GENERAL DISCUSSION
The heme biosynthetic pathway in the animal cells has been studied extensively in the past and the regulatory role of ALA synthetase on this pathway has been elucidated (15,65). In addition, ALA formation is crucial in the regulation of heme biosynthesis under different conditions, e.g., normal (61,178,179), various genetic defects (7,8,65) and chemical porphyria (9-12).

Though the regulatory role of ALA synthetase in heme biosynthesis has been accepted, its presence in the plants has not been demonstrated. However, it was shown that in plant ALA is formed exclusively by the enzyme alanine:DOVA transaminase, suggesting that it might be an important step for heme biosynthesis. Recent report of Varticovski et al. (21) and others (142) about the presence of this new enzyme in bovine mitochondria suggests an additional route for ALA formation in mammalian system. Though, alanine:DOVA transaminase is a promising candidate for regulating heme biosynthesis in mammalian system, not much work has been done on the nature and characterization of this enzyme. Thus, in the present study we examined the role of this enzyme on heme biosynthesis.

Initially we studied the presence of alanine:DOVA transaminase in various tissues, e.g., kidney, liver, brain, heart, testis, spleen, adrenal gland and skeletal
muscle of rats. The enzyme activity is found to be widely distributed and associated with the mitochondria from all the tissues, though the levels vary. The alanine:DOVA transaminase activity is observed to be high in kidney and liver, which have high content of heme proteins, e.g., cytochrome P-450 (62,165). Our results can also be extrapolated to correlate the less activity of alanine:DOVA transaminase in brain with the low content of heme and cytochrome P-450, as observed earlier (166). We also report that the capacity of alanine:DOVA transaminase to form ALA appears to be greater than that of ALA synthetase from the same mitochondria suggesting a significant role of this enzyme on heme biosynthesis in mammalian system in vivo. These results are in agreement with the recent observation, made by Morton et al. (23) who were unable to determine spectrophotometrically ALA after incubating respiratory rat hepatocytes with unlabelled glycine and succinate, whereas, they are able to convert alanine and DOVA into porphyrins and heme in amounts readily detectable by standard methods.

As the developmental aspect of heme biosynthesis is known at the level of ALA synthetase (160,163), heme oxygenase (164) and uroporphyrinogen decarboxylase (160) we further studied the effect of age variation on the activity of alanine:DOVA transaminase in liver, kidney, heart, skeletal muscle and brain. From this study, two
important concepts have been generated regarding the regulation of alanine:DOVA transaminase. The first is that alanine:DOVA transaminase activity is regulated in a tissue specific manner since it remains unchanged in kidney and skeletal muscle. The second concept which has been developed by our study is that a significant decrease in the activity of the enzyme in liver, heart and brain is a general feature of aging rats. This observation can be comparable to developmental alteration of hepatic ALA synthetase and heme oxygenase. This can also be correlated with the observation that age is a known factor to regulate hemeprotein synthesis (160,163).

In the mammalian system, now, alanine:DOVA transaminase, is considered to be an important enzyme. Since nothing is known about the nature of this enzyme and to understand its characteristic properties, regulatory role of this enzyme on heme biosynthesis, we have purified alanine:DOVA transaminase to an apparent homogeneity using a new procedure. The alanine:DOVA transaminase is purified 422-fold with a recovery of 20%. The final enzyme preparation has a specific activity of 59.5 U/mg protein. This was significantly greater than that obtained for the purified enzyme from bovine liver 0.753 units/mg. (142).

The SDS-PAGE suggests that the enzyme is monomeric
in nature. The native enzyme of rat had a molecular weight of 232,000 ± 3,000 and appeared to be composed of identical subunits of M_r 41,000 ± 2000 daltons, indicating that the enzyme is a homohexamer.

The interpretation of the significance of apparent Km values is always difficult. Generally, low values are commonly expected for the natural substrates of biosynthetic enzymes. The apparent Km of 0.28 mM for 4,5-dioxovalerate is compatible with this expectation but the apparent Km for L-alanine of 3.3 mM is not. However, it is important to point out that the apparent Km for glycine of ALA synthetase is 10 mM (188). Mechanistically, transaminases reactions are ping-pong in type (189) and our studies in which L-alanine and 4,5-dioxovalerate concentrations are varied suggests a ping-pong mechanism for alanine:DOVA transaminase (190).

The specificity of this enzyme with regard to the amino donor is examined and the enzyme is quite specific for L-alanine. The pyridoxal phosphate functions at the active site and is expected to find this cofactor associated with transaminases. Alanine:DOVA transaminase is shown to contain 10 n mole of PLP/mg of purified protein. This observation confirms the report of Varticovski et al. (21) that the cofactor is tightly bound to the bovine liver alanine:DOVA transaminase.
The enzyme is markedly inhibited by pyruvic acid, reaction product, or substrate analogs (α-ketoglutarate, and methylglyoxal), cofactor pyridoxal phosphate, sulfhydryl inhibitors (pCMB, iodoacetate and NEM) and DOVA, one of the substrates. The inhibitory effect of DOVA on mammalian alanine:DOVA transaminase supports the earlier finding of alanine:DOVA transaminase inhibition by DOVA in Rhodopseudomonas spheroides (139).

If a significant role in heme biosynthesis is to be assigned to alanine:DOVA transaminase, then the availability of alanine and DOVA must be considered. L-alanine is, of course, readily available in all cells. The enzymatic reduction of α-ketoglutarate to DOVA has been reported in corn leaf extract (32) and DOVA has been detected in algae (191). Though it seems reasonable to assume that DOVA might also be a naturally occurring metabolite within the mitochondria of mammalian cell, in future, we propose to detect DOVA in mammalian system and to study whether alanine:DOVA transaminase is regulated with DOVA in vivo under normal and abnormal heme biosynthesis.

The present study further reveals the regulatory role of hemin on alanine:DOVA transaminase. Purified enzyme is shown to be inhibited by 5 μM hemin, the end product of the pathway. This is supported by our
other observation that intravenous hemin administration (1.2 mg/kg b.w.) significantly inhibits the activity of this enzyme. We have shown that maximum inhibition of alanine:DOVA transaminase is obtained with 1.2 mg/kg.b.w. of hemin injection which is required to achieve saturation of tryptophan pyrrolase with heme according to Welch and Badawy (187). They present an evidence suggesting that tryptophan pyrrolase may be a very sensitive marker to assess delicate changes in liver heme concentration since the degree of heme saturation of this enzyme significantly increases after the injection of hemin (29). This may suggest that hemin administered may exert a negative feedback regulation on the level of alanine:DOVA transaminase in mitochondria. There is ample evidence that porphyrin biosynthesis in animals is subject to feedback control by heme and several investigators (66,115-119) have discussed the concept of the "regulatory heme" pool, which is supposed to be responsible for regulation of ALA synthetase.

This is the first report where we can show hemin, the end product of the pathway inhibits alanine:DOVA transaminase activity, thus suggesting the regulatory role of this enzyme on heme biosynthesis. The importance of this observation is that since, heme is synthesized intramitochondrially by heme synthetase (34), and alanine:DOVA transaminase is a mitochondrial enzyme, the
potential exists for end product inhibition of alanine:DOVA transaminase, as well as heme repression as a controlling factor of heme biosynthesis in mammalian liver. Thus, generation of heme within the mitochondria may provide a local concentration sufficient to inhibit alanine:DOVA transaminase.

To examine this possibility, the present study is further extended to make dual assay for these two enzymes in same mitochondria. The unique feature of the study is that heme synthesis and alanine:DOVA transaminase activities are assayed in the same incubation mixture. Our results clearly indicate that excess generation of heme in the mitochondria decreases the activity of alanine:DOVA transaminase. This is an important observation which indicates that end product inhibition is the physiological mechanism for regulation of hepatic alanine:DOVA transaminase.

Hemin is known as the inhibitor of translocation of ALA synthetase from cytosol to mitochondria (127). Nothing is known about the mechanism of hemin inhibition of alanine:DOVA transaminase. In future we propose to study hemin inhibition of alanine:DOVA transaminase in detail. We recently reported an interesting results that styrene exposure increases heme level, although it inhibits ALA synthetase, (25,192). We assume that this
will provide us an ideal system to examine the role of alanine:DOVA transaminase on heme biosynthesis is styrene treated conditions. Moreover, unpublished observations from our laboratory define another interesting aspect of alanine:DOVA transaminase. Phenobarbital and 20-methylcholanthrene, the known inducers of cytochrome P-450 can induce alanine:DOVA transaminase in rat liver. Thus, the availability of large quantity of purified enzyme will allow the production of antibody against the protein and we expect to obtain specific IgG fractions directed against alanine:DOVA transaminase. Thus, in future, by immunotitration technique, we will be able to determine the actual amount of the enzyme present during induction of cytochrome P-450 by different drugs e.g., styrene, phenobarbital and 20-methylcholanthrene and also in different erythropoietic conditions. This study will further enlighten the role of alanine:DOVA transaminase on heme biosynthesis.