GENERAL CONSIDERATIONS

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Growth of chicks after hatching concerns primarily with traversing the developmental gulf between the neonate and adult and could justifiably involve many adjustments and growth patterns aimed at attaining the adult attributes. The problem of post-natal development is now more fully appreciated by developmental biologists in all its complexity and subtlety. Early studies of post-natal development in birds were mostly descriptive and confined to growth of species in the wild (Bergtold, 1913; Stanwood, 1913). Latimer's work (1924, 1925a, b, 1927) in the same line in domestic fowl though, thorough, is long and tedious. Thus since early times, chicks of domestic fowl proved to be a good experimental model for neonatal studies. As far as the development and growth of chick is concerned, lot of descriptive information is available while, the changes at physiological level still remain less well known. Chicks are in this sense, convenient subjects for experimental physiology and the observations of Pembrey et al. (1895) on the metabolic response of chicks to different temperatures constitute an early attempt on this line.

The role of hormones during the ontogenetic development, specifically, the post-hatched development should be expectedly more dynamic and interesting, primarily because the avian post-natal development phase serves as a link between hormone independent embryonic phase and hormone dependent adult phase and, secondarily because the organism shows varying sensitivity to quantitatively and qualitatively changing

endocrine principles, and gradually adapt for later stages of life. Thus the role of hormones in avian growth becomes significant. Experimental work by various researchers from 1947-1974 have shown that growth hormone and thyroid hormone play important roles in avian post-natal development. It is obvious that the circulating levels of hormones would affect the growth and metabolism of various developing tissues. Investigations on the hormonal regulation of growth have remained restricted to only the effects of growth hormone and thyroid hormone. Role of growth hormone is indicated by the observations of increased growth rate, nitrogen retention and bone growth in hypophysectomised chicks given chicken pituitary extracts (Libby et al., 1955; Glick, 1960b; Nalbandov, 1966). Disruption of thyroid activity by surgical thyroidectomy, radiothyroidectomy or chemical inhibition of thyroid hormones has been shown to have severe retarding effect on growth (Blivaiss, 1947; Winchester and Davis, 1952; Marks, 1971; King and King, 1973; Howarth and Marks, 1973). Blivaiss (1947) has observed 35% reduction in weight, retarded bone and feather growth and occurrence of obesity in thyroidectomised fowl. Age specific effects of thyroidectomy on developmental retardation has also been demonstrated (Voitkevich, 1966). Thyroidectomised chicks were found to be fat with retarded skeletal ossification and feather growth. The liver, adrenal glands and kidneys were however found to be four times larger than those of controls based on percentage body weights. Thyroid secretion levels are not only correlated with growth rate (Tanabe, 1965; Voitkevich, 1966) but also with the development of homeothermy (Spiers

et al., 1974). King and King (1973) observed that severe hypothyroidism reduced the muscle mass during growth and decreased the DNA level thereby suggesting a major effect on cell proliferation. Provision of exogenous thyroid hormone usually reverses the effects of thyroidectomy (Voitkevich, 1966; Raheja and Snedecor, 1970; King and King, 1973). It was also observed that providing supplementary thyroxine in moderate doses (2-4 µg/100 g/day) to intact chicks accelerated growth slightly while slightly higher doses (6 µg/100 g/day) depressed growth (Singh et al., 1968). Tanabe (1965) reported variations in secretion levels of thyroxine during post-natal growth. Accordingly, the levels decreased from about 2 µg/100 g/day at two weeks to about 0.5 µg/100 g/day at hundred days. In the experiment by Singh et al. (1968) the maximum increase in growth rate of about 5% occurred between 7 and 39 days. In general, study of hormones and growth tended to show that though the inadequacy of growth and thyroid hormones have severe retarding effects, administration of either of the two hormones to normal animals was unable to stimulate growth appreciably. Apparently, growth is regulated by a complex and subtle endocrine milieu, and not under the purview of any single hormone per se. Another reported role of thyroxine in neonatal chick is its thermogenic ability. Rectal temperatures were significantly increased by 30 minutes by intraperitoneal injections of thyroxine and triiodothyronine (Freeman, 1971). Both hormones were also effective in delaying the fall in rectal temperature when the chicks were exposed to cold. The stimulatory effect of thyroid hormones on oxygen consumption and tissue metabolism especially carbohydrate metabolism and also on early morphogenesis of epidermis and feather growth and molting have been reviewed by Assenmacher (1973).

A scan of the literature on hormonal regulation of post-natal growth of birds reveals that apart from growth hormone and thyroid hormone, other hormones have been generally neglected. Of the various hormones, the probable role of adrenocortical hormones in post-natal development of birds would require attention in the light of the known effects on metabolism in adult animals (Riddle, 1937; Golden and Long, 1942; Stamler et al., 1954; Dulin, 1956; Brown et al., 1958; Baum and Meyer, 1960; Greenman and Zarrow, 1961). Apart from their direct effect on various facets of intermediary metabolism, corticosteroids also exert permissive effects on secretions and functions of other hormones as well (Mialhe, 1958, 1969). In this light, the need to study the role of adrenocortical steroids in the post-natal growth and maturation of chicks need not be overemphasized. The significance of glucocorticoids in the post-hatched phase of avian development can be gleaned from the reports of altered adrenocortical cell steroidogenic capacity during the transition from embryonic to post-embryonic phase of domestic fowl (Carsia et al., 1987), of increased circulating levels of corticosterone at the end of incubation (Kalliecharan and Hall, 1974; Marie, 1981) and of the ability of administered glucocorticoids to stimulate hepatic T_4-5' monodeiodinase activity and the resultant increase in the concentration of T_3 (Borges et al., 1981; Decuypere et al., 1983) in chick embryos and the reversed action of glucocorticoids to decrease T_3 with concomitant increase in reverse T_3 (rT_3) by stimulation of T_4-5 monodeiodinase activity in the post-natal phase (Kühn et al., 1984; Buyse et al., 1987). Though the functions of adrenocortical

hormone on carbohydrate, lipid, protein and electrolyte metabolism have been studied in greater detail in adult birds, the possible role of corticosteroids in post-hatched phase of development, especially in the wake of reported age dependent changes in endocrine milieu (Kuhn et al., 1984; Sinsigalli et al., 1987), needs careful evaluation. The effects of body weight loss and growth inhibition have been noted in birds during cortisol treatment (Kowalewsky, 1962). The growth depressing properties of glucocorticoids have also been demonstrated in chicks (Gavora and Kondra, 1970; Gavora and Hodgson, 1970). Injection of corticosterone to chicks and castrated pheasants has been reported to depress body weight and to increase carcass fat content (Baum and Meyer, 1960; Nagra and Meyer, 1963; Nagra et al., 1963). Magdi and Hutson (1974) reported decreased body wieght in their studies on three week old male chicks treated with dexamethasone and corticosterone. They further showed that dexamethasone caused a significant reduction in ^{22}Na retention while corticosterone treatment lowered adrenal weight. Corticosterone markedly depressed growth and increased feed consumption and fat accumulation in the carcass as well as in the liver of broiler chicks (Bartov et al., 1980a). Injections of steroids decreased growth rate (Sato and Glick, 1970) and lymphoid numbers and size (Dougherty et al., 1964). De la Cruz et al. (1981) reported that cortisol and corticosterone (4 mg/100 g b.w. for 7 days) treatment in laying quails decreased body weight and increased uric acid excretion, liver weight and hepatic glycogen content. Gross et al. (1980) reported that feeding chickens with corticosterone (5-80 ppm) for 10 days resulted in dose related decrease in weight gain, reduced size

of lymphoid organs - spleen, thymus and bursa of Fabricius - testis, adrenals and breast muscle; and increased body and liver fat. The influence of cortisol has been observed in the chick (Bellamy and Leonard, 1965; Adams, 1968) where it caused an inhibition of growth. With high doses, animals lose weight; the liver however continued to increase in size. The size increase could be due to uptake of extra material, rather than any increase in growth rate (Mangnall and Bartley, 1973). Brake et al. (1988) reported reduction in body weight, relative bursa and spleen weights while adrenal weights increased significantly in 6 week old chicks treated with cortisol. Saadoun et al. (1987) showed that daily injections of two doses of corticosterone (1 or 5 mg/bird), depressed body weight gain, increased liver lipid and abdominal fat along with a dose-dependent increase in uric acid, glucose and insulin in genetically selected lines of fat (FL) and lean (LL) chickens. Most of the above studies have principally concentrated on the influence of corticosterone on growth of organs as well as chick as a whole and on carcass fat and lymphoid structures. In spite of the known influence of adrenal steroids on various facets of metabolism, its functional involvement in post-hatched chick development has not been studied.

In this context, in the present study, parameters such as growth in terms of body weight and organ weights, histological structure of adrenal, thyroid and testis, metabolic features in terms of levels of activity of enzymes and metabolites and histochemical distribution of steroid dehydrogenases in the testis have been evaluated in chicks

chronically rendered hypo or hyper active in adrenocortical functions. Besides, glucose tolerance test and, insulin, glucagon and adrenaline response tests have also been carried out in chicks under induced functional hypocorticalism by DXM.

Retardatory influence of both hypocorticalism as well as hypercorticalism on body weight gain during post-natal development is clearly revealed (Chapter II). Differential effects in terms of organ growth have been observed under DXM and corticosterone treatments. Whereas certain organs responded identically under the two treatments, others showed distinctly opposite responses. The post-natal growth in terms of relative weight of liver and kidney was found to be retarded under hypercorticalism and stimulated under hypocorticalism while that of spleen and bursa were suppressed under both conditions (Chapter II). Though there are number of reports relating the influence of altered adrenocortical functions to body growth and organ weights in mammals, the few studies available in birds have essentially evaluated only body weight changes. The observed effects of DXM and corticosterone on decreased relative weights of bursa and spleen is in accordance with the known antagonistic relationship between corticosteroids and lymphoid organs (Glick, 1957 a, b, 1959, 1960a,b, 1967, 1972; Zarrow et al., 1961; Bellamy and Leonard, 1965; Sato and Glick, 1964, 1970; Dieter and Breitenbach, 1970, 1971). An evaluation of the role of corticosteroids on metabolic features of post-natal chicks is greatly warranted by the known effects of corticosteroids on intermediary metabolism of adult animals and their permissive influence on the secretion and functions of thyroid hormones

(Borges et al., 1981; Decuypere et al., 1983; Kühn et al., 1984; Buyse et al., 1987).

Many studies have indicated the definite influence of corticosteroids on carbohydrate metabolism (Conn and Fajans, 1956; Kitabachi et al., 1968, 1973; Olefsky and Kimmerling, 1976; Ivarsson et al., 1983; Sistare and Haynes, Jr., 1985) and lipid metabolism (Baum and Meyer, 1960; Nagra and Meyer, 1963; Bartov et al., 1980 a, b; Bartov, 1982; Davison et al., 1983; Buyse et al., 1987). Limited studies have also highlighted the role of corticosteroids on protein turnover in muscles (Griminger and Scanes, 1986). The present study in this context has evaluated the effect of hypo- and hypercorticalism on carbohydrate, protein and lipid metabolisms in developing post-natal chicks. The observations indicate a functional interrelationship between corticosteroids and, pancreatic and thyroid hormones, essentially in regulating carbohydrate and lipid metabolisms. The influence of corticosteroids on protein metabolism can be gauged by the reported depressed synthesis of DNA, RNA and proteins in tissues of chicks by cortisol (Bellamy and Leonard, 1965); by the high rate of amino acid deamination in chicks by cortisol (Bellamy and Leonard, 1964; Goodlad and Munro, 1968) and by decreased nitrogen balance and increased nitrogen excretion and uric acid by glucocorticoid treatment in chickens and quails (Adams, 1968; De la Cruz et al., 1981). Obviously, an overall reduction in protein content under glucocorticoid excess can be inferred. This reduction in protein content has been related to glucocorticoid induced gluconeogenesis (Harvey et al., 1986). In the course of the present study, corticosterone

induced hypercorticalism has induced significant reduction in the protein content of liver and muscle (Chapter IV). The possible utilization of proteins in gluconeogenesis finds support from the observed hyperglycemia and increased hepatic glucose-6-phosphatase activity in these experimental chicks (Chapter III). However, the concomitantly decreased hepatic glycogen content is contrary to the known glycogenic action of corticosterone but well supported by the increased phosphorylase activity (Chapter III). These two observations can be taken together to speculate the possible glucagonic effect. An increased glucagon action in chicks chronically treated with corticosterone may result from an altered insulin:glucagon molar ratio and can be either due to increased glucagon secretion/action and/or decreased insulin secretion/action. The reported activity of glucocorticoids to potentiate glucagon action (Exton et al., 1973; O'Neill and Langslow, 1978) or to induce glucagon release (Marco et al., 1972; O'Neill and Langslow, 1978) as well as to antagonize insulin action (Pierluissi et al., 1986; Natarajan et al., 1987) are compelling evidences in this context to support the contention for the prevalence of a physiological state of hypoinsulinemia and hyperglucagonemia.

This contention finds credence from the observed reversed set of changes under DXM induced hypocorticalism. Accordingly, protein and glycogen contents were increased and phosphorylase activity and blood glucose level were decreased (Chapter III & IV). This suggests that hypocorticalism could increase insulin action/release by probably minimising the glucocorticoid antagonism to insulin and potentiation of glucagon thereby increasing the insulin:glucagon molar/activity ratio. Under such an

induced insulinemic condition, increased hepatic and peripheral uptake of glucose (leading to hypoglycemia), glycogen deposition (by decreasing phosphorylase activity and inducing glycogenesis) and protein increment (by increased protein anabolism) can be expected.

Many reports have equivocally established the role of glucocorticoids in inducing lipogenesis and fattening in birds in general and the domestic fowl in particular (Baum and Meyer, 1960; Nagra and Meyer, 1963; Nagra et al., 1963; Bartov et al., 1980 a, b; Bartov, 1982; Davison et al., 1983; Buyse et al., 1987). Increased fat deposition in the visceral and neck region has been noticed in both dexamethasonised and corticosterone treated chicks in the present study with the tendency for deposition being relatively more in the former. A generalized increase in cholesterol and lipids in liver and serum of both DXM and corticosterone treated chicks (Chapter V) is suggestive of the lipogenic and steatogenic influence prevailing in these chicks. However, the greater potency of DXM in the above process in relation to corticosterone seems to have some relation with the possible alteration in the action of other endocrine secretions (either quantitatively or qualitatively) on a prevailing background of hypo or hypercorticalism. The increased fat deposition obtained with corticosterone is justifiable as Nagra and Meyer (1963) had shown that glucocorticoids convert glucose carbon into more lipids and less proteins and carbohydrates. The recorded decreased hepatic glycogen content and hypoglycemic condition (Chapter III) and decreased tissue protein contents (Chapter IV) in corticosterone treated chicks corroborate the same. An aspect related to the possibility of altering the action of

other endocrine secretions, is the likelihood of lowered T_3 levels in chicks with functional hypercorticalism as Decuypere et al. (1983) and Buyse et al. (1987) have reported depressed T_3 levels in corticosterone treated chicks and fowls. Conversely, chicks rendered hypothyroidic have depicted hepatic glycogen and triglyceride storage syndrome (Raheja et al., 1980). Further, a negative correlation between T_3 levels and carcass fat have also been derived for chicks (Stewart and Washburn, 1983). This negative correlation has led to the suggestion that low levels of circulating T_3 may be associated with a higher degree of fatness and the lipogenic effects of corticosterone may also partly be mediated by a decreased T_3 level (Ringer, 1976; Buyse et al., 1987). Hence the presently observed fat deposition in corticosterone treated chicks could be facilitated by lowered T_3 levels and as such corticosterone has been shown to decrease circulating T_3 level and increase reverse T_3 level in post-hatched chicks by suppressing T_4-5' monodeiodinase activity and activating T_4-5 monodeiodinase activity (Borges et al., 1981; Decuypere et al., 1983; Kühn et al., 1984; Buyse et al., 1987). Again, DXM induced increased steatogenesis and fattening could also be taken to validate the concept of functional alteration of other hormonal principles to provide a favourable permissive influence under hypocorticalism. The previous contention that hypocorticalism leads to an increased insulin:glucagon molar/activity ratio could be the factor involved in increased fat deposition in dexamethasonised chicks. Validity to this presumption is provided by the known role of insulin as the potent lipogenic hormone in birds (Touchburn et al., 1981; Yanaihara et al., 1983; Griminger, 1986). Additionally, the

possible role of DXM to mimic the action of corticosterone in lowering T_3 level by suppressing 5' monodeiodinase activity could again explain the relatively more fat deposition observed in DXM treated chicks as compared to corticosterone treated ones.

Induced adrenocortical insufficiency and excess have also revealed characteristic alterations in cholesterol and lipid metabolism of adrenal gland. Some intriguing observations made in this connection include increased adrenal lipid content with both the low and high doses of DXM, decreased lipid content with corticosterone, increased total and esterified cholesterol content of both fractions with DXM(H) and no change with corticosterone (Chapter V). In the light of the established role of DXM to suppress ACTH secretion (D'Angelo, 1966; Chowers et al., 1967; Yates, 1967; Dallman and Yates, 1968; Fleisher and Battenbee, 1968; Kendall and Allen, 1968; Purves and Sirett, 1968; Arimura et al., 1969; Russel et al., 1969; Sirett and Gibbs, 1969; de Kloet et al., 1974; Obara et al., 1984; Macharg et al., 1985; Radke et al., 1985; Smoak and Birrenkott, 1986; Carnes et al., 1987; De Greef and Van der Schoot, 1987; Dupouy et al., 1987; Kloeti et al., 1987; Juniewicz et al., 1987; Medleau et al., 1987; Smith and Feldman, 1987; Brody and Black, 1988; Brooks et al., 1988; Katano, 1988; Wilson et al., 1988) the observed increase in both the total and esterified cholesterol fractions in the adrenal of chicks treated with low dose of DXM is understandable as ACTH treatment has been reported to bring about depletion of adrenal stores of cholesterol in fowls (Howard and Constable, 1958; Siegel, 1962 a, b; Siegel and Siegel, 1966; Freeman and Manning, 1975). It

is established that steroidogenesis in ACTH sensitive adrenal cells of mammals is modulated by the flux of cholesterol through the stored pool of esterified cholesterol and is essentially governed by two enzymes: acyl coenzyme A: cholesterol acyl transferase (ACAT), which esterifies free cholesterol, and cholesterol ester hydrolase (CEH), which releases free cholesterol from esterified cholesterol (Brody and Black, 1988). It is also reported that in guinea pigs, dexamethasone induced adrenal suppression leads to repression of both these enzymes (Brody and Black, 1988). In this context, a possible direct dose dependent suppressive effects of DXM on ACTH release in chicks could be envisaged. It is also speculated that the regulatory sensitivities of ACAT and CEH may be quantitatively related to ACTH levels, with CEH being more sensitive than ACAT to DXM induced ACTH suppression. The increased cholesterol ester content obtained with DXM(L) might indicate reduced breakdown of cholesterol ester as a result of CEH repression brought about by the submaximal suppression of ACTH. On the contrary, the decreased ester content in chicks treated with DXM(H) suggests the repression of both ACAT and CEH brought about by the maximal suppression of ACTH. This dosage effect of DXM on ACTH suppression is further indirectly corroborated by the previously observed dose dependent effect of DXM on hepatic glycogen deposition and hypoglycemia (Chapter III). The reduced total cholesterol content in chicks treated with DXM(H) could also indicate a possible inhibition of cholesterol biogenesis by way of suppression of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMGCOA-reductase; Black et al., 1988) and/or cholesterol uptake. It is also clear from the present study that

exogenous corticosterone (at least in the dosage used in the present study) is without effect on adrenal cholesterol metabolism. It is also inferable, that at the present dosage, corticosterone does not lead to any feed back inhibition of ACTH in neonatal chicks. In fact a stimulatory influence either on the hypophyseal-adrenal axis or, on the adrenal cortical cells directly, is denoted by the significantly decreased total lipid content in contrast to the significantly increased levels in DXM treated chicks. The results indicate that lipid content may serve as a better index of assessing steroidogenic activity of the adrenal gland in chicks.

The earlier presumed idea of modulatory influence of corticosteroid on the functioning of pancreatic hormones was tested by studying the chronological glycemic response to glucose loading, as well as, insulin, glucagon and adrenaline injections. The permissive influence that corticosterone can exert on the actions of pancreatic hormone has been hinted at since quite sometime (Mialhe, 1958, 1969). In this respect, the above evaluation of alterations in glucose level in the blood subsequent to glucose, insulin, adrenaline and glucagon administration in dexamethasonised chicks has revealed specific alterations. Based on the above study it has been concluded that normal chicks in the early post-natal phase is more insulin-sensitive. Evidences supporting this inference are the pronounced percentage fall in glycemic level in response to insulin and the relatively poor percentage increment after glucagon administration (Chapter IX). Increasing glucagon sensitivity as denoted by the increasing hyperglycemia and glucose elevation rate

from day 1 to 30 with corresponding increasing Eg/Kg values and, fluctuations in circulating insulin level in its sensitive phase during the first month of post-natal development, as revealed by decreased glucose elevation on 10th day and 30th days as related to 1st and 20th day subsequent to glucose loading, are further inference drawn therein. It appears likely that relatively more insulin is present during 2nd and 4th week. Another interesting revelation is the more potent action of adrenaline as glycogenolytic agent than glucagon in the first fortnight of post-natal chick development. This is confirmed by the observed greater glucose elevation rate with adrenaline on 1st and 10th day and with glucagon on 20th and 30th days. Apparently, in neonatal chicks, adrenaline may function as the more potent glycogenolytic agent during the first fortnight after hatch, a period corresponding to low glucagon responsiveness as inferred above. DXM induced hypocorticalism, increased insulin-sensitivity and decreased glucagon responsiveness. This aspect is established by the observed attenuated hyperglycemia on glucose loading and better glucose tolerance, and pronounced hypoglycemia in response to insulin injection as compared to control chicks. Further corroborative evidences strengthening this contention are the decreased glucose elevation rate on glucose loading with resultant low E/K values, greater clearance rate subsequent to glucagon induced glucose elevation rate with corresponding low Eg/Kg values and greater glucose clearance in response to insulin followed by slower normalization rate with resultant higher Ki/Ni values. All these taken together indicate stimulated insulin-sensitivity together with attenuated glucagon responsiveness in chicks with hypocorticalism during the first

30 days of post-natal development. The purported role of corticosterone in potentiating the action of glucagon and blunting that of insulin as is being inferred from this study finds adequate support from the observations of Mialhe (1958, 1969), of the corrective influence of corticosterone on insulin-hypersensitiveness and markedly impaired hyperglycemic effect of glucagon in hypophysectomised chicks. The observation of significantly increased hepatic glycogen content and hypoglycemia occurring in response to DXM treatment in chicks (Chapter III) lend further evidence to the concept of permissive role of corticosteroids in establishing and maintaining carbohydrate homeostasis by way of the modulatory action on pancreatic hormones. Another inference that can be drawn from the present obseravtion is the potentiated glycogenolytic action of adrenaline in chicks with functional adrenocortical insufficiency. Observations that provide evidence to this are the adrenaline induced markedly higher glucose elevation rate and double the Ea/Ka values obtained in dexamethasonised chicks relative to the controls (Chapter IX). The more or less identical glucose clearance rate recorded in both control and DXM treated chicks subsequent to adrenaline induced hyperglycemia also suggest the immunity of post-natal chicks to the antagonistic effect of adrenaline on insulin action reported for in adult mammals and birds (Ensinck and Williams, 1981; Patel and Ramachandran, 1989). Based on the glycemic response to hormonal challenges (Chapter IX) and, alterations in carbohydrate metabolism observed in dexamethasonised and corticosterone treated chicks (Chapter III), it has been concluded that corticosteroids during the post-natal phase of chick development do perform a definite

modulatory role in the chronological set of events leading to the establishment of homeostatic interrelationship between pancreatic hormones vis a vis carbohydrate metabolism charactertistic of the adult state.

In keeping with the inferred alteration in hormonal sensitivities/inter-relationships and resultant effects on metabolite levels in tissues, it becomes pertinent to assess the possible implication on the activity of enzymes involved in intermediary and oxidative metabolisms during chronically induced hypo and hypercorticalism in chicks. Changes in the activity levels of aldolase and LDH (glycolytic enzymes) and SDH (oxidative enzyme) assayed in this connection implicate corticosterone in stimulating both glycolytic and oxidative metabolisms. In fact, the importance of corticosterone in oxidative metabolism and oxygen uptake of various tissues in rats has been documented (Bottoms and Goetsch, 1968). The reduced aldolase and SDH activities in liver and muscle of chicks with DXM induced hypocorticalism and the reversed changes under corticosterone induced hypercorticalism bear ample testimony to the above inference (Chapter VI). Relatively more decrement in total LDH activity under hypoadrenocorticalism than under hypercorticalism (Chapter VI) could be related to a generalized decrement of both anaerobic and aerobic LDH isozymic forms under the former as opposed to a decrement of only anaerobic LDH isozymic subunits under the latter condition. Viewed in this perspective, corticosterone induced increased oxidative metabolism as denoted by the stimulated SDH activity could be complemented by the increased ratio of aerobic to anaerobic LDH

isozymic subunits. Pertinently, Rehiman et al. (1986) has correlated cortisol level and serum LDH activity in human subjects with increased oxidative LDH isozymic forms. The report of Amando et al. (1988) is contradictory to the present one in that they had observed increased LDH activity coupled with decreased SDH activity under DXM treatment and decreased activity of both the enzymes under corticosterone treatment in rats. Based on these observations, they had suggested a functional duality of the two types of glucocorticoids at the hepatic level in rat. However, the present findings tend to corroborate the same but in a reverse sense in that both LDH and SDH activities were decreased under DXM treatment and SDH activity increased and LDH decreased under corticosterone treatment in post-natal chicks. Postulated alterations in LDH isozymic forms suggested herein due to functional manipulation of adrenocortical activity in developing post-hatched chicks is strengthened by the reported developmental changes in total LDH activity in liver and kdiney of rat neonates during the first 56 days (Beebee and Carty, 1983). The decreased glycolytic and oxidative metabolisms of liver and muscle in DXM induced hypoadrenocorticalism as observed in the present study is well reflected in the observed decrease in phosphorylase activity and increased glycogen content (Chapter III) and increased lipid content (Chapter V). However, the increased SDH activity in liver and muscle of corticosterone treated chicks associated with increased lipid content and depleted protein and glycogen contents could suggest increased oxidation of glycogen and protein reserves. In the wake of previously inferred common action

of both DXM and corticosterone in decreasing T_3 formation (Chapter II) and the known role of thyroid hormone to induce SDH (Hulbert et al., 1976), the present findings provide evidence for a possible direct action of corticosterone in inducing SDH activity and oxidative metabolism in chicks.

Besides these, other enzymes having bearing on metabolic activities, albeit indirect (phosphomonoesterases), energy flux and transport mechanisms (ATPases) and hormonal actions (phosphodiesterase) have also been studied (Chapter VII). The observations indicate that hypocorticalism per se has no effect on acid phosphatase activity of liver and muscle while hypercorticalism exerts a definite stimulatory influence. The collateral observations of decreased protein contents in liver and muscle of corticosterone treated chicks (Chapter IV) finds valid explanation in the reported correlation of high acid phosphatase activity in the muscle of rat with regressive changes (see Lojda and Gutmann, 1976) and increased proteolytic activity (see Gutmann et al., 1976). The observations made with reference to ATPase activity indicate that corticosterone increases total ATPase activity essentially by increasing the Ca^{++}-Mg^{++}-ATPase activity with reciprocal decrease in Na^{+}-K^{+}-ATPase activity. This is in contrast to the reported role of adrenocortical steroids to stimulate Na^{+}-K^{+}-ATPase activity in vertebrate tissues invovled in Na transport (Pickford et al., 1970; Epstein et al., 1971; Forrest et al., 1973; Saunders and Henderson, 1978; Geering et al., 1982). In concurrence with the present inference, Langdon et al. (1984) have

suggested the pituitary interrenal axis to be only partly responsible for increasing Na^{+}-K^{+}-ATPase activity in the Atlantic Salmon. Alternative conclusion that can be drawn in this context is that in developing chicks, corticosterone regulation of ATPase activity is probably immature and differential. Though a direct correlation between corticosteroids and Ca^{++}-Mg^{++}-ATPase can be deduced, with reference to Na^{+}-K^{+}-ATPase it appears that corticosterone excess suppresses its activity while its deficiency has no effect. Glucocorticoids have been identified as important agents modulating cAMP content by their suppressive effect on phosphodiesterase activity (Manganiello and Vaughan, 1972; Lee and Reed, 1977; Ross et al., 1977; Elks et al., 1983; Durand et al., 1983). Further confirmation is provided by the reported increase in phosphodiesterase activity after adrenalectomy (Allen and Beck, 1972). In the present study both DXM(H) and corticosterone decreased phosphodiesterase activity in muscle, in keeping with the above mentioned phosphodiesterase activity lowering effect of corticosteroids. The hepatic phosphodiesterase activity was however decreased under both DXM(L) and DXM(H) treatments and increased under corticosterone treatment. These observations may be correlated with the observed increased and decreased glycogen content respectively in DXM and corticosterone treated chicks (Chapter III).

In the case of mature fowl, a relationship between gonadal functions and adrenocortical activity has revealed both parallel as well as antagonistic relationships. Accordingly, adrenalectomy has been shown to induce pronounced atrophy of the testis (Herrick and Finerty, 1941;

Hewitt 1947) and to inhibit the development of the right gonad in ovariectomised birds (Taber et al., 1956). Conversely, administration of corticosterone acetate has been shown to induce testicular atrophy (Selye and Freedman, 1941). Based on these observations, it can be inferred that both corticosterone insufficiency as well as excess can inhibit gonadal function in adult fowl, thereby suggesting the requirement for an optimum concentration of corticosterone in maintaining normal adult gonadal functions. However, the influence of adrenocortical activity on gonadal maturation and differentiation during post-natal development of chicks has not been clearly assessed. Investigations carried out in this context in the present study in terms of testicular weight and histology (Chapter II and VIII) reveal a stimulatory influence of hypocorticalism with a concomitant retardatory influence of hypercorticalism. Supporting this inference are the observations of increased relative weight of testes in dexamethasonised chicks and decreased relative weight in corticosterone treated ones with corresponding histological alterations in the form of well organized lumenating seminiferous tubules and interstitium in the former and inactive cords with degenerating epithelium with poorly organized interstitium in the latter. These observations suggest that corticosterone has a retardatory influence on the functional differentiation and maturation of testes in the early post-hatched developmental phase of chicks. The observations of increased 3β-HSDH activity in both the tubules and interstitium with increased 17β-HSDH activity in the central part of the tubules and decreased lipid contents and 3α-HSDH in both the components of the testis of chicks treated with low dose of DXM (hypocorticalism;

Chapter VIII) provide further supportive evidence to the possible favourable influence of low circulating titre of corticosterone in early post-natal maturation of testes in chicks. Apart from the compact organization of seminiferous cords and the interstitium, the prominent histological observation is the early lumenation of cords transforming them into tubules by 30 days in dexamethasonised chicks as opposed to the same occurring by 42-45 days in normal chicks (see Johnson, 1986). These histological alterations are paralleled by increased 3β-HSDH activity in both the tubules and interstitium, increased 17β-HSDH activity in the central part of the tubules and, decreased lipid contents and 3∝-HSDH activity in both the components of the testis. It is inferable that lumenation of the seminiferous cords and the resultant transformation into seminiferous tubules is associated with increased intratubular 3β- and 17β-HSDH activities. A possible role for testosterone synthesized locally within the central part of seminiferous cords (albeit in small qunatities) prior to and concomitant to degeneration of the central germ cells and resultant formation of seminiferous tubules in avian testes is assumable in the context of the present observations; a process which is greatly facilitated or enhanced under DXM treatment. The present observations also indicate that DXM(L) induces increased 3β-HSDH activity in the interstitium as well, with both the Δ^4 and Δ^5 pathways being more or less equally active. The increased production of progesterone and androstenedione by the interstitial cells coupled with reduced breakdown of androstenedione (due to reduced 3∝-HSDH) could contribute to a local build up of these two steroids leading to organization and differentiation of the testicular components during

post-natal development. It is presumable from these that androstenedione and probably also progesterone could have a tubular differentiation and organization capacity during the post-hatched avian development.

It is debatable as to how DXM can accelerate or hasten these processes in the post-hatched avian testicular development. Interestingly, neither DXM(H) nor corticosterone could induce such changes and the activities of 3β-and 17β-HSDH. remained similar to that of controls with concomitant increase in 3∝-HSDH activity. These observations suggest no enhancement in either androstenedione or progesterone production but increased degradation of androstenedione under DXM(H) and corticosterone treatments. Increased lipid content together with unchanged 3β- and 17β-HSDH activities and increased 3∝-HSDH activity, bear testimony to the fact that normal testicular differentiation and development are retarded
under these treatments. Increased AA content in testis of corticosterone treated chicks also lend support in this connection (Chapter IV). It is presumable from these observations that DXM(L)-induced hypocorticalism is somehow linked with early maturational changes affecting the post-hatched chick testes development; while hypercorticalism or DXM(H) might retard the same. In this context, it is assumable that hypocorticalism might provide favourable milieu for early development in the immature male chicks. The possibility of DXM increasing LH secretion from the pituitary at this stage of development is overruled by the fact that 17β-HSDH activity was not increased under this treatment and thus did not lead to significant testosterone production.

Metabolic changes seen under induced functional alterations in adrenocortical activity also tend to suggest hypocorticalism to be more favourable to early differentiation and maturation of testes in chicks. Metabolic activation of the testes in dexamethasonised chicks is well reflected by the increased glycolytic activity denoted by increased aldolase and LDH activities (Chapter VI). The prevailing hypoglycemic condition and increased glycogen content with decreased phosphorylase activity also suggest utilization of blood glucose as the metabolic source for energy metabolism and accumulation of carbohydrate reserve as a physiological response to the precocial maturational changes. The reverse changes of decreased SDH activity with no increase in aldolase and LDH activities in the testis of corticosterone treated chicks corroborate the fact that corticosterone excess in the early post-natal development is retardatory to metabolic maturation and development of testes (Chapters II, VIII). These are in contrast to the reported influence of DXM on rat testicular and epididymal enzyme activities (Valivullah et al., 1981, 1983, 1985; Balasubramanian et al., 1987). In all the above studies, DXM has been shown to be acting as corticosterone while, in the present study on chicks, DXM and corticosterone seem to have differential effect in keeping with the purported function of DXM as adrenocortical suppressant and corticosterone as contributing to adrenocortical excess. Apparently, native corticosteroids from the adrenal cortex somehow retards testicular development and maturation in the early phase of post-natal development in chicks. In this context, it is reasonable to ponder as to whether DXM induced adrenocortical insufficiency in post-hatched chicks has a favourable influence on

FSH/PRL release or their action? However, corticosterone seems to exert a maturation delaying effect on testis of immature chicks as has been noted with reference to other observations as well in the course of the present study (Chapter II, III, IV, V, VI, VII, VIII).

Another aspect of relevance that has been brought out by some of the observations made in the present study is the possible occurrence of two distinct population of corticosteroid receptors. Based on the observed differential response of some organs and, similar response of some to DXM and corticosterone (Chapter II), it can be surmised that corticosterone receptors are of two types : one which is specific and responding to only native corticoids and other which is nonspecific and incapable of distinguishing between native or synthetic corticoid thereby responding to both. Such a conclusion had been drawn also by Ayyar and Ramachandran (1990) based on their studies on pigeon. An alternative explanation could be that there are distinct corticosterone and DXM receptors (referred to as type I and type II receptors), a concept which has been established at least for the corticosterone sensitive centres of nervous system (Mc Ewen et al., 1986). This would entail the necessity to presume the occurrence of either only corticosterone receptors in some organs (leading to differential response in terms of hyper or hypocorticalism) and a mixture of both corticosterone and DXM receptors in some (thereby inducing identical response either with corticosterone or DXM). Though these two different possibilities cannot be resolved at present; it is nevertheless to be accepted that, there exists two distinct population of corticosteroid receptors and

that their distribution in sensitive target organs is variable. This problem can however be solved only with specific refined techniques involving isolation and analysis of receptors and also by competitive binding studies. But less sophisticated techniques of analysis such as RIA with specific antibodies can provide confirmatory evidences for the other concepts developed during the course of the present investigation. RIA of corticosterone levels in both DXM and corticosterone treated chicks together with those of insulin, glucagon and T_4 and T_3 would be profitable in confirming the inferred alterations (either quantitative or qualitative) in hormonal actions and interactions under conditions of adrenocortical insufficiency or excess. A simpler study of pancreas by differential staining of the islet tissue could also provide a clue to the possible alterations, if any, on the secretory activity of the α and β cells. Moreover, a careful analysis is also warranted to identify the level (receptor or postreceptor) at which the potentiating or antagonistic action of corticosterone is exerted on pancreatic and thyroid hormones. In the light of the observed testicular maturation effect under induced hypocorticalism, it would be worthwhile to study the possible effect of such a condition on a still prolonged basis extending to 2 to 3 months and see whether reproductive maturation of cockerels could be achieved at a much earlier age. On the whole, the present study has succeeded in developing some concepts and has also opened up avenues of further searching investigations in chicks vis a vis adrenocortical hormones and hormonal interactions and post-natal development.