AIM AND SCOPE
Iron-protoporphyrin-IX, commonly referred to as 'heme' is the most ubiquitous metalloporphyrin chelate of the animal kingdom. It constitutes the prosthetic group of biologically active hemeproteins. Thus, heme is vital to maintain the structural and functional integrity of heme-proteins.

A major fraction of heme, synthesized in the liver, specifically serves as a prosthetic group of cytochrome P-450. The cytochrome P-450, a group of proteins play an important role in the detoxification and activation of foreign chemicals in the liver (1-3). The underlying theme of this project is to understand the mechanism and involvement of alanine: 4,5-dioxovalerate transaminase in heme biosynthesis. Such studies will not only broaden our understanding of a number of fundamental problems related to regulatory mechanism of heme biosynthesis and cytochrome P-450 formation, but also have useful application in toxicology and health hazard problems.

6-Aminolevulinic acid (ALA) is the first committed precursor of porphyrin and heme (4). The biosynthesis of ALA in mammalian cells has been thought to occur exclusively through a condensation reaction involving glycine and succinyl Co-A, which is mediated by a mitochondrial matrix enzyme ALA synthetase (5,6). It is known that altered regulation at the level of ALA synthetase is associated with various genetic defects, e.g., acute intermittent porphyria and
sideroblastic anemia (7-12), which are biochemically characterized with the over-production of moieties and porphyrins. It is also established that certain drugs, e.g., phenobarbital and 3-methyl-cholanthrene which stimulate two distinct species of cytochrome P-450, also induce ALA synthetase (13,14). Information so far available, strongly suggests that the primary factor regulating the overall activity of heme biosynthesis is at the level of ALA synthetase in mitochondria (15). However, several points raise the question about the sole responsibility of ALA synthetase for the synthesis of ALA and for the regulation of porphyrins and heme synthesis. The first point is observed initially by Shemin et al. (4) who reported the incorporation of 5-\textsuperscript{14}C-ALA into the purine. They proposed a pathway of glycine-succinate cycle, which was involved with the synthesis of ALA from an intact 5-C atom skeleton. Another important point is the absence of ALA synthetase in green plants (16,17) even though it has been established that ALA is an obligatory precursor in the biosynthesis of chlorophyll. Ultimately, evidence continues to accumulate that in plant leaves, ALA is synthesized exclusively via a transaminase reaction between L-alanine and 4,5-dioxovalerate (18-20). The discovery of the new pathway in the plant gives a new dimension to the problem of heme biosynthesis. Several lines of evidence have led to the conclusion that not all ALA, synthesized in
mammalian system is mediated by ALA synthetase, condensing glycine and succinyl Co-A. Recently, an additional pathway of ALA formation from DOVA has been reported to be present in mammalian liver by Varticovski et al. (21,22). They have isolated and partially characterized alanine:DOVA transaminase from bovine liver, which catalyzes the transamination reaction between L-alanine and 4,5-dioxo-valerate, yielding ALA. More likely, the synthesis of ALA by alanine DOVA transaminase determines the contribution of this alternate pathway to the overall synthesis of porphyrins and heme in vivo (23). In the wake of the discovery of this enzyme in the mammalian system there is a scope of research into its possible role, mechanism of action and overall physiological significance. Therefore, as a prerequisite to further studies, our attention has been mainly focussed on tissue distribution and cellular localization of the alanine:DOVA transaminase in rat. We also compared the capacity of ALA formation mediated by two different pathways.

The enzymes, which are generally termed regulatory or 'key' enzymes are often placed at strategic points, such as at the beginning of a pathway (24), thus limiting the flux through the pathway to which they belong, and are sensitive to control by factors in addition to substrate concentration. Factors which regulate the key
enzyme of the main metabolic pathways include hormones, dietary components, age, environmental agents, effector metabolites etc. The regulation and levels of such enzymes at different physiological states and age may be of great significance. The above facts prompted us to study the changes of alanine:DOVA transaminase activity in different tissues of aging rats.

As a part of our programme, we isolated a homogeneous preparation of alanine DOVA transaminase using new procedure from rat liver mitochondria. The purified enzyme has been utilized in further understanding of kinetics, specificity and mechanism. Furthermore, enzyme inhibition studies have been valuable tools and are used widely to study isolated enzymes and various aspects of cellular metabolism. Our objective is to present concisely the basic principles of inhibition to describe the actions and mechanisms of the most important inhibitors, to correlate the actions at the enzyme level with the changes observed in cellular function. In order to carry out such study, we describe the inhibition of alanine DOVA transaminase by various substances, e.g., the sulfhydryl inhibitors, excess DOVA, substrate analogs, reaction products, pyridoxal phosphate, etc. Furthermore, attention has been centered to see the inhibitory effect of some intermediary compounds of heme metabolism.
In many multi-enzyme systems generally, the end product of the pathway act as a specific inhibitor of an enzyme at or near the beginning of the sequence of reaction. This is determined by the steady-state concentration of the end product (24). An attempt has been made to elucidate the regulatory effect of hemin, an end product of the pathway, on alanine:DOVA transaminase activity.

The enzyme alanine: DOVA transaminase was observed to be inhibited in vivo and in vitro by hemin. And the last but of crucial importance, this study examined the possibility that generation of heme within mitochondria provide a local concentration sufficient to inhibit the activity of alanine:DOVA transaminase. We conclude that end product inhibition of alanine:DOVA transaminase activity by heme is an important physiological mechanism of hepatic heme biosynthesis regulation.

The interesting aspect of the regulation of alanine:DOVA transaminase has been studied in our laboratory and 20-methyl cholangrene (20-MC) inducer of cytochrome P-450, has been shown to stimulate significantly hepatic alanine:DOVA transaminase (unpublished observation). Recently, we also reported (25) that treatment of styrene, a cytochrome P-450 inducer, does not stimulate ALA synthetase, questioning it's role on heme biosynthesis. Thus, we also propose to utilize this model in future to understand the role of
alanine:DOVA transaminase on heme synthesis. In general, two major approaches have been followed to understand the molecular basis of enzyme induction:

(1) monitoring the metabolism of total mRNA;
(2) the assay of specific gene products by immunoprecipitation. We propose to continue our work in future by quantifying this enzyme with immunotitration.

This purified enzyme would be an extremely useful for future work to raise an antibody against alanine:DOVA transaminase in rabbit and by immunotitrating, we are planning to study the role of specific alanine:DOVA transaminase in different erythropoietic conditions or with phenobarbital and styrene treatment, where induction of cytochrome P-450 and other hemeproteins occurs. This study will enlighten further the regulatory role of alanine:DOVA transaminase on heme biosynthesis.