The process of life itself is a state originating from the inorganic elements kept in precise organisation by the enzyme systems. Enzyme system is the primary machinery through which a living organism continues its process of self-assembly, self-regulation and self-replication in the isothermal open system expressed as life. In an organism, particular enzyme system is the primary determinant of its cellular behaviour. Variation from a normal behaviour of the cell reflecting its altered enzyme system results a cancerous state.

Enzyme, as a subject, has a special interest because it lies on the boundary where biological and physical sciences meet. Scientific exploration have already established a supreme significance of enzymes in biological sciences. The existence of life lies in chemical reactions brought about by specific enzymes and by their activities. Any change or modification in enzyme systems may have far-reaching consequences in living systems. Enzymes are thus receiving increasing attention from physical chemists as biological catalysts. The mechanism of action of enzyme is in itself one of the most fascinating field of scientific investigations gaining increasing attention. One of the most striking characteristics of enzyme is their high specificity and strict limitation of action to a substance or to a group of closely related substances. This specificity is one of the most important
biological phenomena without which the ordered metabolism of living matters and the very existence of life itself would have been impossible.

Each enzyme behaves qualitatively in much the same way in all tissues, however, the distribution of enzymes vary quantitatively in different tissues. This quantitative distribution, on the other hand, governs the pattern of individual tissue. Enzymes being interrelated, ultimately regulate the direction of the metabolism of a tissue.

Neoplastic growths are considered as progressive process of cell proliferation and abnormal formation of living materials, but this in no way, is in the normal organisation of the body. These are either in the form of benign tumours or the dangerous and aggressive malignant outgrowths.

Enzymes, which govern the metabolic processes of organisms, occupy a place near the centre of oncology in living organisms. It is commonly believed that the uncontrolled growth of cancer cell is due to some metabolic or enzymatic peculiarities giving rise to abnormal biochemical composition of cells. Recent findings in enzyme chemistry is thus thought to provide a rational basis for the control or annihilation of this dreaded disease. The invasive growth of cancer cell, depends on the high synthetic capacity of the cell. This has directed much attention to the mechanism by which energy is made available for the rapid anabolic processes. The main source of this high requirement of energy is oxidation of
carbohydrates and fats. It is in this field that biochemical exploration of the cancer cell has been probed most deeply.

According to Greenstein (1954), tumours like normal tissues from which they arise, possess chemical substances, enzymes etc. and their existence too must be at least partially explicable in terms of a chemical and metabolic pattern. A comparison of the pattern of a tumour with that of the normal tissue from which it arose may be expected to reveal the metabolic and chemical changes which are the consequence of neoplasia. Such a comparison has its limitations for among other things, the normal tissue under scrutiny is, unlike the tumour, a resting non-growing tissue. It has, further, been suggested that for a better understanding of the chemical changes involved, a fetal tissue should be included (Greenstein, 1954).

As neoplastic growth is associated with the abnormal metabolic processes, the enzyme differences between cancerous and non-cancerous tissues are believed to be quantitative rather than qualitative (Greenstein, 1954). Further it has been suggested that the pattern of alteration in the enzyme system is a gradual and continuous one changing with successive cell divisions.

Nearly or completely loss of some enzymes specially the enzyme involved in specific activities in the normal tissues is observed in the neoplastic growth - this deletion
or loss is more particularly true in case of tumours of the liver - the chief organ of metabolic processes. There are many other instances of the loss of specific metabolic pathways and the enzymes when a normal tissue becomes cancerous. The loss of the enzymes may be associated either in the primary tumours or in the process of successive transplantation. This indicates that a tumour is not necessarily to be of a fixed biochemical entity - with characteristic invariant pattern of enzyme activities but is a continuous process of variation in the enzymes in successive cell division and transplantation. This phenomenon also explains the sudden transfer of long standing benign tumours to malignancy. Studies of Skipper et al. (1959) in fact revealed that loss of the specific enzyme transphosphorylase causes failure in conversion of purine analogue to the corresponding ribonucleotide and thus a tumour sensitive to 8-azaguanine and 6-mercaptopurine becomes resistant to these toxins.

The "deletion hypothesis" of carcinogenesis is even fundamentally based on the loss or totally absence of the enzyme/s activity/activities though no specific enzyme has todate been identified.

Though sufficient caution and warning have been sounded "to refrain from an all too prevalent tendency to regard each newly discovered enzyme deletion as the
explanation for the neoplastic process" by Weinhouse (1960) but changes in the surface membrane glycoproteins and glycolipids as observed by Nachbar et al. (1974) in the electron transport (Pedersen, 1972) and changes in the transport into the cell of sugars and amino acids (Nachbar et al., 1974) all imply that the enzymes responsible for these events are all modified and changed in the growth behaviour and the modification of the response of enzyme to intracellular messenger molecules and/or the enzyme modification of surface molecules affect cell-cell interactions. Quigley (1979) has suggested a number of empirically defined properties as the characteristic of malignant cells and these properties are (i) loss of growth control, (ii) invasiveness and metastasis and the enzyme system like proteases, is directly or indirectly interlinked to bring these characteristic of cancer cells. Not only the alteration of the enzymes and isoenzymes is associated with the neoplasm but also a reversion towards foetal isoenzymes is noted in the malignancy (Schwartz, 1973, Shapira, 1973).

It has been pointed out times and again by different groups of workers that alteration of one enzyme or even a battery of enzyme system is of no significance in neoplastic growth. Further, comparative observations of the enzymes of normal and neoplastic material suffer from the problems like (i) in whole tissue, an enzyme may be
preferentially associated with one type of cell which may be more in the neoplasm than in the normal tissues, (ii) changes in enzyme specific activity may merely reflect an alteration of total protein, (iii) differential adsorption of serum or cytoplasmic proteins on to the surfaces of normal and neoplastic cells could influence surface enzyme activities, (iv) the enzyme may be present in both soluble and membrane bound forms; assays on whole tissue or whole cells may thus fail to detect a change in one compartment or an import distributional change.

Carcinogens are agents which transform normal cell or tissue into neoplasia. These agents are known to regulate the enzyme patterns and other components of the tissue or cells in varying manner. This prompted considerable researches throughout the world on the carcinogenic effect on enzyme pattern system/s. Nades et al. (1956) observed an almost complete loss in the ability to synthesise ketone bodies to store glycogen in animals fed with a high carbohydrate diet and to synthesise fatty acids from acetate loss of specific metabolic pathways. Changes in enzyme pattern has also been observed by others when a normal tissue becomes cancerous. Tissue culture studies reveal similar changes when compared with developing embryonic tissue (Nades et al. 1956).

The induced alteration in the enzyme pattern are dependent on the nature of carcinogens. Inspite of their
diversity, the vitally important action of inducing agent is the interaction with the control of cellular growth and multiplication in some way or other. The ionizing radiations and some chemical agents are important carcinogenic factors producing alteration of enzymatic pattern of a normal cell.

Many investigators have observed that the content of nicotinamide adenine dinucleotide (NAD) in the liver of rats fed with 4-dimethylaminoazobenzene (4-DAB) increased considerably and ultimately resulted into hepatoma. Mensler et al. (1940) considered it to be in part, the effect of the dye on neoplastic transformation in the liver cells by effecting certain enzyme systems whose activity depended on NAD. In tumours the activity of lactate dehydrogenase (LDH) is more resulting into rapid catalysis of pyruvate to lactate (Mensler et al., 1940).

Further, normal rat liver has relatively low alkaline phosphatase activity whereas an extremely high alkaline phosphatase activity is found in rats fed with 4-DAB (Woodward, 1943).

In chick embryo, estimation of some enzyme patterns have been made in normal tissues e.g. glutamic dehydrogenase has been found in various stages of development. Similar results have also been obtained by different investigators on LDH, malate dehydrogenase, citric acid dehydrogenase in various developmental stages (Solomon, 1958).
Recent studies on tissue culture techniques have revealed similar patterns on human tumours and mouse sarcoma, demonstrating clearly the enzymatic changes under laboratory conditions. Similarly, alteration in enzyme constitution have been reported in the embryonic tissues both in man and chicken. In some cases enzyme changes have been associated with neoplastic transformation. Comparatively recently Wilson (1973) has suggested similarities between embryonic and malignant tissues based on the studies of antigens and malignants, including glucose-6-phosphatase, phosphohexose isomerase, tRNA methylase, LDH and aldolase in both rat and human tissues.

The behaviour of malignant tumours and developing embryos shows interesting similarities as mentioned earlier. But majority of the earlier investigations were based on the enzymatic changes in the liver cells of rats and other mammals. The present investigation have been conducted on the developing chick embryos, as chick being one of the easily monitored organisms in embryological studies, as well as easily manipulated embryos, it served as an ideal test material.

The present study has been designed to study the effect of 4-DAB on the pattern of enzyme activity, with emphasis to two enzymes namely LDH and alkaline phosphatase, along the following lines:
1) To study the effects of 4-DAB on alkaline phosphatase on developing chick embryo and embryonic livers and brain at different stages.

2) To study the effects of 4-DAB on lactate dehydrogenase (LDH) on developing chick embryo and embryonic livers and brains at different stages.

3) To study the effect of 4-DAB on the isoenzyme pattern of lactate dehydrogenase on developing embryonic liver and brain tissues.