Development is a continuous process by which organisms grow. The process of development onsets as soon as the fertilized egg begins to grow. It may be divided into two main periods, prenatal and postnatal. The prenatal development involves differentiation and encompasses three main stages—ovum, embryonic and fetal stages. Postnatal development begins with birth and continues into neonatal, infancy, childhood, adolescence and adulthood. Differentiation and development are programmed processes which occur due to sequential activation and repression of genes causing alterations in the levels of enzymes. The activities of several enzymes decrease and of several others increase as a function of age of an organism. Age leads to alterations in the levels of enzymes and their inducibility by certain hormones. The rate of synthesis of enzymes usually changes in response to changes in the extracellular environment. The regulatory mechanisms which control the level of enzymes at the biochemical level involve a change in the rate of synthesis and/or degradation and post-translational modifications. A number of hormones and growth factors exert an intricate but judicious control on the process of development in aves. The divergent morphology and physiology observed during development with many distinct complex and coordinated processes involve the control of the programmed circuits of gene expression. Organisms have certain genes which control specific events during development. Gene expression in organisms is regulated at essentially all possible levels—transcription, pre-mRNA processing, mRNA transport, mRNA stability, translation, and post-translational protein processing. The regulatory mechanisms with largest effects on phenotype have been shown to act at the levels of transcription and mRNA processing.

Adenosine deaminase (ADA; EC 3.5.4.4), a key enzyme participating in the purine salvage pathway, catalyzes the irreversible hydrolytic deamination of the substrates adenosine and 2’-deoxyadenosine to yield the products inosine and 2’-deoxyinosine, respectively. ADA is a well-characterized enzyme involved in the depletion of adenosine. It is essentially required for lymphocyte proliferation and differentiation. The physiological function of ADA is critical for controlling the levels of adenosine and 2’-deoxyadenosine in immunological, neurological and cardiovascular systems. ADA activity is widely distributed in human tissues and is highest in lymphoid tissues. In humans, rats and mice, the highest level of enzyme activity is found in
thymus, spleen, placenta, and in organs comprising the gastrointestinal tract, whereas low activity is found in muscle, lung and kidneys.

A correlation has been drawn with the failure of both B- and T-lymphocytes mediated functions due to deficiency of ADA because of different missense mutations in exon 4 of its gene. The absence of ADA in humans results in severe combined immunodeficiency (SCID), which is characterized by hypoplastic thymus, T lymphocyte depletion and autoimmunity. Abnormalities of this enzyme have also been reported in other diseases of immune system including AIDS, lymphomas, leukemias, anemia and several other unrelated disorders like short-limbed dwarfism, hepatitis and jaundice. Increased serum ADA activity has been reported in patients with liver diseases like chronic hepatitis and liver cirrhosis.

Various avian species provide models of evolution, development and differentiation, behaviour and ecology. Avian genomes appear to show relatively high levels of conservation, and the genome sequence and related information that are available for chicken provide added benefits for the genetic analysis of all wild and domestic birds. The immune system of chicken has the thymus, a paired lobulated gland along the neck of the chicken which is considered to be the source of T cells or small lymphocytes which mediate the rejection of hemografts, graft-virus-host reaction and delayed hypersensitivity. They also have the bursa of fabricus, an organ situated dorsally to the cloaca which is considered to be the source of B cells which form plasma cells and produce antibody. In chicken, the gene for ADA is located on chromosome 20 and is approximately 64 kb in length. Sp1 protein is essential for both enhancer-mediated and basal activation of ADA promoter.

Keeping in mind the role of ADA in the regulation of a wide array of physiological processes, the present work embodied in this thesis has been directed towards the following:

1. To determine the normal endogenous activity level of ADA in different tissues of GIT (esophagus, crop, proventriculus, small intestine) and spleen at various postnatal ages (day 1, 10, 30, 60 and 90) of male chicken.
To assess the role of hormones and their analogues like corticosterone (adrenalcortical hormone), testosterone (sex hormone), triiodothyronine (thyroid hormone) and a membrane permeable analog of cAMP, dibutyryl cAMP, on the activity of adenosine deaminase in different tissues of chicken at two specific postnatal ages (day 10 and 60) ascertained, and to postulate the effect of these hormones, if any, in modulation of ADA in a tissue- and age-specific pattern.

To purify ADA from small intestine of two select age groups (day 1 and 90) of chicken and characterize its physicochemical and kinetic properties, with a view to analyze the alterations, if any, in these parameters of the enzyme during the course of development.

NORMAL ENDOGENOUS LEVEL OF ADA

ADA activity in the male chicken exhibits tissue specificity as well as age-related changes. The normal endogenous level of ADA activity (U/mg protein) has been ascertained for esophagus, crop, proventriculus, small intestine and spleen at various postnatal ages. The data indicate the highest level of activity in small intestine, followed by proventriculus, esophagus, crop and low activity of ADA in spleen. Our findings reveal that the normal endogenous level of ADA in the GIT of chicken is highest on the day of hatching. Amongst the regions of the GIT studied, the highest level of ADA activity is found in the small intestine followed by the esophagus, proventriculus and crop. In the esophagus, the activity is highest in day 1 and is seen to decrease significantly at day 10 and thereafter shows a slight decrease. In the crop, ADA activity is highest in day 1 showing a decrease at day 10 and then remains constant. The proventriculus is also seen to have a very high level of ADA activity at day 1 which shows a significant decline at day 30 and 90. Region specific studies indicate that in the small intestine, the level of activity is highest in day 1 followed by a sharp decrease at day 10 and then remains almost constant. Unlike all the tissues of GIT, the activity of ADA in the spleen is seen to increase maximally at day 30 after which there was a decline. In all the studies, our interest was to find out if there was a change in the level of protein of the enzyme for which the slot and Western blots were done for the two ages where differences were
observed in almost all cases. Representative data of the blots show that the level of the protein of ADA is indeed seen to change or remain constant for the ages studied. The avian gastrointestinal mucosal immune system has evolved with specialized features that reflect their role as the first line of defense on mucosal surfaces. High level of ADA on the day of hatching may ensure lower adenosine and better survival of lymphoid cells. Thus, a high ADA activity at early ages of chicken probably helps the tissues to cope with the increasing need for immunological competence, thus decreasing adenosine, which could otherwise exert unwanted physiologic effects.

**HORMONAL REGULATION OF ADA**

Our studies also show that corticosterone significantly inhibits the ADA activity in all the regions of GIT except proventriculus, in an age- and region-specific manner. In the spleen, corticosterone decreases the activity of ADA at both day 10 and day 60. The magnitude of inhibition is more pronounced at the later stage of chicken development (60-day) compared to a very young age (10-day). The findings of age- and tissue-specific inhibition may be correlated to the differential adaptive role and maturation of corticosterone action mechanism, its receptor and post-receptor events. Since corticosterone is immunosuppressive, it may be acting through the inhibitory action of ADA activity, leading to an accumulation of adenosine and 2’-deoxyadenosine, producing lymphotoxicity, leading to an immunosuppressive action. The inhibition of ADA activity level by corticosterone was also ascertained using slot and Western blot analyses which confirmed the inhibition of ADA activity at protein expression level. The immunosuppressive actions of corticosterone may thus control the host’s immune response to a great extent. Pronounced inhibition at a later stage may be attributed to a greater maturation of corticosterone receptors and post-receptor events, thus facilitating greater binding of the hormone to its receptor and/or hormone-receptor binding to ADA gene promoter leading to inhibition of cognate gene expression.

\[ \text{Bt}_2\text{cAMP} \]

which is a membrane permeable analog of cAMP, is found to increase the activity of ADA in all regions of GIT studied except crop. Like the tissues of GIT, in the spleen
too, Bt2cAMP increases the activity of ADA at day 60 but unlike the tissues of GIT, there was no effect at day 10. This stimulation is seen to be age and region specific. The substrate adenosine influences the intracellular concentration of cAMP. Deficiency of ADA leads to an accumulation of adenosine and 2'-deoxyadenosine which are reported to be lyphotoxic. Thus, cAMP may increase the immune responses by stimulating ADA activity, lowering the level of adenosine and 2'-deoxyadenosine, to ensure better survival of lymphocytes. The immunoinducing role of cAMP may be because of enhancing the activity of ADA, thus decreasing the intracellular concentration of adenosine, which ensures an environment better suited for lymphocyte proliferation. In the GIT, the activity level of ADA was found to be greatly enhanced at a later age (day 60) of chicken development when compared to the younger age (day 10). This may be because of the differential expression of secondary messenger cascade at the later stage of GIT development. Such induction of ADA activity level by Bt2cAMP was also reaffirmed using slot and Western blot analyses confirming that ADA activity is indeed induced at the protein expression level.

Thyroid hormones are known to decrease the activity of ADA in humans. However, in our course of study, there is no significant effect of thyroid hormone on the activity of adenosine deaminase in the tissues studied. The slot/Western blots also do not show any change in the level of the enzyme. This may be because of the difference in the physiology of humans and chicken, thyroid hormone receptors and post-receptor events or the lack of the regulatory elements of thyroid hormone in the chicken ADA gene.

Testosterone is also seen to decrease the activity of human ADA. But, no significant decrease in the activity level of ADA is seen in the tissues studied at the two postnatal ages, upon administration of testosterone. It could be due to a difference in the testosterone receptor and/or post-receptor events in human and chicken. There could also be a possibility of lack of testosterone/androgen regulatory elements (AREs) in the ADA gene of chicken. The slot and Western blot analyses performed also indicate no change in the activity at the protein expression level.
PURIFICATION AND PHYSICOCHEMICAL CHARACTERISATION OF ADA

Intestinal ADA from immature (1-day) and mature (90-day) chicken was purified using identical procedures. ADA preparations from both the ages were passed through sephadex G-100 gel filtration and DEAE-cellulose columns. The elution profiles indicated that both ADA, from immature and mature chicken, have similar molecular weights and ionic net charges. Gel filtration, PAGE and SDS-PAGE analyses indicate that both immature and mature ADA have similar molecular weight of 100 kDa, a similar overall charge and consist of a single molecular form. From the Michaelis-Menten equation and Lineweaver-Burk transformation, both the ADA show similar $K_m$ for adenosine of 33.3 μM and 34.2 μM, respectively, for 1-day and 90-day old chicken. The computed $K_m$ values of the immature and mature small intestinal ADA for 2'-deoxyadenosine are 14.3 μM and 14.3 μM, respectively. Analyses of the data indicate that there is no age-related difference in the affinity of ADA for adenosine as well as 2'-deoxyadenosine. However, both ADA from immature and mature chicken show more affinity towards 2'-deoxyadenosine than adenosine, indicating that a lower concentration of 2'-deoxyadenosine is required to reach a similar reaction velocity. Analysis of data indicates no significant difference between $K_m$, $V_{max}$ and $K_{cat}$ values of the enzyme for both substrates in the two age groups. There is also no significant difference observed in the $K_i$ of immature and mature ADA for purine riboside. Hence, analyses of the data indicate that purine riboside is a strong competitive inhibitor of both ADA with a similar $K_i$ of 6 μM and 7 μM, respectively. The pH stability studies indicate that ADA from immature and mature chicken is most stable in the broad range of pH from 5.5 to 8.0, after which slight instability starts in both the ADA. Thus, it implies that the salt bridge contributes to both immature and mature ADA by the same degree. The inactivation in the alkaline or in the urea solution can be derived from a slight deformation of the active site which cannot hold the entire activity due to a change in the molecular form at sites distant from the active site, whereas, acidic pH is capable of producing a direct change in or around the active site. When purified ADA from immature and mature chicken is assayed at different temperatures, the enzyme is stable till 45°C, after which a drop in the activity is seen in both the ages, with almost all activity lost at 70°C. This is because the increase in temperature after this does not increase the kinetic energy of the enzyme but instead
disrupts the forces maintaining the shape of the molecule. Modulation studies on the activity of 
ADA show that DTT, β-mercaptoethanol, DTNB all inhibit the activity of purified ADA albeit 
to a similar degree in both the immature and mature ages. The extent of inhibition by these 
sulfhydryl modifying agents is in the range of 25-35%. Amongst the divalent cations, Ca²⁺ is 
found to be less inhibitory (15%) to ADA followed by Mg²⁺ (50%) and the Hg²⁺ (88%). In 
addition to these, caffeine is also found to be inhibitory to ADA activity to the extent of 45%.

From the findings embodied in this thesis, it is concluded that:

➤ ADA activity and its level expresses in a tissue- and age- specific manner during 
postnatal development of chicken to ensure its better suited physiological roles.

➤ Corticosterone inhibits ADA activity in a tissue- and age- specific pattern, indicating 
that ADA is under the tonic inhibition by circulating corticosterone. Age-related 
difference in the magnitude of ADA inhibition is correlated to a differential adaptive 
role and maturation of corticosterone action mechanism, its receptor and post-receptor 
events.

➤ Bt₂cAMP is found to be stimulatory to the activity of chicken ADA, thereby exhibiting 
an immunoinducing role of cAMP.

➤ T₃ and testosterone fail to produce any significant change in ADA activity of chicken in 
either tissues or ages studied.

➤ Physicochemical and kinetic properties of purified ADA from small intestine of chicken 
remain the same at both the immature and mature ages indicating that there is no 
alteration in these properties as a function of age in chicken during postnatal 
development.
The studies compiled in the present thesis provide an insight into the basic role of ADA during postnatal development of chicken and also pave the way for using corticosterone and Bt2cAMP in inhibiting and inducing, respectively, the activity of ADA in various ADA-related diseases.