	6.67	--
Thyroid weight	C	0.155	0.049	0.455	0.051	0.628	0.084	0.413	0.063
	HPR	0.144	0.041	0.378	0.060	0.957	0.294	0.493	0.074
	HPO	0.155	0.037	0.329	0.047	0.789	0.088	0.433	0.064
Adrenal weight	C	0.263	0.084	0.611	0.068	0.555	0.074	0.476	0.073
	HPR	0.199	0.034	0.822	0.131	1.00	0.099	0.647	0.098
	HPO	0.341	0.082	0.833	0.119	0.866	0.097	0.680	0.102
Ovary weight	C	0.433	0.138	4.27	0.480	0.822	0.110	1.84	0.283
	HPR	0.678	0.197	2.26	0.362	3.71	0.367	2.22	0.336
	HPO	0.833	0.202	3.53	0.504	2.18	0.244	2.18	0.326
Oviduct weight	C	0.408	0.130	1.83	0.206	0.811	0.109	1.01	0.155
	HPR	0.541	0.157	1.83	0.197	0.668	0.066	0.814	0.123
	HPO	0.441	0.107	1.12	0.160	2.22	0.249	1.26	0.188

Values : Mean

Table 4. Growth rate and growth indices of liver and lymphoid organs (in gms) of HPR and HPO pullets under LP.

		0-30 days		30-60 days		60-90 days		Overall	
		growth rate	growth index	growth rate	growth index	growth rate	growth index	growth rate	growth index
Liver weight	**C**	0.131	0.041	0.146	0.0164	0.057	0.0076	0.111	0.017
	HPR	0.141	0.040	0.092	0.014	0.135	0.0133	0.123	0.0186
	HPO	0.158	0.038	0.103	0.014	0.171	0.019	0.144	0.021
Thymus weight	**C**	0.0021	0.0006	0.045	0.005	0.0015	0.0002	0.016	0.0024
	HPR	0.0025	0.0007	0.019	0.003	0.030	0.0029	0.017	0.0025
	HPO	0.0034	0.0008	0.039	0.0055	0.031	0.0003	0.024	0.0035
Bursa weight	**C**	0.0004	0.0001	0.046	0.0051	0.0051	0.0006	0.017	0.0002
	HPR	0.0043	0.0012	0.023	0.0036	0.018	0.0017	0.015	0.0022
	HPO	0.0044	0.0010	0.019	0.0027	0.058	0.0006	0.027	0.0040
Spleen weight	**C**	0.0021	0.0006	0.016	0.0018	0.0051	0.0006	0.0066	0.0010
	HPR	0.0003	0.0008	0.012	0.0019	0.007	0.0069	0.0063	0.0010
	HPO	0.0022	0.0053	0.0083	0.0118	0.028	0.0031	0.012	0.0017

Values : Mean

Table **5a.** Changes in serum corticosterone, T_3, T_4, and progesterone of HPR and HPO pullets under **LP.**

		30 days	**60 days**	**90 days**
Corticosterone	**C**	3.59 ±.22	4.1 ±.17	1.46 ± .04
	HPR	3.74 ±.17	1.28 ±.09	2.56 ±.03
	HPO	2.63 ±.21	0.71 ±.02	1.56 ±.07
T_3	**C**	1.21 ±.05	0.617 ±.04	0.821 ±.05
	HPR	0.764 ±.03	0.798 ±.06	0.940 ±.04
	HPO	0.790 ±.07	1.26 ±.09	0.760 ±.05
T_4	**C**	4.65 ±.12	4.13 ±.13	3.99 ±.22
	HPR	1.78 ±.07	3.03 ±.18	4.30 ±.21
	HPO	3.43 ±.19	4.62 ±.17	4.49 ±.24
Progesterone	**C**	0.293 ±.03	0.207 ±.03	0.340 ±.04
	HPR	0.234 ±.05	0.125 ±.02	0.316 ±.06
	HPO	0.225 ±.06	0.220 ±.07	0.260 ±.05

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

Table 5b. Ratios of serum hormones in HPR and HPO pullets under LP.

		30 days	60 days	90 days
$T_3 : T_4$	C	0.260	0.149	0.205
	HPR	0.429	0.263	0.218
	HPO	0.230	0.272	0.169
T_3 : CORT	C	0.320	0.801	0.562
	HPR	0.204	0.623	0.367
	HPO	0.300	1.77	0.487
T_4 : CORT	C	1.23	5.36	2.73
	HPR	0.475	2.36	1.67
	HPO	1.30	6.50	2.87
T_3 : Body wt.	C	0.01	0.0015	0.0013
	HPR	0.0058	0.0025	0.0015
	HPO	0.0052	0.0035	0.0012
T_4 : Body wt.	C	0.038	0.010	0.0065
	HPR	0.013	0.0095	0.0069
	HPO	0.022	0.012	0.0071
T_3 : Thyroid wt.	C	0.134	0.027	0.019
	HPR	0.088	0.039	0.019
	HPO	0.037	0.064	0.017
T_4 : Thyroid wt.	C	0.516	0.182	0.096
	HPR	0.205	0.151	0.088
	HPO	0.381	0.234	0.103
CORT : Body wt.	C	0.031	0.0019	0.0023
	HPR	0.028	0.0040	0.0041
	HPO	0.017	0.0019	0.0024
CORT : Adrenal wt.	C	0.182	0.019	0.026
	HPR	0.229	0.064	0.052
	HPO	0.144	0.014	0.021

Values : Mean

Table 6. Histometrics of the ovarian follicles in ovary of HPR and HPO pullets under LP.

			S_1 (<30µm)	S_2 (31-90µm)	S_3 (91-120µm)	B1 (121-240µm)	B2 (241-440µm)	S (6-200µm)	B (200-300µm)	L (>300µm)	Total
30 days	C	POF	10 (13.5%)	54 (72%)	10 (13.5%)	--	--	74	--	--	74
		AF	4 (40%)	12 (22.2%)	2 (20%)	--	--	--	--	--	18 (24%)
	HPR	POF	34 (54.8%)	23 (37%)	5 (8.06%)	--	--	62	--	--	62
		AF	2 (5.8%)	2 (8.6%)	1 (20%)	--	--	--	--	--	5 (8%)
	HPO	POF	3 (5%)	42 (70%)	11 (18%)	4 (6.6%)	--	59	1	--	60
		AF	--	6 (14.2%)	2 (18%)	--	--	--	--	--	8 (13.3%)
60 days	C	POF	10 (21.2%)	18 (38.2%)	4 (8.5%)	13 (27.6%)	2 (4.2%)	32	10	5	47
		AF	--	5 (28.7%)	1 (25%)	4 (30%)	--	--	--	--	10 (21.2%)
	HPR	POF	35 (63.6%)	8 (14.5%)	4 (7.2%)	5 (9%)	3 (5.4%)	47	4	4	55
		AF	--	1 (12.5%)	--	--	--	--	--	--	1 (1.8%)
	HPO	POF	28 (42.4%)	7 (10.6%)	16 (24.2%)	15 (22.7%)	--	61	5	--	66
		AF	--	4 (57%)	3 (18.7%)	2 (13.3%)	--	--	--	--	9 (13.6%)

Values : Mean .

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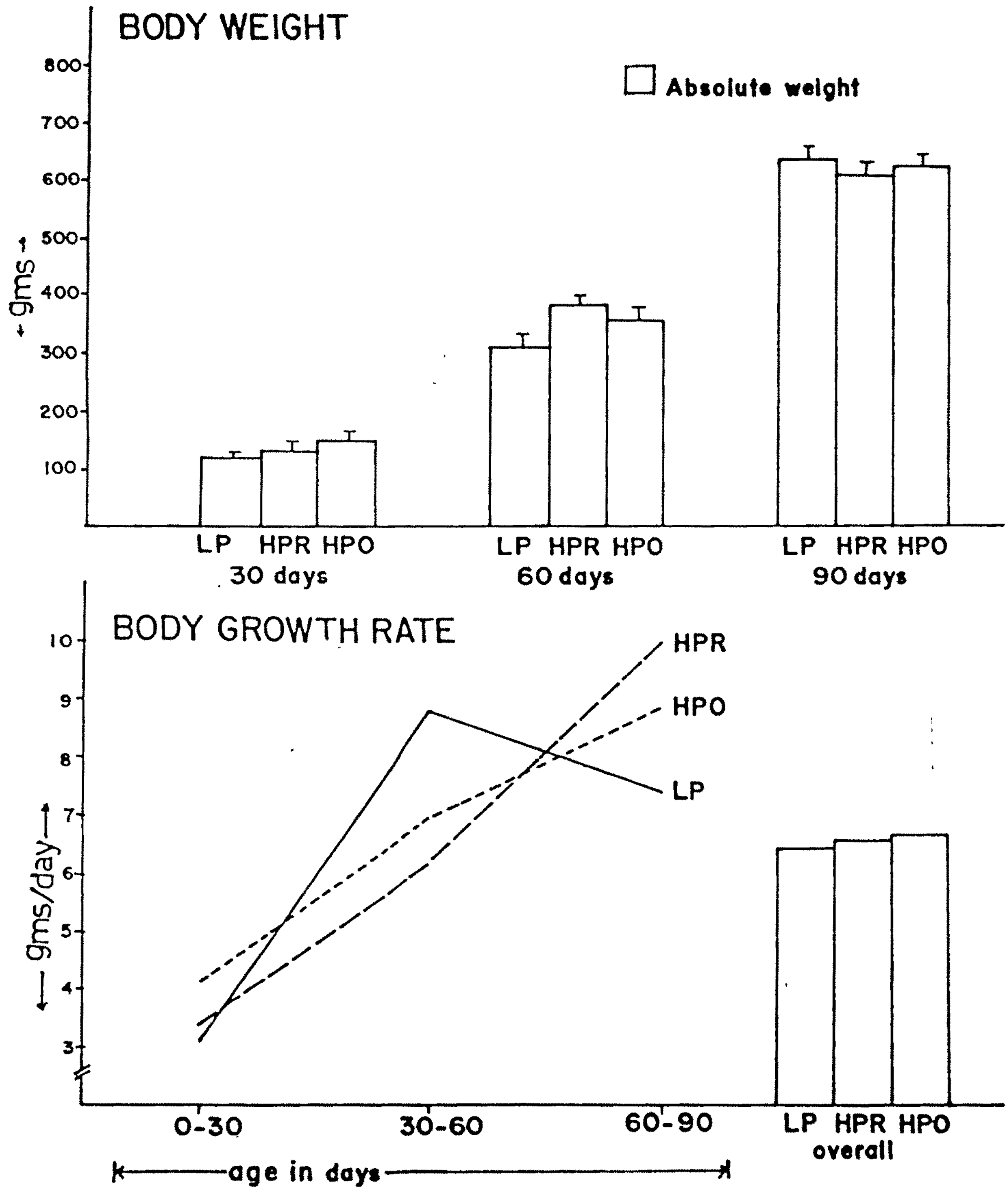
			S_1 (<30µm)	S_2 (31-90µm)	S_3 (91-120µm)	B1 (121-240µm)	B2 (241-440µm)	S (6-200µm)	B (200-300µm)	L (>300µm)	Total
90 days	C	POF	29 (34.9%)	37 (44.5%)	4 (4.8%)	8 (9.6%)	5 (6%)	73	5	5	83
		AF	--	--	2 (50%)	2 (25%)	--	--	--	--	4 (4.8%)
	HPR	POF	31 (60.7%)	3 (5.8%)	2 (3.9%)	9 (17.6%)	6 (11.7%)	39	8	4	51
		AF	--	1 (33%)	--	2 (22.1%)	--	--	--	--	3 (5.8%)
	HPO	POF	2 (4.2%)	17 (36.1%)	9 (19.1%)	11 (23.4%)	8 (17.02%)	33	9	5	47
		AF	--	5 (29%)	2 (22%)	--	6 (12.5%)	--	--	--	8 (17.2%)

Values : Mean

Fig. 1. Body weight gain upto 180 days (6 months) of **HPR** and **HPO** hens under **LP.**



*-P<.05, **-P<.005, ***-P<.0005

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

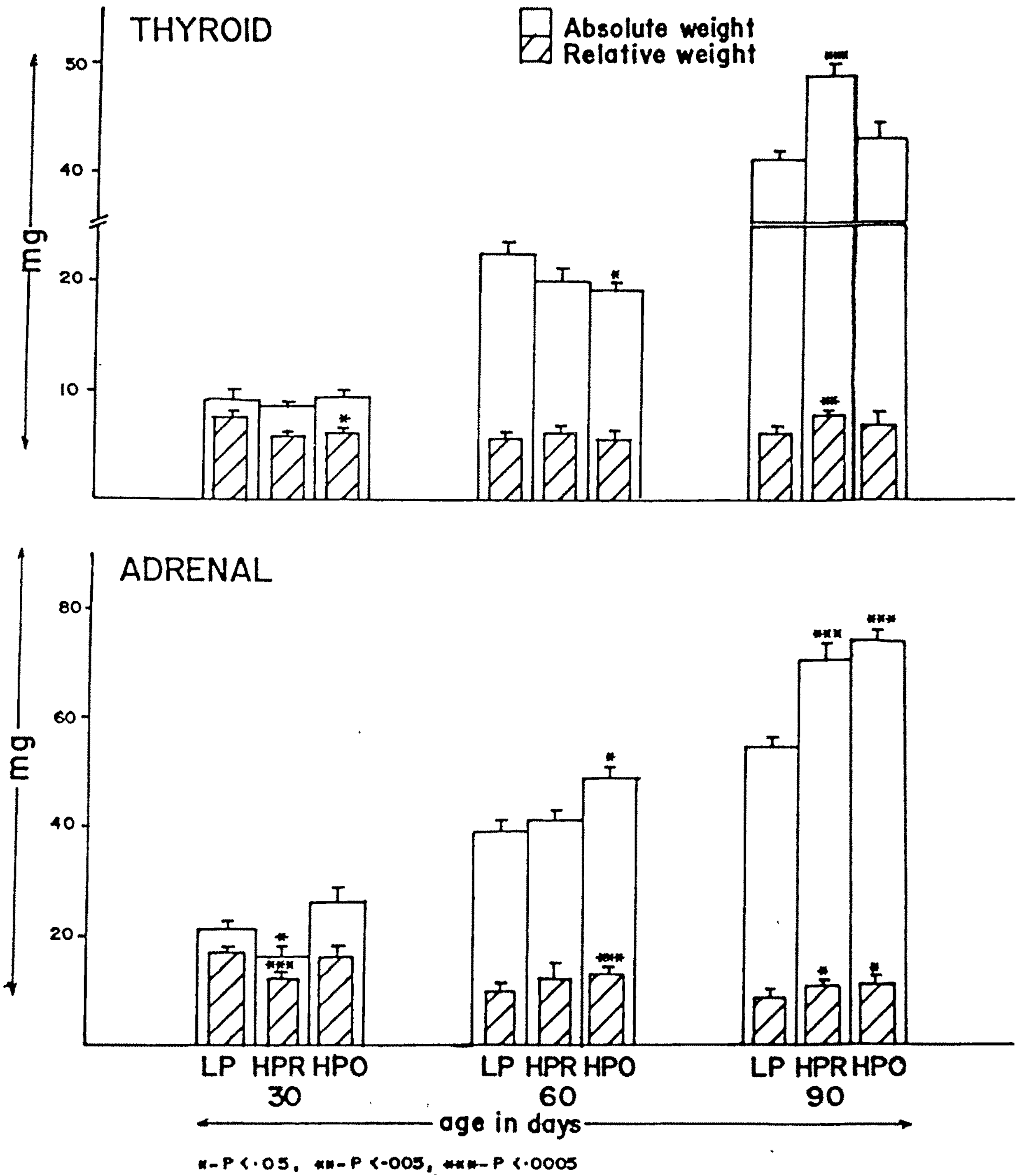


Fig. 2b. Absolute and relative weights of ovary and oviduct (in mg) of HPR and HPO chicks under LP.

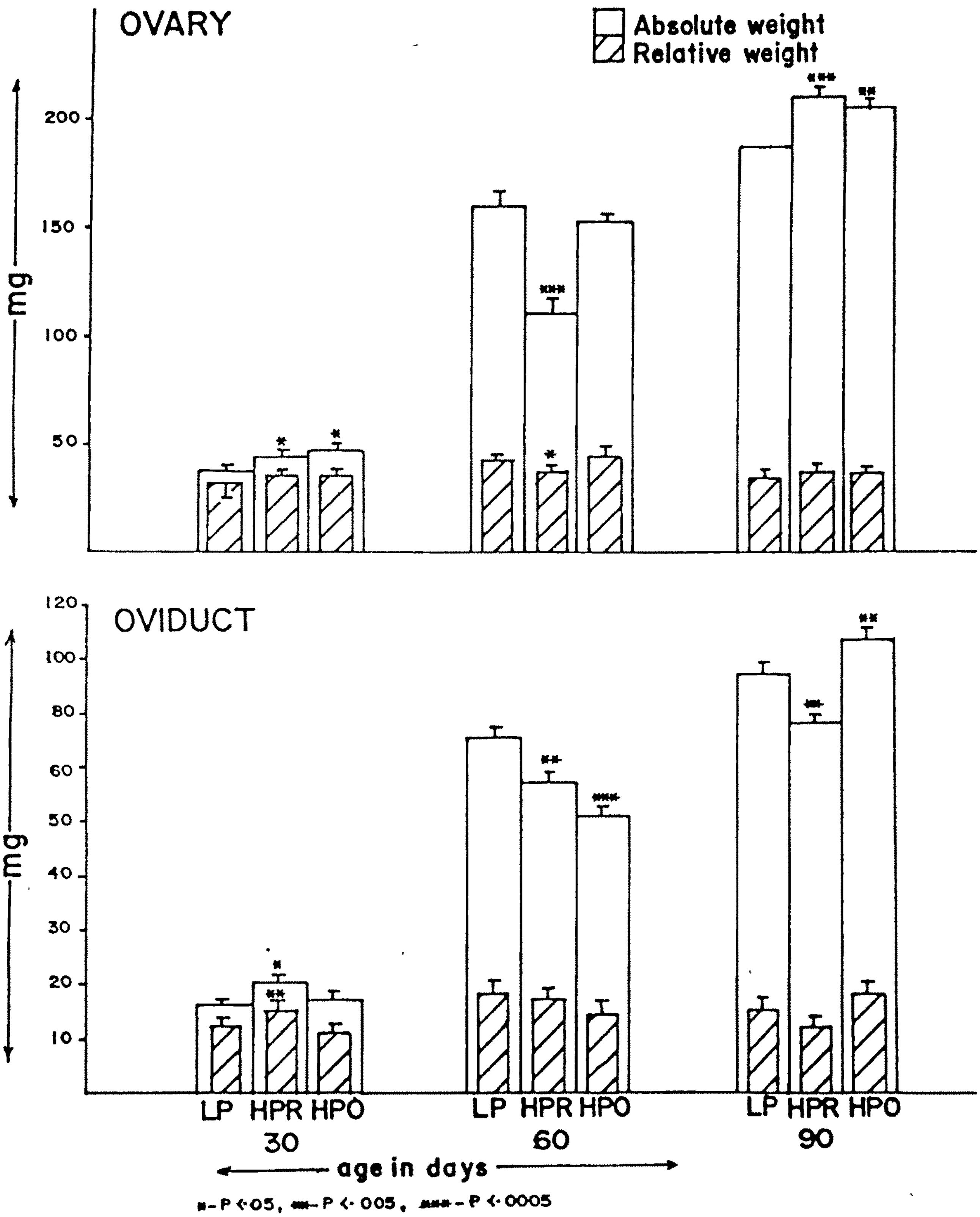
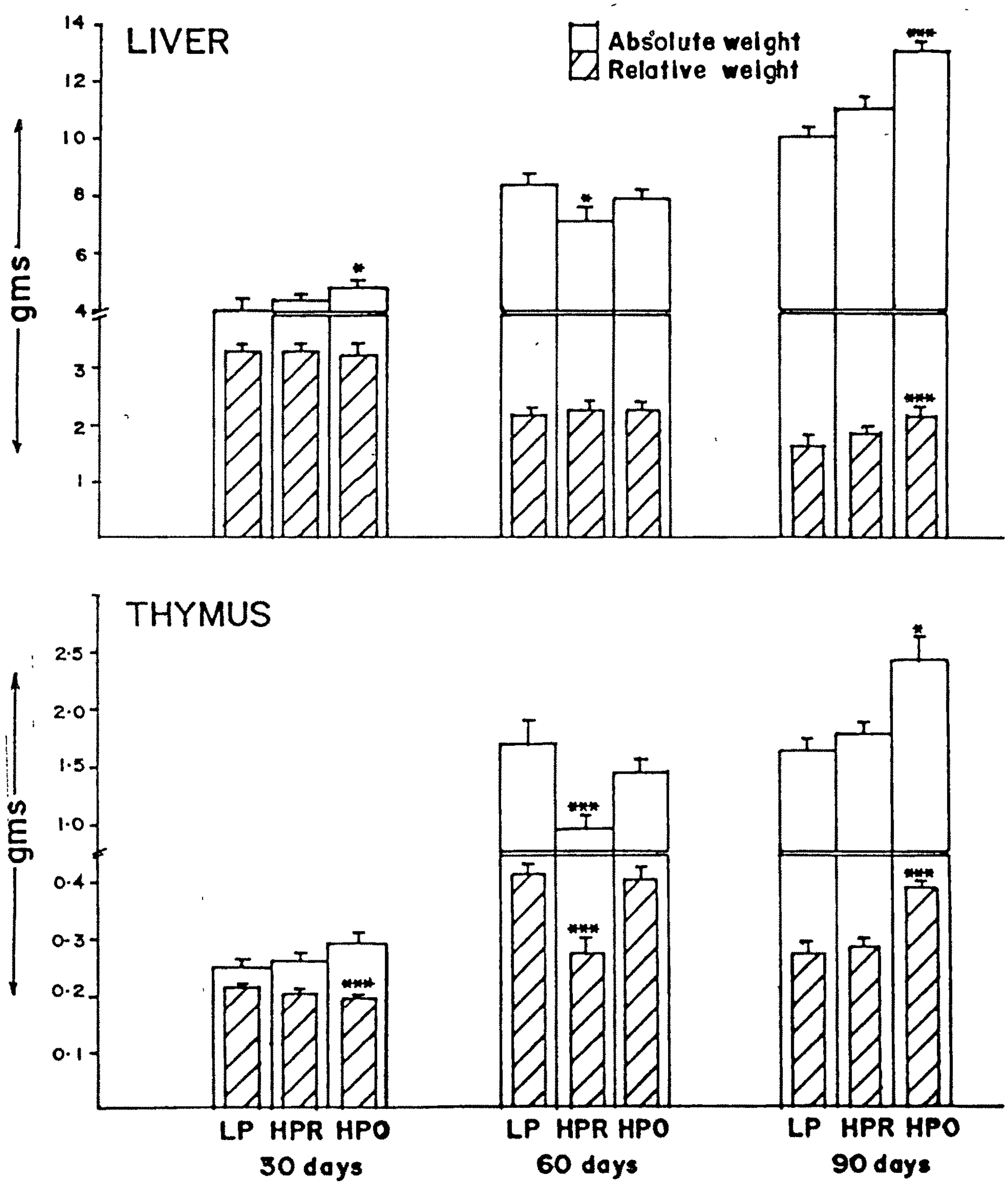
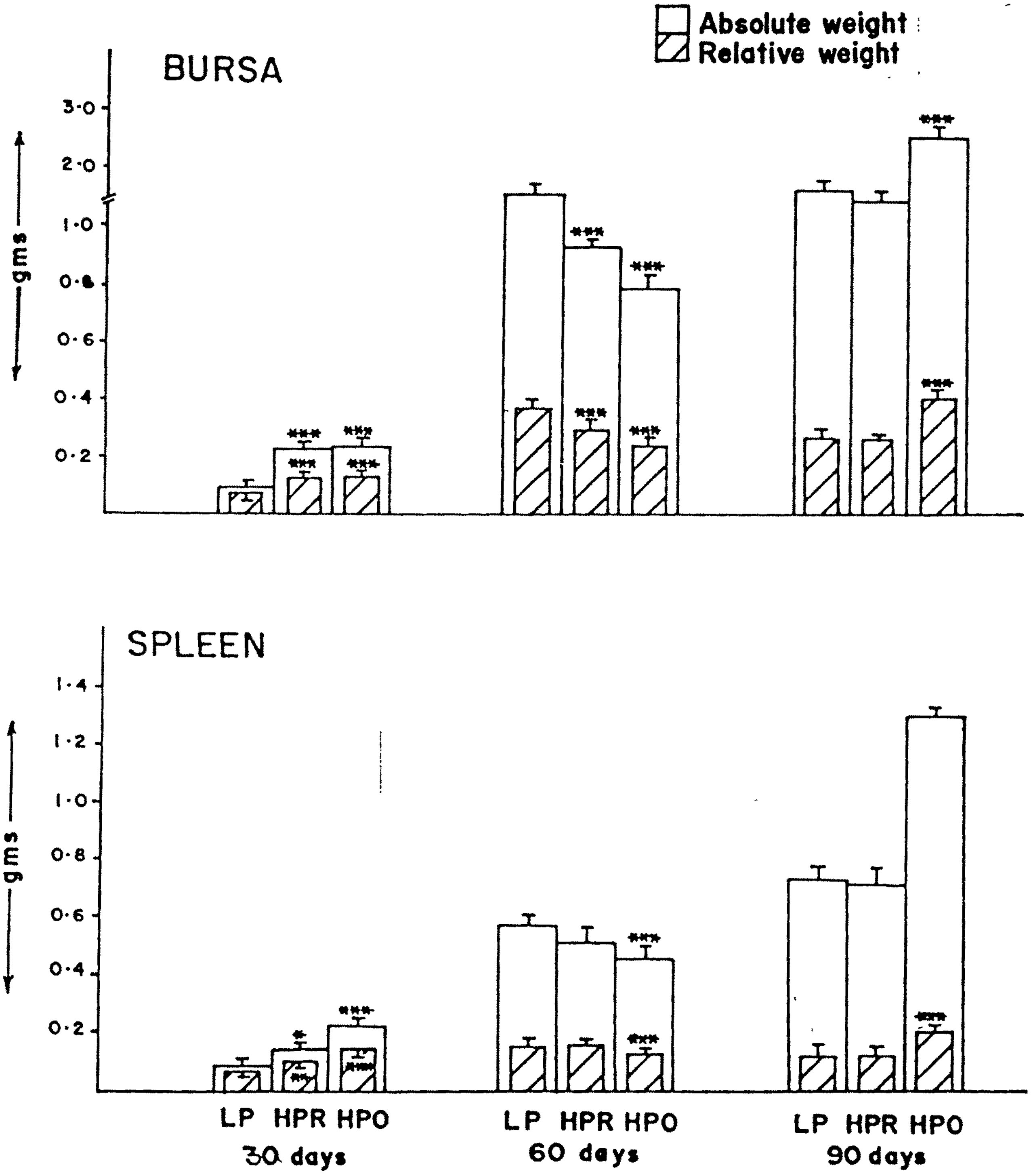


Fig. 2c. Absolute and relative weights of liver and thymus (in gms) of HPR and HPO chicks under LP.



*-P<.05, **-P<.005, ***-P<.0005

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



*-P<.05, **-P<.005, ***-P<.0005

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

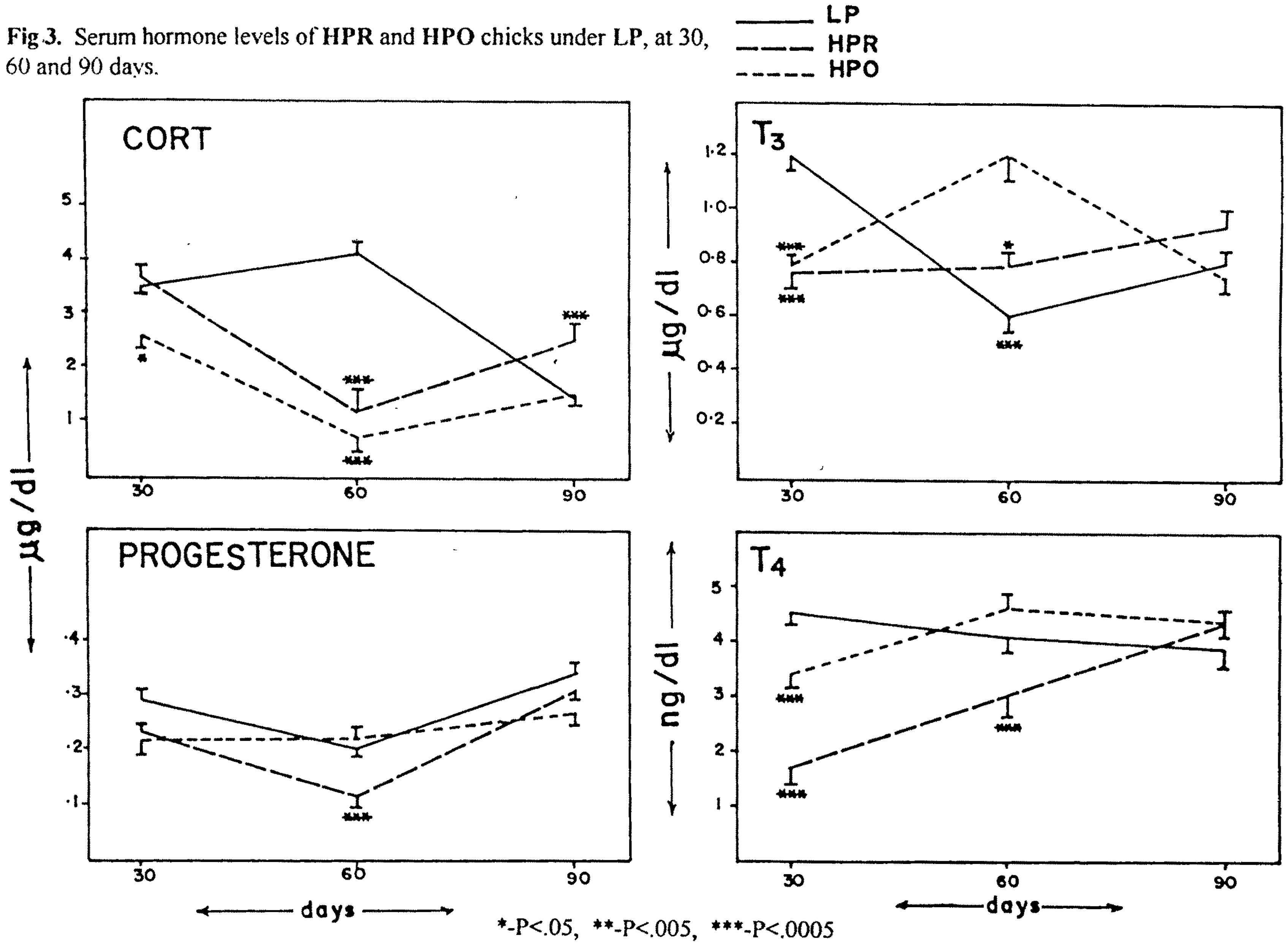


Plate 1 (Figs. 1-9)

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

Fig. 1. Thyroid of 30 day HPR chick. Note the increased follicular cell height and colloid depletion.

Figs. 2-3. Thyroid of 60 and 90 day HPR chick showing large to medium sized follicles and reduced height of follicular epithelium. Follicles show moderate colloid depletion.

Fig. 4. Thyroid of 30 day control chick showing medium to large sized follicles with varying contents of colloid and a cuboidal follicular epithelium.

Figs. 5-6. Thyroid of 60 and 90 day control chick showing a flat follicular epithelium and overall colloid retention.

Figs. 7-8. Thyroid of 30 and 60 days HPO chick showing follicles with varying degrees of colloid content and cuboidal follicular epithelium.

Fig. 9. Thyroid of 90 day old HPO chick showing low epithelium with prominent nucleus. Follicles showing overall colloid retention.

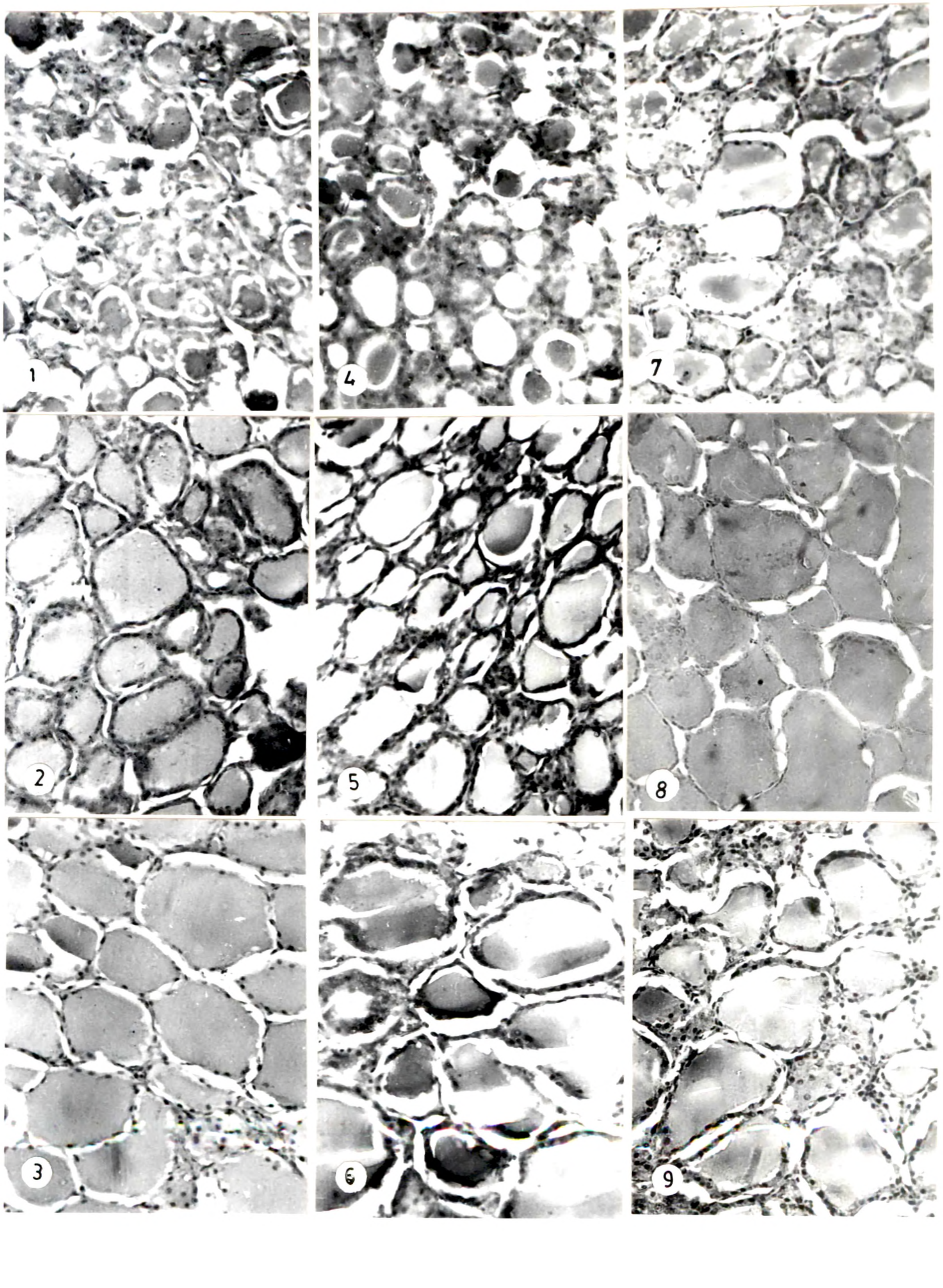

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Plate 2 (Figs. 10-18)

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

Figs. 10-12. Adrenal of 30, 60 and 90 days old HPR chick showing prominent and well formed cortical cords. Note the vacoulization and secretory exhaustion. Medullary cells prominent showing secretory exhaustion.

Figs. 13-15. Adrenal of 30, 60 and 90 days old control chick showing prominent cortical cords with secretory exhaustion. Medullary cells also appear to be active.

Figs. 16-18. Adrenal of 30, 60 and 90 days old HPO chick showing prominent cortical cords. But the cortical cells appear to be relatively inactive from nuclear characteristics. Medulla hypertrophied and active.

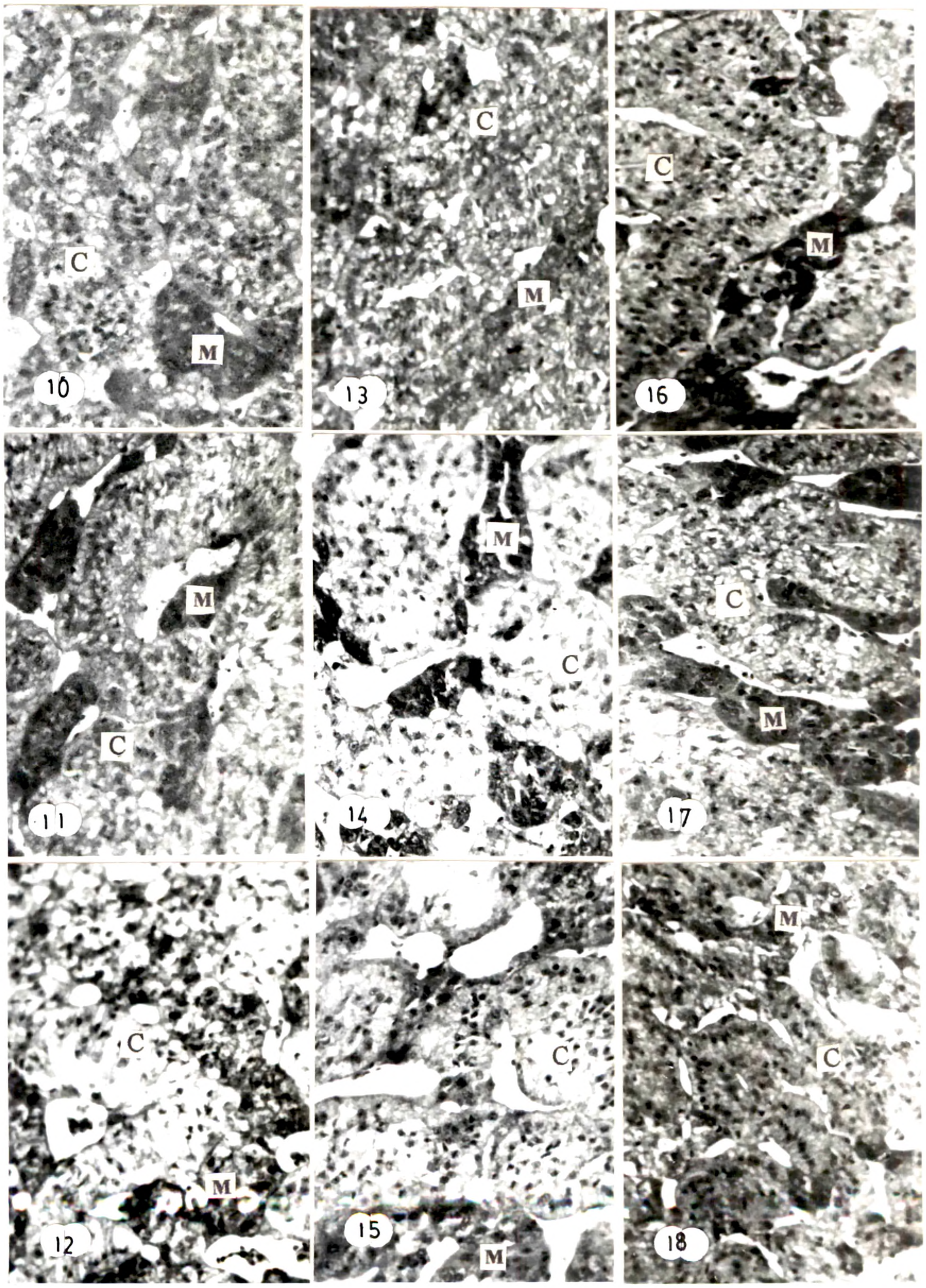
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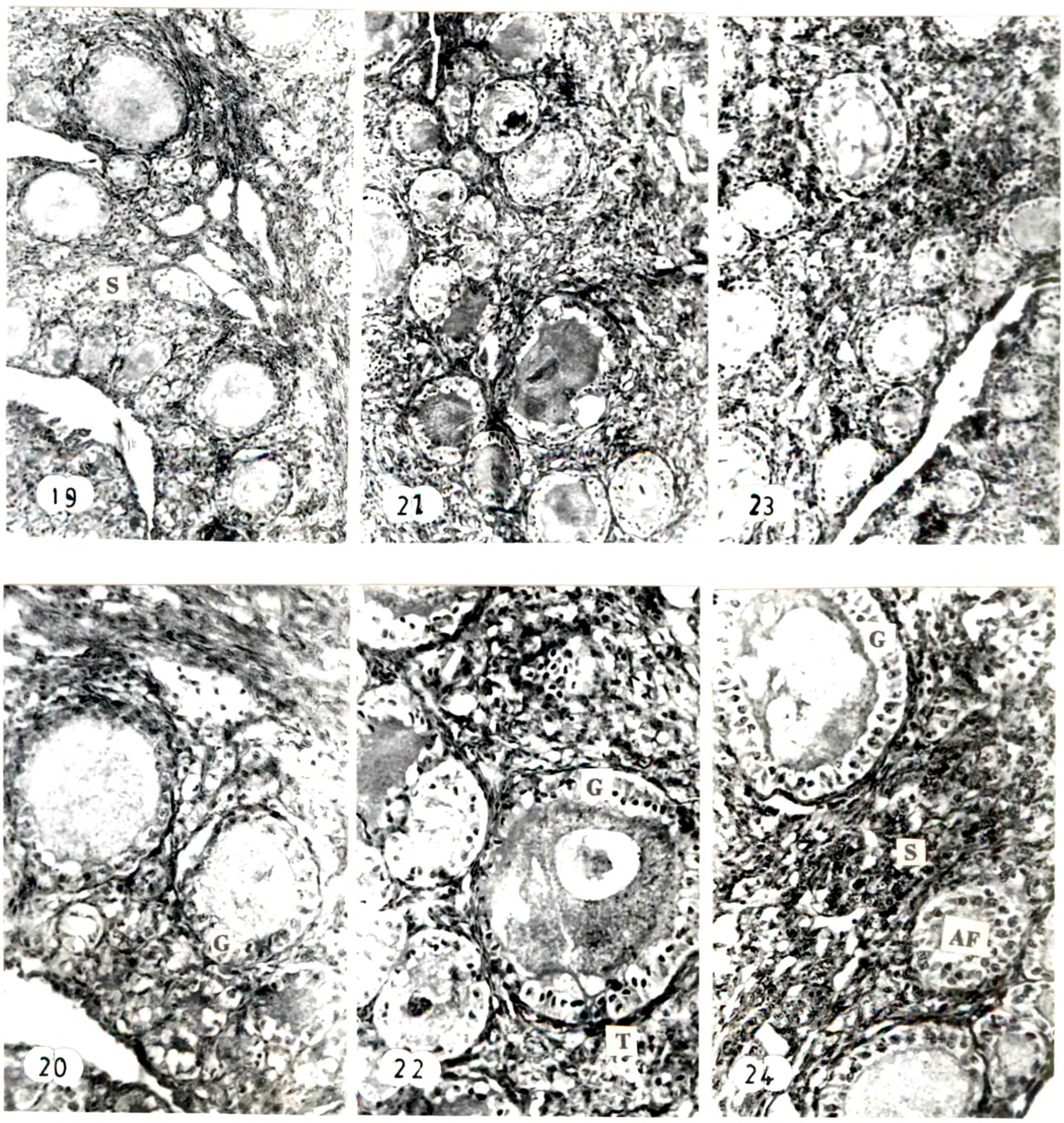
Plate 3 (Figs. 19-24)

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

Figs. 19-20. Ovary of HPR chick showing overall more number of follicles Note the prominent and hypertrophied granulosa. Stromal differentiation into interstitial glands evident.

Figs. 21-22. Ovary of control chick showing many primary and primordial follicles with hypertrophied granulosa. Note the thecal condensation around larger follicles.

Figs. 23-24. Ovary of HPO chick showing small to medium-sized follicles with hypertrophied granulosa. Stroma hypertrophied and differentiation into interstitial glands evident. Larger follicles show disproportionate yolk deposition. Note the follicular atresia.


19
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21
23
20
G
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24
G
S
AF

Plate 4 (Figs. 25-30)

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

Figs. 25-26. Ovary of HPR chick showing many large follicles and well developed stroma.

Figs. 27-28. Ovary of control chick showing prominent and active theca surrounding large follicles. Some follicles are atretic.

Figs. 29-30. Ovary of HPO chick showing many small to medium sized follicles, some are atretic. Hypertrophy of granulosa cells, and stromal differentiation into interstitial glands are evident.

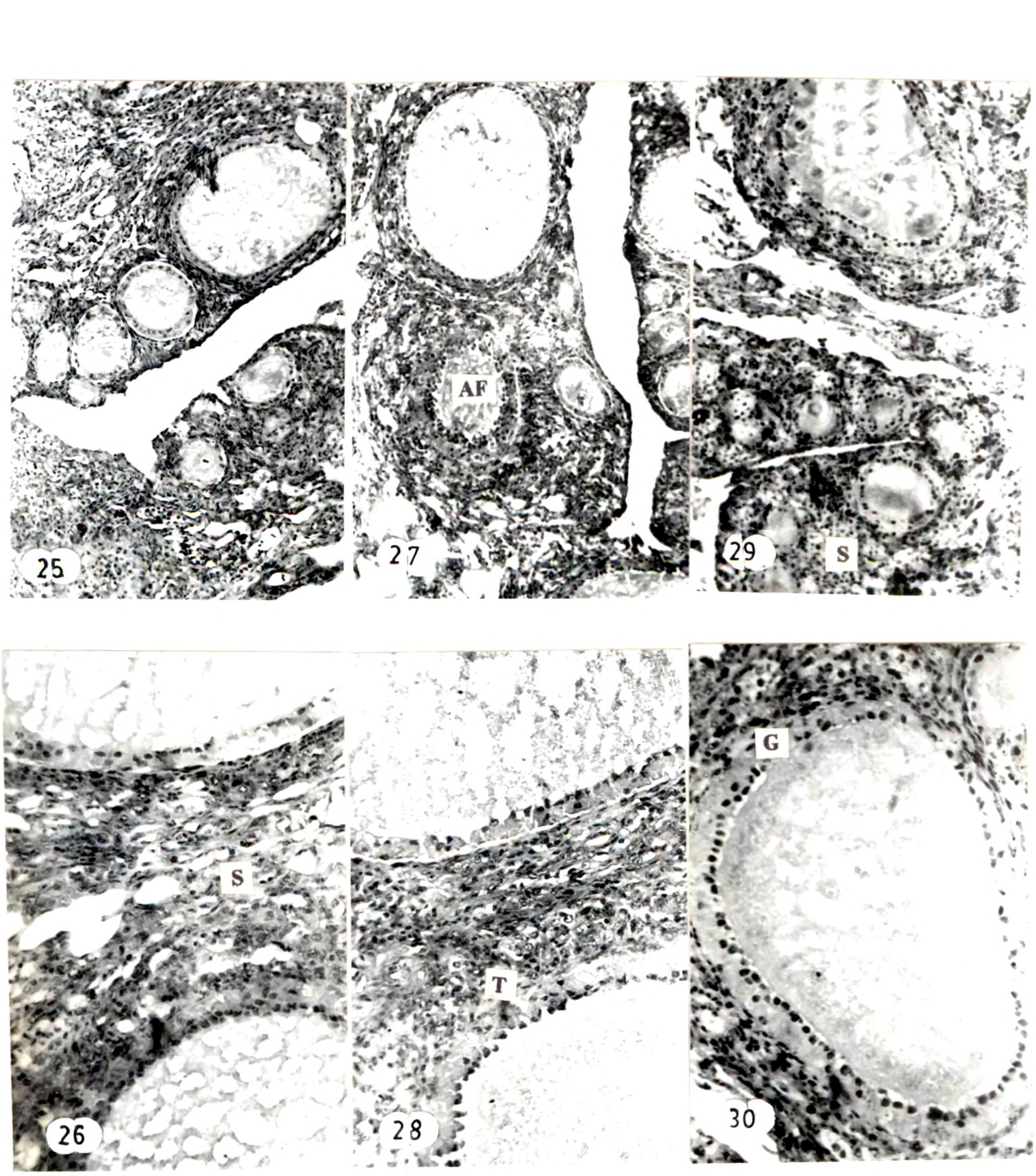
25
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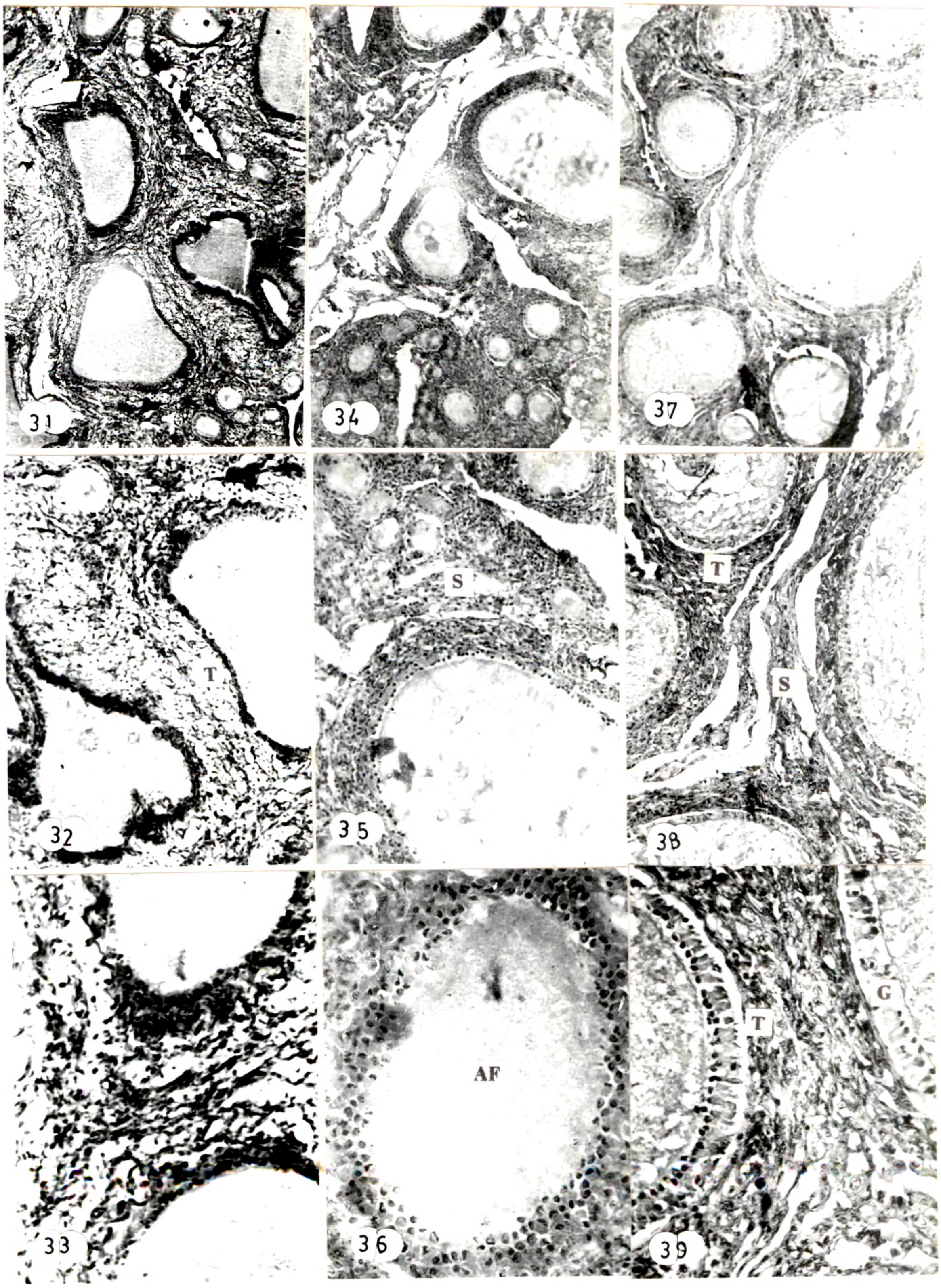
Plate 5 (Figs. 31-39)

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

Figs. 31-33. Ovary of HPR chick showing many small to medium sized follicles. Large follicles few in number. Theca and granulosa cells show differential activity. Note the hyperplastic changes affecting granulosa.

Figs. 34-36. Ovary of control chick showing many medium to large sized follicles with prominent hypertrophied granulosa. Note the atretic changes in some follicles.

Figs. 37-39. Ovary of HPO chick showing overall lesser number of small follicles and greater number of medium to large sized follicles with prominent granulosa. Theca well differentiated, stromal tissue loose and hypertrophied.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chicks throughout. Whereas in the ovary of control chicks, the degree of atresia tended to decrease progressively from a higher percentage from 30-90 days, in the ovary of HPO chicks, there was a constant average level of atresia throughout. Whereas the ovary of 90 day old control and HPR chicks showed a persistent higher number of follicles of 6-30µm size, that of HPO chicks showed an almost complete depletion of such smaller follicles (table 6).

Discussion :

Neither HPR nor HPO has any influence on the increase in body weight though, a marginally insignificant favourable influence is indicated, and in general, is quite similar to that of NLD chicks. Clearly, the body weight and growth rate are not altered by a sustained long photoperiod or superimposed HPR or HPO. However, the weight of organs and their growth kinetics are differentially altered in the three groups of chicks. The relative weight of liver, lymphoid organs, adrenal and oviduct is significantly reduced in LP chicks, while that of thyroid and ovary is significantly increased. These differences in relative weight are well reflected in the overall growth indices of these organs (table 4). Though the relative weight of liver showed an increment in HPR chicks, that of the lymphoid organs did not show any change and, the overall growth indices of the lymphoid organs were similar in both LP and HPR chicks. But the relative weight and growth indices of liver and lymphoid organs were significantly more in the HPO chicks. Apparently, a superimposed HPO on LP, nullifies the retardatory influence of LP. In this respect, whereas the relative weight and growth index of thymus became similar to that of NLD, those of liver, bursa and spleen were significantly more than even NLD. Except for the oviduct, which showed significantly reduced relative weight and growth index in HPR chicks, thyroid, adrenal and ovary showed significantly increased relative weight and growth indices in both HPR and HPO chicks, with relatively greater values in the former (table 3). The recorded data clearly show differential chronological alterations in terms of relative weight, growth rate and growth indices of various organs in the three experimental groups. The increased relative weight and growth index of liver in the HPR and HPO groups of chicks, are essentially due to minimised chronological decrease in relative weight during the three months, more prominently in the HPO group. The highest relative weight and growth indices of lymphoid organs in the LP chicks are attained during the second month followed by a decrement during the third month. Differential effects of HPR and HPO are well reflected in the relative weight and growth indices recorded for the three lymphoid organs. Whereas the relative weight and growth indices of both spleen and bursa showed a decrease in the third month in HPR chicks, like in the control, they showed a continuous increment in HPO chicks. In the case of thymus, whereas the HPR chicks did not record an increase characteristic of second month, the HPR chicks registered only a marginal decrement during the third month. Overall, HPO seems to exert a favourable influence on the growth of all organs while, HPR has a differential effect with a favourable influence being manifested by non-lymphoid organs other than liver.

Changes in the relative levels of CORT, T_3, and T_4 do not seem to show any clear cut pattern in HPR and HPO group of chicks compared to LP controls (table 5a)(fig. 3). Though there are some differential alterations during the three months, the only definite change is an increased T_4 : CORT ratio in the HPO chicks (table 5b). The induction of mild HPR and HPO in the two experimental groups is clearly indicated not only by the absolute levels of the hormones but also by the CORT : adrenal weight and CORT : body weight ratios. It is clear that the absolute levels of CORT and thyroid hormones and their changes during the 90 days of experimentation, do not permit any meaningful correlations between these hormones and body and organ growth. It is very likely that, even mild alterations in corticosteroid status under LP, have, as yet undefinable consequences in terms of differential organ growth, by altered neuroendocrine mechanisms, which might affect, the free hormone levels, the metabolic clearance of the hormone, as well as the sensitivity/responsiveness towards these hormones. It is clear from the present results, that the growth kinetics of body and organs under superimposed HPR or HPO in LP may not bear any relation with the absolute levels of thyroid and adrenal hormones, as had been inferred earlier for chicks reared under LP compared to those reared under NLD (see chapter 8). More studies are needed on this line to understand the photoperiod-endocrine interactions as related to post-natal growth.

Previously it was inferred that, exposure to LP in the juvenile period has a stimulatory influence on the HHG axis (see chapter 8). This is confirmed by the recorded higher growth rate and growth index of the ovary and significantly higher proportion of big and large follicles during the second month. A comparison of the histometric data of the ovary reveals a very low progression of follicular development during the first two months followed by an augmented follicular development during the third month in the HPO chicks, as marked by the significantly higher proportion of big and large follicles (table 6). The ovary of HPR chicks also shows higher percentage of big and large follicles (relatively lesser than HPO) though, with a greater rate of follicular transition in the second month relative to HPO chicks, but lower than the control chicks. The HPO chicks have been recorded to give an overall poor egg yield like LP control (see chapter 7). The presently recorded constant higher rate of follicular atresia and the depletion in the number of follicles by third month could be the raison d'etre for the lesser egg yield. In contrast, the HPR chicks have been recorded to lay more eggs (25 eggs) than the LP controls which may be due to the presently observed lesser degree of follicular atresia and, the presence of a higher pool of small follicles even during the third month. Previously, it was speculated that a step down photoschedule reduces egg yield by a probable higher incidence of follicular atresia (see chapter 8). Apparently, a superimposed HPR on LP nullifies the above purported high rate of follicular atresia, which accounts for the increased egg yield.

In terms of initiation of egg laying, it was shown previously that HPO chicks initiated egg laying significantly earlier (by as much as 76 days) while, the HPR chicks also initiated egg laying earlier though by only 13 days (see chapter 4). An integrated model, based on photoperiodic response of birds, has proposed both stimulatory and inhibitory input to hypothalamic GnRH neurons by long and short photoperiod respectively (Sharp, 1993). In the above model, prolonged exposure to long days is suggested to induce photorefractoriness by way of increased negative input to the GnRH neurons. In the present study, exposure of chicks to LP from the day of hatch till 90 days, also seems to induce juvenile photorefractoriness during the second and third, month preceded by nevertheless increased activation of the hypothalamo-hypophyseal-ovarial axis. This inference is substantiated by the growth rate and growth index of the ovary which were greater during 0-30 and 30-60 days and, significantly reduced between 60 and 90 days in LP chicks compared to NLD chicks. Further, follicular development as marked by the percentage of follicles undergoing transition from small to big and big to large follicles was significantly increased by 60 days and decreased by 90 days in LP chicks. The shifting of these chicks to a short photoschedule at the end of 90 days initially strengthens the LP induced negative input to the hypothalamus, subsequently followed by dissipation of this negative input resulting in gradual activation of the hypothalamo-hypophyseal-ovarial axis. This, probably provides adequate explanation for the reported delay in the initiation of egg laying in these birds (see chapter 4). Based on the growth rates and growth indices of ovary and, on the rate of follicular transition into higher size hierarchy in HPR and HPO chicks, it is surmisable that, while in HPR chicks the setting in of the photorefractoriness is delayed and, probably corresponds with early periods of exposure to LP, in the HPR chicks photorefractoriness is totally prevented and probably even weakens the purported negative input due to exposure to LP. The slightly earlier initiation of egg laying in HPR chicks and, significant advancement of egg laying in HPO chicks, probably find correlation in the above concept. Overall, the present study tends to indicate differential effects of superimposed HPR or HPO over LP on the hypothalamo-hypophyseal axis and, on intraovarian functions (though by as yet unknown mechanisms) resulting in higher egg yield with slightly early initiation in the former and, reduced egg yield with significant advanced initiation in the latter. These observations suggest some intricate interactions between corticosteroids and hypothalamo-hypophyseal-ovarian axis and other events related with ovarian functions, which need to be evaluated in greater detail.