2.1 Chemical Carcinogenesis

2.1.1 Possible Mechanisms of Chemical Carcinogenesis:

Bernblum in 1964 studied the two stage mechanism of carcinogenesis and the carcinogenic activity of aromatic compounds in relation to their molecular geometry has been discussed by Arcos and Argus (1975). Miller (1970) propounded a theory to explain chemical carcinogenesis which had a significant impact on cancer research. According to him most of the chemical carcinogens which are not chemically reactive must be metabolically converted into a reactive form. The activated metabolites are electrophilic and react with nucleophilic groups in cellular macromolecules to initiate carcinogenesis. This theory was corroborated a few years later by Miller and Miller (1974) who suggested that carcinogens or their metabolic products act as electrophiles initiating tumor formation through covalent interaction with nucleic acids or proteins of cells. This theory had acquired support from the studies of Heidelberger (1970) showing that amines and other alkaline compounds can protect the cells from carcinogenesis by breaking the linkage of carcinogens to proteins. Pullman and Pullman (1955) suggested that in order to be carcinogenic a molecule must possess an active K-region and if the molecule has an active
L-region, this region must be somewhat inactive. According to this theory, a region is an assembly of two carbon atoms placed in such a way that they may undergo an addition reaction. A reactive centre is a single reactive carbon atom with an affinity to undergo a substitution reaction. The reactive centre may or may not be on a reactive region.

The K-region is defined as a bond of a conjugated molecule possessing a low ortholocalization energy. The carcinogenic activity of a chemical more or less follows the ability of a molecule to fix a double-bond reagent. The presence of a bond which is active for addition reaction (K-region) favours the appearance of carcinogenic activity. Two atoms with a low paralocalization energy are called an L-region. The presence of two reactive para positions (L-region) is unfavourable for carcinogenic activity. Substituents which decrease the electronic charge of K-region also decrease the carcinogenic power, and substituents which increase the electronic charge usually increase the carcinogenic power as well (Daudel and Daudel, 1966).

The interaction between carcinogens and biorceptors at the molecular level has been ascribed to involve either charge transfer (Hoffman and Ladik, 1961; Mason, 1958) or chemical reactions (Pullman and Pullman, 1955).
or energy transfer (Birks, 1961). Popp (1976, 1977) has propounded his resonance hypothesis to explain carcinogenesis.

Several hypotheses have been put forward for tumor induction with carcinogens but the foremost important ones are: (i) direct interaction of the carcinogens with vital cellular constituents forming precancerous/cancerous cells; (ii) metabolic conversion of polycyclic aromatic hydrocarbons into their carcinogenic forms; (iii) activation of a carcinogen in the presence of a co-carcinogenic agent, such as Croton oil (Hecker, 1971); and (iv) conversion of viruses into tumorigenic agents by carcinogens, like methyl cholantherene or benzpyrene, which catalyze the induction of mammary tumor in mice.

Most of the carcinogenic agents react with sulphydryl groups (Harington, 1967) and inhibit cellular adhesiveness (Grinnol and Srere, 1971). Interaction of carcinogens with sulphhydryl groups can therefore temporarily or permanently block cell division. When cell division is inhibited the initiation process of carcinogenesis might start.
THEORETICAL MODELS DEMONSTRATING POSSIBLE REACTIONS OF THIOLS AND UNSATURATED ALIPHATIC ACIDS WITH THE K-REGION OF 3,4-BENZOPYRENE (BENZO (a) PYRENE).
2.1.2. Detection of carcinogenicity of aromatic compounds by structural analysis: Comparison of the molecular structures of chemical compounds with those of known carcinogens can be used for the detection of potential carcinogenicity of environmental pollutants. Unfortunately the structure–activity patterns observed for one series of carcinogens does not, in general, apply to, superficially related series. This lack of inter-series consistency can be illustrated by the carcinogenicity of 4-aminobiphenyl (I) and the low or non-carcinogenicity of its 2-methyl homologue (II). The loss of activity is thought to be due to the abolition of co-planarity required for carcinogenicity which the methyl group disturbs.

![Structural formulas](I) (II)

In sharp contrast to the above 2-methylbenzidine is considered to be a more potent carcinogen than benzidine. A similar situation is encountered when comparing the effect of O-methylation across several series of aromatic-amino carcinogens. These types of
inconsistencies in structure-activity relationship can be understood if we consider the metabolic transformations suggested by Miller and Miller (1975). To correlate metabolic transformations in molecules and structural requirements for carcinogenicity the use of short term detection techniques could be extremely meaningful.

On the basis of structure-activity studies, the following main series of carcinogens could be grouped. Within these groups, there are generalizations in the structures of various compounds.

2.1.2.1 Aromatic amines: This group constitutes one of the largest family of carcinogens. The most important members are aminobiphenyl, benzidines and amino azobenzenes, the di, and tri (para-aminophenyl)-methanes, the amino-stilbenes, some amino derivatives of fluorene, dibenzothiophene, dibenzofuran and 2-napthylamine and its analogues. These series are linked in at least some stages during their carcinogenic conversions. E.g., ring hydroxylation and conjugation is a usual method for detoxification and most of them have been shown to undergo carcinogenic activation via N-hydroxylation. The N-hydroxy compounds further undergo esterification followed by their reaction with nuclear material, either purines or proteins.
However, the absence of any reliable rules governing activity within these series means that the four generic structures (III to VI) should be considered as representing them when attempting to detect future carcinogens in this class.

2.1.2. 4-nitroquinoline-N-Oxide (VII): This compound and its active analogues are distinct from aromatic amines.
One reason is the surprising inactivity of the corresponding 4-amino-quinoline N-Oxide. Moreover, susceptibility of the 4-nitrogroup to nucleophilic displacement has added a complication to the study of metabolic carcinogen activation in this series which is not encountered in aromatic amines.

2.1.2.3. Polycyclic aromatic hydrocarbons: A large number of compounds in this series are known carcinogens and any new compounds must undergo some form of evaluation before being released for human use. Many structure-activity hypotheses have been advanced to explain the carcinogenicity of these compounds. It has been suggested that successive replacement of the fused benzene ring of these compounds with methyl or alkyl groups often introduce carcinogenic potential. In this way potent carcinogens like 3-methyl cholantherene (VIII).

![VIII](image)

and 7,12 dimethyl benzanthracene (IX) can be derived. Apart from simulating a benzene ring, the methyl groups themselves may play a part in the carcinogenic sequence. Whatever their function the methyl groups seem to be instrumental in converting the non-carcinogen anthracene
into the carcinogen 9,10-dimethyl anthracene (X).

Correctly positioned methyl group, or substituted methyl groups, must therefore, be considered capable of replacing a fused benzene ring in some carcinogens, and perhaps more important be capable of transforming other non-carcinogenic polycyclic aromatic compounds into carcinogens (Boyland, 1952). Such an effect can be further disguised by the appropriate and simultaneous iso-steric replacement of a fused benzene ring by, for example, a thiophene ring.

The polycyclic aromatic carcinogens have a putative relationship to antitumor compounds such as ellipticene (XI) and olivacine (XII). Ellipticene structurally resembles with the carcinogen 7,12-dimethyl benzanthracene.

Haddow's paradox (Haddow, 1947) concerns the association between antitumor and carcinogenic properties in some
substances. This paradox advocates that a given antitumor compound may under special circumstances exhibit a carcinogenic effect, e.g., 4-dimethylamino stilbene (Haddow et al., 1948) and 4-nitro-quinoline-N-oxide (Sakai et al., 1955) were previously regarded as antitumor compounds but later on, they were found to be active carcinogens. Similarly, 4-chloro-quinoline-N-oxide possesses both carcinolytic and carcinogenic properties under appropriate circumstances (Searle, 1967, 1968). Haddow's concept is self evident from the fact that alkylating agents have been used in antitumor therapy.

2.1.2.4. Nitrosamines, Nitrosamides, hydrazines and azoxyalkyl compounds: Nitrosamines are formed by the reaction of nitrites with secondary amines over a range of pH conditions (Challis and Kyrtopoulos, 1976). The possibility of this reaction under in vivo conditions has led to increased interest in nitrosamines as carcinogens. A clear link between occupational exposures to nitrosamines and human cancer has not been established. But it has been shown that dimethyl nitrosamine is activated as mutagen by both human and rat liver microsomes (Bartsche and Montesano, 1975). α-Hydroxylation and B-hydroxylation seem to be the probable routes for the activation of nitrosamine to potential carcinogens.
The nitrosourea and the nitrosoguanidine are representatives of the nitrosamide family of carcinogens (Ward and Weisburger, 1975) and their several derivatives are employed as antitumor agents (Wheeler, 1975). Thus although there is now a much better understanding of structural requirements for the carcinogenicity of nitrosamines, all such compounds should be considered carcinogens with caution.

Two further groups of carcinogens are worth consideration in the context of nitrosamines, the first of these is the azoxylalkane group, of which ethylazoxyethane (Druckrey et al., 1963) and elaiomycin (Schoental, 1967) are representatives. The second class of compounds is that of hydrazines which are proved carcinogens (Toth, 1975). The metabolic oxidation of hydrazines to azo or azoxy compounds may precede their carcinogenic response (Preussman et al., 1969). It has been suggested that the carcinogenic activation of nitrosamine may also involve the formation of hydrazine as an intermediate (Suss, 1965).

2.1.2.5. Alkylating agents: This group includes compounds which can react with nucleophiles by alkylation. Most of the alkylating activity of the carcinogens appears to be
related with the ring strain present in a particular compound. For example \(\beta\)-propiolactone (XIII) (Roe and Salaman, 1955) is a carcinogen due to the ring strain which is much less in its homologue \(\gamma\)-butyrolactone (XIV) (Dicken and Jones, 1961), a noncarcinogen. The carcinogenic effects of alkylating agents under \textit{in vivo} conditions seem to be determined by their partition co-efficients, chemical half-life and nucleophilic reactivity.

2.1.2.6. Miscellaneous groups: Many miscellaneous carcinogens can be grouped together because each possesses an activated carbon-carbon double bond which is known to react with biologically occurring nucleophiles. This type of reaction can be detected by the isolation of cysteine or methionine adducts which could indicate the carcinogenicity of these compounds. But certainly all compounds that react with biological sulfur-containing compounds are not carcinogens (Harington, 1967). Several classes of antitumor agents also fall into this general classification of activated double bonds e.g. Chalcones etc.
2.2. **Isatins**: Isatins are a group of heterocyclic compounds with indole nucleus. The parent compound, isatin (2,3-dioxo-indoline) was discovered independently by Erdmann and Laurent in 1841 during the course of their studies on oxidation of indigo. Subsequent studies showed isatin to be a very reactive molecule that can be converted into many derivatives (Sumpter, 1944).

2.2.1. **Physico-chemical properties of Isatin**: Isatin dissolves in sodium or potassium hydroxide solution forming sodium or potassium salts that on heating result in ring opening with the formation of salt of isatic acid. Acidification results in ring closure and isatin precipitates. At higher pH isatin has been shown to undergo ring cleavage (Sumpter et al., 1949; Korshunov et al., 1950).

Oxidation-reduction potentials of isatins have been studied (Cassebaum, 1954). These studies show that the catalytic properties of these compounds could be related to their oxidation-reduction potentials. The ultra violet-spectral studies have led many workers to establish the structure of complexes formed by the interaction of isatin-3-oxime with cations. Similarly Chen et al. (1966) using ultra violet-spectrum of isatin were successful in showing that its auxin like activity was due to the conversion of isatin to isatate. Sadler et al. (1959) on the basis of
their infra red spectral studies of isatins could show that the L-amino acid dehydrogenase activity of these compounds was related to their infra red spectra.

Again, it is the highly reactive 3-oxo group of isatin on the planar structure of o-amino-phenylglyoxylic acid lactam that is responsible for its interaction with a host of diverse molecules, such as amines, amino acids, guanidines, glycols and thioglycols (Sumpter, 1959; Spande and Witkop, 1967). Sumpter, et al. (1944) reported that with mercaptans isatin forms 1 : 1 addition products with the following structure (XV).

\[
\text{XV}
\]

and with ammonia the product formed has the structure XVI. Similarly, isatin reacts with alcoholic ethylamine to give isatin-β-ethyamide (XVII)

\[
\text{XVI} \quad \text{XVII}
\]
and with phenol, isatin forms phenol-isatin (XVIII)
Isatins have been reported to undergo condensation with acetones and related ketones (Popp et al., 1980) to give compounds of general structure (XIX).

![Chemical structures](image)

The presence of such and related functional groups in the side chains of amino acids of proteins and enzymes and the reactivity of the 3-oxo-group of isatin as given above have been of special interest to enzymologists and protein chemists.

2.2.2. Biological properties of Isatin: Isatin and its derivatives have been of immense biological interest due to their diverse biological properties, some of which are reviewed below:

2.2.2.1. Central Nervous System (CNS) affecting properties: Muller (1962) reported that isatin could antagonise electrical or pentetrazol tonic seizures in mice. But indole was found to be highly toxic and did not manifest anticonvulsant activity. Sareen et al. (1962) also reported potent
anticonvulsant activity of isatin against electro-shock seizures in rats. They also showed that on N-alkylation or acylation isatin lost its anti-convulsant property. The anti-epileptic potency of isatin was found to be less than Dilantin but due to its lower toxicity it had an edge over the other drugs (Issacson, 1956 and Livingston, 1966). By virtue of its ability to cross the blood brain barrier it was thought to bind with ammonia, that may have accumulated in excessive amounts in brain of the epileptic patient, resulting in seizures. Klingberg and Muller (1968) studied the anti-extensor effect of isatin with respect to various behavioral parameters. The influence of isatin on the extinction of a passive avoidance reaction in rats has been studied by Petschnick et al. (1975). These studies showed that depending upon the dose of isatin used and the parameters of the unconditional stimulus; a facilitation of reaction and a delay of extinction were observed. The positive effect of isatin upon consolidation is discussed in connection with the influence of the vigilance level of the central nervous system. The effect of an anticonvulsant dose of isatin which produces spindle activity, influences photocally evoked potential and photocally evoked rhythms was also investigated by Ruergerie et al. (1974). The peak latencies of all components of the photocally evoked potentials were prolonged proportionally indicating a general effect of isatin upon axons, slowing down the conduction
velocity besides the effect on F and I cells of the lateral geniculate body.

Recently the effects of isatin on a behavioral syndrome after tryptamine in Pargyline-treated mice have been studied by Kastner and Muller (1981). They have reported that when mice were injected subcutaneously with 50 mg/kg body weight pargyline and after 10 min, 141 mg/kg body weight tryptamine (intravenously) they died within a maximum of 60 seconds, but survived partly after pre-treatment with isatin. The effect was found to be dose dependent. They observed that the different components of the syndrome were gradually more or less suppressed by isatin, showing that effects of isatin are partly mediated by an action on indolaminergic receptors.

2.2.2.2. Isatin and Cancer: Isatins have been reported to be associated with antineoplastic activities (Varma and Khan, 1978). Isatin thio-semicarbazone (ITSC) inactivates the transforming ability of murine sarcoma virus (Lavy et al., 1976), and replication of leukaemia virus upon direct contact in vitro. The drug inhibits RNA dependant DNA polymerase.

The effects of condensation products of thiazolinone-4 with isatin have been studied on malignant diseases (Lozyuk, 1979). The compound has been shown to possess the most pronounced anti-tumor effect on the model of Harding-Passey melanoma (mouse). The sensitivity of the melanoma
to the preparation may be connected with its being potential antimetabolite of phenyl alanine, the metabolism of which in the presence of melanoma is greatly disturbed.

Leukaemia in drug treated mice was enhanced due to inhibition of immune response by the drug. Using counter-current distribution and chromatographic techniques Matsumoto (1958) was able to identify the compound from the urine of cancer patients as dihydroxyindole. Subsequently Maki (1959) confirmed that along with indigo and indirubin, isatin was also excreted in the urine of cancer patients. Yartessova et al. (1975) found that thiosemicarbazone of 1-glucofuranosyl isatin or 1-glucopyranosyl methyl-isatin stimulates the growth of mouse adeno carcinoma.

2.2.2.3. Effect on blood sugar level: There are conflicting reports in literature on the effect of isatin on blood sugar level. Subcutaneous and intraperitoneal administration of isatin to rats in doses ranging from 60-200 mg/kg body weight did not show any diabetogenic activity (Hidy, 1946). A similar study by Bruckman and Wertheimer (1947) showed that isatin in doses of 50-80 mg/kg body weight in rats was neither toxic nor diabetogenic. On the other hand Gaede and Frischner (1948) observed a temporary but slight fall in blood sugar level of rabbits injected intravenously 25 mg/kg body weight of isatin followed by a steady rise.
reaching a maximum of 170 mg per cent in 4 h. The blood sugar level of these rabbits was found to return to normal after 24 h. Repeated intravenous injection of isatin on alternate days for over two months resulted in elevated fasting level and enhanced intolerance to glucose. When such animals were given 2 g of glucose, their blood sugar level went up to 300 mg per cent in 4 h. There was no damage to liver and kidney and hence it was inferred that isatin possibly caused a selective damage or necrosis of the pancreatic B-cells. Muller and Schultrich (1966) failed to observe any significant change in blood sugar level on intraperitoneal administration of isatin (50-200 mg/kg body weight) to rats, guinea pigs and rabbits. Oral administration of isatin for 129 days to rats showed no increase in body weights of rats. Light and electron-microscopy of isatin administered animals also did not show any untoward effect of the drug. However, Hort and Mcall (1967) observed that isatin diminished the adsorption of glucose from perfused rat small intestine but in this respect it was not less effective than oxyphen-isatin which prevented the active transport of glucose. Administration of 400 mg of isatin to human volunteers produced a statistically significant fall in their blood sugar level (Amma and Singh, 1968).
2.2.2.4 Antimicrobial properties:

Isatins have been widely studied for their antiviral, antitubercular, antifungal and other antimicrobial properties. Isatin thiosemicarbazone was shown to be effective against intracerebral vaccinia infection. It was subsequently found to be active against rabbit pox, cow pox, Aalastrim Columbia and Variola viruses (Thomson et al. 1953). Bauer (1955) found this compound to be effective also against vaccinia in mice but not against neurotropic yellow fever influenza and other viruses. Bock (1957) demonstrated its inactivity against ectromelia.

Derivatives of isatin-3-thiosemicarbazone furnish an elegant example of structure-activity relationship. Bauer and Sadler (1960) found that substitution at N-1 enhanced activity against vaccinia virus, but these compounds had no influence on ectromelia virus. On the other hand, substitution at N-4 yielded derivatives which were active against ectromelia virus but were ineffective against vaccinia virus (Bauer and Sadler, 1961). N-methyl isatin-3-thiosemicarbazone (XX) has been found to be an excellent agent against smallpox; it proved highly efficacious during an epidemic (Bauer et al, 1963).
Several workers have tried to delineate the mechanism of antiviral activity of 1-methyl isatin thiosemicarbazone. Furasawa et al. (1965) showed that N-methyl isatin thiosemicarbazone inhibited the viral specific protein synthesis and virus maturation step (assembly of the DNA and the protein coat). In the same year Appleyard et al. (1965) showed that N-methyl isatin thiosemicarbazone did not prevent the synthesis of viral DNA. Woodson et al. (1965) added isatin-B-thiosemicarbazone at different times and in different concentrations to preparations of HeLa cells infected with vaccinia virus. Although no effects were observed up to 3 h after infection, the presence of ITSC resulted in a reduction of the functional half-life of viral m-RNA by 15-60 min to 5 h so that synthesis of protein programmed to be translated later
than 3 h drastically reduced and consequently little (if any) mature viral progeny was formed. Magee and Back (1965) studied the antiviral activity of ITSC and its analogues with HeLa cells. It did not affect the early biological and enzymic activities. The N-methyl derivative of ITSC inhibited viral induced DNA synthesis. N-methyl-4, 4-dimethyl ITSC was much more inhibitory than ITSC or its N-methyl derivative. Wolfgang et al. (1966) reported that ITSC inhibited the synthesis of early enzymes and arrested vaccinia virus multiplication. Further, it was demonstrated that in the presence of ITSC, certain proteins were altered allosterically and these in turn prevented the functioning of m-RNA. In addition there are many other reports in literature on isatin-3-thiosemicarbazone and its derivatives as potent antiviral agents (Hermann, 1968; Sidwell et al. 1968; Jornef and Gunner, 1972).

Tomchin and Fusachov (1974) suggested that B-thiosemicarbazone-5-sulfamido-isatin had preventive action against vaccinia virus. Also isatin B-semicarbazone and its derivatives have been examined for their ability to inhibit wild type and IBT resistant mutants of vaccinia virus and for promoting the growth of IBT dependant mutant. Out of 13 compounds tested only 2 inhibited the growth of all the three types of viruses and thus differed from IBT. These compounds were found to interfere with
DNA synthesis of the virus. This difference is important in view of the substantial similarity in the chemical structure of the two compounds. Similarly isatin-N-Mannich bases have been studied for their antiviral, antibacterial and antifungal activities (Varma and Lewis, 1975; Kupinic et al., 1979).

Lately Zgorviak et al. (1976) have demonstrated a protective effect of ITSC on mice infected with neurovaccinia virus. Again the studies on antiviral activity of certain isatin isothiosemicarbazones against mengo and vaccinia virus infection showed that out of 11 compounds tested only 1-ethyl isatin-S-n-butylisothiosemicarbazone was significantly effective against lethal mengo and neurovaccinia virus induced encephalitis in mice (Veckenstedt, 1979). Further in a cell free preparation isatin isothiosemicarbazone has been shown to inhibit RNA-dependent RNA polymerase of mengo virus (Toneu et al., 1980).

A large number of hydrazones including isatin-3-hydrazone were tested for their anti-tubercular activity (Bau-Hoi et al., 1953; Kashimura et al., 1954). Subsequently Knotz (1970) reported that acylhydrazones and semicarbzones of l-isatin acetic acid derivatives were quite effective antiviral and antimicrobial agents.
Similarly, antifungal activity (Claude, 1967), anti-candida albicans (Sasaki, 1957) activity and anti-brucella activity of isatins (Jeney and Zsolnai, 1955) have also been reported.

### 2.2.2.5 Other miscellaneous properties:

Isatins have also been studied for their analgesic, cathartic, plant growth promoting and herbicidal properties. Isatin has been shown to potentiate the analgesic action of morphine (Razdan and Razdan, 1966) and Jacques (1970) demonstrated that 5-bromo isatin possessed analgesic and sedative properties and it was shown to have an edge over aspirin in that it did not show any side reactions such as lengthening of the blood clotting time.

Several derivatives of isatin have been found to possess cathartic activity. Bergel (1926) found that phenyl isatin was endowed with cathartic activity while other isatin derivatives such as pentaacetylresorcin isatin, diacetyl guaiicol isatin, dimetacresol isatin acetate and diorthocresol isatin (Yamamoto and Kawahara, 1937) and diacetyl bis (p-hydroxy-phenyl) isatin have also been shown to exhibit cathartic activity (Demande, 1971).

Isatin has been shown to possess auxin-like activity (Galstone and Chen, 1965, Milen and Galstone, 1968). Chen et al. (1966) confirmed the above findings.
and further observed that it was due to the gradual conversion of isatin to isatic acid. James and Wain (1968) investigated the auxin-like activity of isatic acid and 26 derivatives obtained by substitution in the benzene ring using the wheat cylinder, the pea segment and the pea curvature tests. Isatin along with L-tryptophan and gibberellins prevented the induction of flowers from the buds of Plumbago indica var Angkor (Nitsch, 1967).

Isatins (alkyl and halogen derivatives) have been found to be quite effective as pre and post emergence herbicidal agents for killing dicotyledonous plants, but they do not attack grasses (Hoechst, 1957).

2.2.3. **Enzyme like activity of Isatin:**

Isatin, along with alloxan, ninhydrin and quinones oxidises L-amino acids to one carbon lower aldehydes, CO₂ and NH₃ (Traube, 1911; Adberhalden, 1948). However, it was Langenbeck (1927), who embarked upon a comprehensive study of dehydrogenase like activity of isatin.

The mechanism of oxidation (dehydrogenation) of L-amino acids suggested by Langenbeck was based on the reversible reduction of isatin to isatide in which atmospheric oxygen or methylene blue could serve as hydrogen acceptors.
Langenbeck (1928) further observed that the dehydrogenase like activity of isatin was relatively small as compared to that of isatin-5-sulphonic acid. This catalytic activity of isatin was found to increase between 70-100°C. Langenbeck et al. (1937) found that 3-amino-naptho indole also exhibited this activity. Amongst these compounds, 6-hydroxy and 6-methyl derivatives were more active than 3-amino napthoindole. The catalytic activities of these compounds were higher when studied in dilute pyridine solution. Introduction of a carboxyl group in 4 and 6 positions also increased the activity, and the carboxylic acid was about 3 folds more active than the yellow enzyme of Warburg and Christian. Later Langenbeck et al. (1956) reported the dehydrogenase activity of isatins in relation to oxidation reduction.
potentials and molecular weights of some complex isatins, viz. phenyl-, styryl-, indolo-, and coumarino-isatins.

Abderhalden (1938) studied the action of isatins on polypeptides. It has been shown that the dehydrogenase activity of isatin is due to its 3-keto group (Giovanini and Portman, 1948, 1957) and that this activity could be modified by proper substitution in the benzene ring. Isatin 4- and 5-carboxylic acids in particular were found to be much more effective as catalysts than the parent compound.

Mix and Krause (1956) tested the dehydrogenase activity of 4-substituted isatins and N-(7-methyl isatin-4-carboxyl)amino acid ethyl esters substituted in the 4-position against methylene blue in 70% dimethylformamide at 40°C. They observed that introduction of a methyl group at position 7 had no effect on the catalytic activity of isatin. In a later communication Mix et al. (1958) tested acid amides of 7-methyl isatin-4-carboxylic acid and other derivatives of the carboxylic group and 7-methyl-4-carboxylic amide of isatin was found to be the most active catalyst; the tertiary amides were less active. The greater activity of these compounds was attributed to hydrogen bonding.

O'Sullivan and Sadler (1957) implicated both the carbonyl groups of isatin in the dehydrogenase activity.
In 1957 they further examined the Sigma values and the stretching frequencies in infra-red spectra in relation to catalytic activities and discussed their implications in predicting the pharmacological actions of such compounds.

Sadler, Mix and Krause (1969) studied the infra-red spectra of isatin derivatives in relation to dehydrogenase activity. They tried to correlate the activity of 7-methyl isatin 4-carboxylic acid with its various possible structures and also discussed the role of hydrogen bonding in this compound.

Cassebaum (1954) observed that the electro-chemical behaviour of isatins was related to their dehydrogenase activity. The dependence of catalytic activity on polar and steric effects of substituents in isatin was also studied (Cassebaum and Liedel, 1960). The role of hydrogen-bonding and the solvents were also examined.
2.2.4. Modification of Enzymes and Proteins by Isatins:

Many enzyme activities have been reported to be modified by isatins. An inhibition of rat liver enzyme by 5,7-dinitro isatin was reported by Felix and Ghaefer (1947). They used this inhibition to detect liver injury by 5,7-dinitro isatin and trinitro toluene. Bruns (1954) found that isatin, alloxan and ninhydrin were inhibitory to milk xanthine oxidase. Isatin (5mM) inhibited the enzyme by 73 per cent while alloxan and ninhydrin in the same concentration could inhibit the enzyme activity by only 43 per cent and 31 per cent respectively. The inhibition was found to be a function of inhibitor concentration and time of reaction. In view of the fact that isatin, ninhydrin and alloxan were known to react with amino acids by a Strecker type reaction, it was concluded that the inhibition was the result of the reaction of the inhibitor with the enzyme protein, possibly resulting in deamination and decarboxylation of its N-terminal amino acid. These inhibitors maintained their inhibition properties even when xanthine was substituted by benzaldehyde as substrate.

Shigyo (1957) studied the effect of isatin, homogentisic and benzoquinone acetic acid on the degradation of tryptophan to kynurenine by rabbit liver extract. It was found that all the three compounds inhibited the breakdown of tryptophan. Inhibition by
isatin and homogentistic acid was overcome by the addition of glutathione, while that by the third compound was not affected.

Calvet and Bozal (1958) studied the inhibition of tryptic digestion by certain drugs including isatin. Muller (1962) reported that isatin inhibited monoamine oxidase activity in mice liver homogenate. In a latter communication (1965) Muller and Schmiedel showed that administration of isatin, N-methyl isatin, dioxindole and oxindole inhibited monoamine oxidase activity of mice liver homogenates. Isatin-B-hydrazone and isatin-B-imide exerted only an insignificant inhibitory effect. On the other hand, 5-bromo-isatin as well as 5-bromo-B-hydrazone were found to be more potent inhibitors in comparison to isatin and isatin-B-hydrazone respectively. After 5 h of in vivo administration isatin and 5-bromo-isatin decreased the activity of liver monoamine oxidase by 3 and 60 per cent respectively. These compounds inhibited the brain enzyme only to an inconsequential degree. Linitskoya et al. (1972) demonstrated that isatin thiosemicarbazone activated viral alkaline DNase and inhibited the acid DNase.

Webb et al. (1974) have reported N-methyl isatin-B-thiosemicarbazone as new phosphodiesterase inhibitor in human lymphocytes. Besides the aforesaid reports, published evidence from this laboratory shows that
isatin (200 mg/kg body weight) administration to rats diminishes the activity of alkaline phosphatase after 5 h but caused an increase in enzyme activity in duodenum and jejunum after 2 h and 5 h of the administration of the drug. The increase in the activity was possibly due to induction of the enzyme by isatin (Singh et al., 1978).

Kumar et al. (1977) have demonstrated the utility of isatin as a valuable tool for the screening of species specificity of testicular hyaluronidase. Singh et al. (1978) have shown isatin to be a non-allosteric uncompetitive inhibitor of rat kidney alkaline phosphatase. The mode of attachment of the drug with the enzyme protein was possibly through essential amino group(s).

Studies on the effect of isatin on rat testicular acid phosphatase (Kumar et al., 1978) show that isatin combines with the enzyme protein at the non-allosteric site and the activation is of mixed type.

Krawczyk (1962) made an interesting use of isatin when he differentiated peptides obtained by tryptic digests of human and horse homoglobins. He showed that peptides obtained from human haemoglobin were mostly stained pink while those from horse haemoglobin stained dark violet or violet pink on treatment with isatin. He has ascribed this difference to possibly different amino acid composition of human and horse haemoglobins.
Atassi and Ząblocki (1975) have reported that 2,3-dioxo-indolinesulfonic acid is a highly specific reagent for modification of tryptophan in peptides and proteins. The reagent offers the advantages of stability, easy handling, high water solubility and high specificity. It affords protein and peptide derivatives that are completely water soluble because of the polar nature of the added group. The yellow color ($\lambda_{\text{max}}$ 367) of the derivatives offers advantage of easy determination of the extent and localization of the modification.

2.3. Alkaline Phosphatase: Alkaline phosphatases (EC 3.1.3.1) are the enzymes which catalyze the hydrolysis of phosphomonoesters, optimally at alkaline pH. Kidney, intestine, bone marrow and placenta are the rich sources of alkaline phosphatases.

2.3.1. Alkaline phosphatases and Cancer: Changes in the levels of alkaline phosphatase have been observed in different carcinomas. Serum alkaline phosphatase was investigated in the course of cancer of several tissues, including bone. Its activity is increased principally in primary bone tumors, osteogenic sarcoma, and in metastasis of bone, carcinoma of prostate or parathyroid, with osteoblastic metastatic lesions. Serum alkaline phosphatase is also raised in liver metastases. It's
level is elevated in nonisophoblastic neoplasm (Huang et al., 1973), cystic fibrosis (Hosh et al., 1976) and osteocarcinoma (Ghanta et al., 1976). High alkaline phosphatase activity in some carcinomas is the basis of action of some antitumor drugs. N-p-di-2-chloroethyl aminophenol is unselective cytotoxic drug but its phosphate ester is not cytotoxic. So in cancerous cells, because of high alkaline phosphatase activity more drug is released which causes the death of the cancerous cells (Workmann et al., 1978).

However, it is reported to decrease significantly in leukocytes of patients with malignant disease (Lokich and Jacob, 1971), bladder cancer (Kunze et al., 1975), spontaneous treatoma (Bernstine and Hooper, 1973), squamous cell carcinoma (Rubin and Levij, 1971), breast cancer (Srivastava et al., 1976) embryonal carcinoma (Chung et al., 1977) and testicular carcinoma (Fishman et al., 1974).

A most important discovery concerning alkaline phosphatase isoenzymes in cancer was made by Fishman et al. (1968a, b). These authors characterised an alkaline phosphatase which was undistinguishable from placental enzyme in serum and tumor of a patient with bronchogenic carcinoma. This type of phosphatase was named "Reagan" after the name of the patient and was extensively investigated (Fishman et al., 1968a, b, 1971; Fishman, 1969; Stolbach et al., 1969). Both
enzymes were sensitive to L-phenylalanine, unaffected by heating during 16 min at 55°C and 5 min at 65°C, and were very active against phenyl phosphate at pH 10.7. Electrophoresis on starch gel, using sodium o-naphthyl phosphate at this pH, and performed on heated serum, revealed a "placental band". Action of neuraminidase, Km optimum, pH, and inhibition by p-hydroxymercuribenzoate were similar. Fishman (1968) was able to demonstrate that Reagan isozyme is identical to the variant "B" of placental isozyme. It has been found in the serum of patients with cancer of various organs: lungs, gastrointestinal tract, genital tract. During therapy, Reagan isoenzyme progressively disappears (Stolbach et al., 1969); but Nathanson and Fishman (1971) found the Reagan isoenzyme in the serum of some patients without cancer — principally during the course of ulcerative colitis, familial polyposis of the colon, and some types of cirrhosis. Warnock and Reisman (1969) have described an alkaline phosphatase, the properties of which resembled those of the placental form. It was found in extracts of tumors in 8 out of 10 patients with hepatocellular cancer; and the sera of 3 of 6 patients showed the same isozyme. Benham et al. (1981) have studied alkaline phosphatase isozymes as possible markers of differentiation in human testicular teratocarcinoma cell lines. Richiko et al. (1981) in their studies on alkaline phosphatase isoenzymes from
spontaneous colon carcinoma of highly inbred rats have found that the specific activity of carcinoma alkaline phosphatase was 10 fold higher than normal.

2.3.2. Molecular characteristics: Denaturation experiments with urea (Butterworth and Moss, 1967) and at low pH (Butterworth, 1967/1968; Scutt and Moss, 1968) have revealed that all the mammalian alkaline phosphatases have two subunits. These subunits are structurally similar and their molecular weights vary depending upon the source, between 65000 to 90000 D. In most of the cases, the subunits have same molecular weights. Pig kidney alkaline phosphatase has been shown to have two subunits of 90000 and 90000 D (Ramaswamy, 1974). The homodimers are coded by one cistron.

Each alkaline phosphatase has several isoenzymes which are identified by their electrochemical and immunological properties, thermolability and differential response to substrate and effectors (Farnley, 1971; Fishman, 1974). The different isoenzymes may be coded by different genes. The isoenzymes may have same protein core with identical catalytic centre(s). Alkaline phosphatase from human kidney contains a minor component of intestinal enzyme. The different isoenzymes when treated with neuraminidase, become identical in their electrophoretic and biochemical properties. Eight multiple forms of placental enzymes after treatment with neuraminidase are reduced to four different forms (Kirsten et al., 1976).
The mammalian alkaline phosphatases have been shown to be glycoproteins (Ostrowski et al., 1976; Kamoda and Sakagishi, 1976; Pasen, 1970; Kamoda and Sakagishi, 1978). They contain glucosamine, galactosamine, galactose, fucose, mannose, glucose and sialic acid. The different alkaline phosphatases differ in their carbohydrate content. Bovine kidney alkaline phosphatase has been reported to have about 15 per cent carbohydrate. Purified intestinal enzyme from adult human contains very little of sialic acid whereas the fetal intestinal enzyme has good amount of sialic acid. This difference has been attributed to the "Sialidase" activity of *E. coli* present in adult intestinal mucosa. Desialation of liver enzyme results in lowering of its molecular weight from 1,75000 D to 1,67000 D and its half life is reduced from 125 h to 22–25 h. Desialation plus degalactosilation or desialiation plus defucosylation result in shortening of half life to 1–2 h of kidney enzyme.

The experiments with sialidase treatment of alkaline phosphatase isoenzymes indicate that a possible role of sialic acids and other carbohydrates may be to protect the active conformation of the enzyme. Possibly the carbohydrate moiety which when present, increases the stability of protein to heating and pronase digestion and maintain the three dimensional structure of the protein. This is supported by the finding that sialidase treatment causes conformational changes in prostatic acid
phosphatase. It has been reported that the sialidase treatment does not significantly effect other properties of human liver alkaline phosphatase e.g. interaction with L-homoarginine and antigenicity. Similarly treatment with mannosidase, exo-N-acetyl-D-glucosaminidase and end-N-acetyl-D-glucosaminidase-L displayed a decrease in enzyme activity.

The mammalian alkaline phosphatases require two types of metal ions, one which are essential for activity as Zn$^{2+}$ and others which are activators, as Mg$^{2+}$ (Cathala and Brunel, 1975; Jan Ahlers, 1975; Linden et al., 1977; Brunel and Cathala, 1971; Brunel and Cathala, 1973; Ohulo and Langerman, 1974; Brieve, 1975). Zn$^{2+}$ is usually associated with native enzyme loosely bound at the active site. Bovine kidney alkaline phosphatase contains 4.5 ± 0.2 g-atoms of Zn$^{2+}$ per mole of protein (Cathala and Brunel, 1975) and cow milk enzyme has 4.9 ± 0.6 g-atoms of Zn$^{2+}$ per mole of protein (Linden et al., 1977). In general number of g-atoms of Zn$^{2+}$ per mole of protein ranges between 2 to 4 (Cathala and Brunel, 1975; Linden et al., 1977; Hiwada and Wachsmith, 1974; Fossel et al.). Zn$^{2+}$ can be easily removed by chelating agents like EDTA and KCN (Ackermann and Ahlers, 1976). Interestingly ovarian alkaline phosphatase incubated with EDTA and amino acids is inactivated, but when incubated with either EDTA or amino acid no inactivation is achieved.
It has been suggested that the active site is unable to accommodate big EDTA molecule but can bind small amino acid to form the complex (E-M-AA). This complex could undergo conformational changes to form a modified enzyme which could interact with EDTA. The extent of inactivation depends on the nature of amino acid used. Glycine was found to be the most effective. Claude et al. (1975) have discussed the mechanism of activation by Mg$^{2+}$ and Zn$^{2+}$ of rat placental enzyme (Brieve, 1975). According to their model, asymmetry is the main feature of alkaline phosphatase active site. Zn$^{2+}$ or Mg$^{2+}$ either induce or prevent the asymmetry of the enzyme and hence their binding to the catalytic site determines the enzyme activity (Scheme I).

Similar activatory effect of Mg$^{2+}$ has been observed in other alkaline phosphatases (Linden et al., 1971; Brunel and Cathala, 1971; Brunel and Cathala, 1973; Ohkubo and Langerman, 1974). In case of cow milk alkaline phosphatase Mg$^{2+}$ can be replaced by Mn$^{2+}$, Ni$^{2+}$, Co$^{2+}$ and Zn$^{2+}$. Mn$^{2+}$ and Ni$^{2+}$ binding at Mg$^{2+}$ binding site cause decrease in activity of the enzyme (Linden et al., 1977). Activation by Mg$^{2+}$ is also dependent on pH. Jan Ahlers (1975), who studied the effect of Mg$^{2+}$ at different pH values, has put forward a scheme to explain the pH effect on stimulation by Mg$^{2+}$. At low pH enzyme species are formed which prevent the binding of Mg$^{2+}$ or of substrate to the enzyme (Jan Ahlers, 1975). (Scheme II).
23.3. **Mechanism of Hydrolysis:** According to Kerishtein et al. (1975) the substrate binding to the enzyme active site via some functional group is the first step in the enzymatic hydrolysis (Vol'Kerishtein et al., 1975). Conformational transformations occur in the enzyme molecule and the structure of the active site changes due to the energy released by interactions of the ester with the active site of the enzyme which results after further interactions into the hydrolysis of the substrate.

To explain the role of Mg$^{2+}$ and Zn$^{2+}$ in the hydrolysis Jan Ahlers (1975) has suggested the mechanism of hydrolysis of phosphomonoesters by pig kidney alkaline phosphatase. Tyrosine residue was found to be present at the active site. Presence of hydroxyl group(s) at the active site has been reported by other workers (Hiwada and Wachsmith, 1974; Sizer, 1942). It is suggested that binding of Mg$^{2+}$ leads to a conformational change which causes the substrate to be shifted into the vicinity of the hydroxyl group conjugated to Zn$^{2+}$. The hydroxyl group liberates the alcoholic residue by nucleophilic attack on the phosphorus atom. The transfer of phosphate group to a serine or a tyrosine residue occurs coincidently and subsequently the enzyme is dephosphorylated. The dephosphorylation reaction being the rate limiting step and is determined by the functional group(s) at the active site. The mechanism proposed also explains how
Scheme of effect of metal ions on the activity of alkaline phosphatase

Scheme of effect of Mg\(^{2+}\) and pH on the activity of alkaline phosphatase

Mechanism of substrate hydrolysis at the active site of pig kidney alkaline phosphatase
dephosphorylation occurs. The phosphate bound to the serine residue is liberated after conformational changes induced by Mg$^{2+}$ and caused by alterations in the conformation of hydroxyl group on induction by Zn$^{2+}$ (Fig. 1).

Mammalian alkaline phosphatases can also hydrolyze orthophosphate and pyrophosphate (Cox and Griffin, 1965; Farnley and Walker, 1967). Moss et al. (1967) studied the pyrophosphatase activity of purified human liver and intestinal enzyme. They confirmed the results of Cox et al. (1965) and Farnley et al. (1967). It has been suggested that a single type of active site is responsible for activity towards both classes of substrates (Eaton and Moss, 1967). Excess of Zn$^{2+}$ has been reported to inhibit the pyrophosphatase activity of alkaline phosphatase. Zn$^{2+}$ forms a complex with pyrophosphate and this complex acts as a competitive inhibitor of pyrophosphatase and orthophosphatase activities (Moss, 1964). Excess of Mg$^{2+}$ also inhibits pyrophosphatase activity by decreasing the concentration of free substrate available for hydrolysis (Eaton and Moss, 1967). Possibly Mg$^{2+}$ inhibits pyrophosphatase activity by forming a complex with substrate which acts as a competitive inhibitor (Moss, 1964). Low concentrations of Mg$^{2+}$ activates both types of hydrolytic activities.

Lumeng et al. (1975) found that pyridoxal phosphate and p-nitro phenylphosphate are hydrolyzed by a membrane
bound enzyme. In both cases same pH profiles, pH-Km profiles and inhibitors were observed suggesting that both the activities are associated with one enzyme. It is suggested that rat liver alkaline phosphatase is related to metabolism of pyridoxal phosphate.

2.3.4. Modifiers of Alkaline Phosphatase Activity:
A number of factors are known to modify the activity of alkaline phosphatase both in vitro and in vivo.

2.3.4.1. Inhibition by Sugars: Komoda et al. (1977) while studying the interactions of human alkaline phosphatases with sugars found that hexosamines N-acetyleneuraminic acid (NANA), N-acetyl neuraminic acid and N-acetyglycosylenuraminic acid caused mixed type inhibition of the enzyme. The sugars having both free NH₂ and COOH groups were more potent inhibitors as compared to those having either one of the two groups. Sialic acid was found to be most potent inhibitor.

Tersa et al. (1976) reported the partial inhibition of milk alkaline phosphatase by sucrose. They suggested the presence of two types of alkaline phosphatase in milk, one sensitive to sucrose and the other resistant to it (Tersa et al., 1976). Dupis (1976) reported the inducing effect of sorbitol on rat jejunum enzyme.

2.3.4.2. Effect of Detergents: Nonionic detergents triton- X100, Tween-20 and the Zwitterionic detergents,
Empigen BB, increased the rate of hydrolysis of p-nitrophenylphosphate by calf thymus alkaline phosphatase without changing the Km (Ey and Ferber, 1977). The bile salts sodium deoxycholate and sodiumcholate decreased the rate of hydrolysis and increased the affinity of the enzyme for nitrophenyl phosphate. It is further reported that the inhibition caused by the deoxycholate and cholate could be reversed by the addition of pure lecithine and lysolecithine. It was concluded that membrane bound alkaline phosphatase is separated from most if not all of its neighbouring lipid moieties by these detergents.

2.3.4.3. Effects of short chain fatty acids: Koyama et al. (1976) reported that the short chain fatty acids such as propionate, n-butyrate, n-valeric, isovaleric, n-capric, and n-caprylic acids induced alkaline phosphatase in cultured mammalian cells. n-Butyrate was found to have maximal inducing effect, whereas long chain fatty acids had no effect on the enzyme activity. n-Butyrate when added alongwith other inducers like c-AMP and 5-bromodeoxy uridine had a synergestic effect. The inducing effect is suggested to be at the transcriptional level.

There seems to be a casual relationship between high alkaline phosphatase activity during lactation and presence of these fatty acids and elevated enzyme levels during the differentiation of the mammary glands (Koyama et al., 1976).
2.3.4.4. Effect of Tris (hydroxy methyl) amino methane: Tris (hydroxy methyl) amino methane has been reported to activate reversibly the alkaline phosphatase (Neumann et al., 1975). The presence of three alcohalic functions in Tris confers nucleophilic properties on the buffer molecules and under certain conditions extensive phosphorylation of the hydroxyl groups can be catalyzed by alkaline phosphatases (Wilson and Dayson, 1964; Neuman, 1969; Reich and Wilson, 1971). Increase in the rate of substrate hydrolysis in the presence of Tris is explained either by this trans-phosphorylation reaction or it forms a more active complex with the enzyme or it can potentiate binding of substrate at second active site due to its ionizing effect on the acidic groups of enzyme at alkaline pH. At high concentrations it can also cause changes in the dielectric constant of the medium and decrease the solvation of the protein and the tertiary structure of the enzyme.

2.3.4.5. Effect of Diet: Alkaline phosphatase activity is also regulated by the dietary factors. Alberts et al., (1976) found that feeding of Zn$^{2+}$ free diet to guinea pigs resulted in lowering of serum alkaline phosphatase. Molyzhera et al. (1976) reported that feeding of protein free diet led to 75 per cent loss of enzyme activity from intestinal mucosa in rats. Feeding the protein rich diet led to 2 fold increase in the intestinal alkaline
phosphatase activity. Deficiency of vitamin D is also reported to be manifested by reduction in alkaline phosphatase activity (McCarthy et al., 1977). It has been reported that dietary Zn$^{2+}$ and vitamin D are more efficient in restoring the levels of the enzyme in serum than the diets rich in either of the two (Kumar and Belavady, 1975). Deficiency of thiamine is also accompanied by a decrease in intestinal alkaline phosphatase activity in rats. Schaler et al. (1974) found that the inhibition of alkaline phosphatase by L-phenylalanine results in a decrease in transport of thiamine across the intestinal membranes. They suggested that intestinal alkaline phosphatase plays an important role in the active thiamine transport across the intestinal membranes. Alimentary B$_1$ avitaminosis also causes a fall in brain enzyme which can be restored to normal levels by injections of oxythiamine (Pron'ko et al., 1975). Low doses of fluoride in diet have no detectable effect on serum enzyme activity (Speirs, 1974). Whanger (1973) observed that the diet containing additional amounts of nickel acetate cause reduction in packed cell volume (PCV), Hb., tissue cytochrome oxidase and alkaline phosphatase in weanling rats.

2.3.4.6. Effect of Amino acids: L-phenylalanine inhibits uncompetitively intestinal alkaline phosphatase whereas D-phenylalanine is ineffective (Fishman and Ghosh, 1967). Histochemical studies also show that L-phenylalanine
inhibits nonspecifically alkaline phosphatase in kidney, urinary bladder, testes and epididymus (Sugai et al., 1974).

Human kidney alkaline phosphatase and rat kidney alkaline phosphatase have been reported to be inhibited by L-cysteine (Cox and Griffen, 1967; Agus et al., 1966; Singh et al., 1978). It was noted by El-Assare et al., (1975) that it is a specific inhibitor of mammalian alkaline phosphatase and has no effect on 5'-nucleotidase. Low concentrations of L-cysteine have been reported to activate the intestinal enzyme (Fishman and Ghosh, 1967). L-homoarginine also inhibits alkaline phosphatase (Kamoda and Sakagishi, 1976). L-tryptophan, B-norleucine and L-leucine have also been reported to inhibit hepatoma alkaline phosphatase (Higashino et al., 1973). Adunts and Sarkisyon (1981) have studied the effect of complex forming amino acid on alkaline phosphatase activity. They have shown that histidine significantly stimulated the activity of purified alkaline phosphatase from chick intestine. Serine at a similar concentration was also stimulatory but to a lesser extent. The stimulatory effect of mixture of histidine and serine was lower than that of each of the amino acid used separately. Zn^{2+} ions were found to block the stimulatory effect of histidine apparently due to conformational changes in the tertiary structure of the alkaline phosphatase.
2.3.4.7, Effects of Metal Ions: Excess of Zn$^{2+}$ (Moss et al., 1967) oxovanadium (iv) ion, Vo$^{2+}$ and oxo vanadium (v) ion, Vo$^{3+}$ are potent competitive inhibitors of alkaline phosphatase. Mg$^{2+}$ has been reported to stimulate a number of alkaline phosphatases (Cathala and Brunel, 1975; Cathala and Brunel, 1975; Jan Ahlers, 1975; Linden et al., 1977; Brunel and Cathala, 1971; Brunel and Cathala, 1973; Ohkulo and Langerman, 1974; Brieve, 1975). It has been reported that alkaline phosphatase activity was inhibited by bismuth (Komoda et al., 1981). These authors have shown that bismuth was a more effective inhibitor of human alkaline phosphatase than of other alkaline phosphatases tested.

2.3.4.8, Effect of Metal Chelating Agents: Metal ion chelating agents like KCN, EDTA and glutathione inactivate the enzyme by removing Zn$^{2+}$ from the active centre of the enzyme (Ackermann and Ahlers, 1976). This inhibition is reversible at low concentrations and irreversible at high concentrations of the chelating agents. A number of (N) heterocyclic carboxaldehyde thiosemicarbazones with metal chelating capacity are reported to inhibit alkaline phosphatase purified from a murine ascitic cell line of sarcoma (Lechi et al., 1974) resistant to antileukemic drugs (Lee et al., 1973). Pokrovskaya (1973) found that a single intraperitoneal injection of disodium EDTA to 6-8 or 15-18 months old male rats had no effect on
alkaline phosphatase activity in liver, heart, kidney or muscle but the enzyme activity was decreased in testicles of mature rats.

2.3.4.9. Effect of Concanavalin A: Interactions of concanavalin A with alkaline phosphatase differ according to the source of the enzyme (Komada and Sakagishi, 1976). It strongly activates the intestinal enzyme but slightly inhibits liver alkaline phosphatase. These interactions are reversible in the presence of methyl-D-mannoside. It is suggested to inhibit enzyme activity by binding at the site needed for L-homoarginine inhibition and do not alter the binding of L-phenylalanine.

2.3.5.10. Effect of levimisol and R 8231: Levimisol and R 8231 are known to be potent organ specific inhibitors of alkaline phosphatase in rat and dogs. It seems that enzyme preparations sensitive to L-phenylalanine are insensitive to levimisol and R 8231 and vice versa. These two compounds have similar organ specificity as that of L-homoarginine (Van Belle, 1976). The inhibition caused by them does not need Mg$^{2+}$ but can be counteracted by the addition of high concentration of N-ethylamino ethanol (NEAE) which is a known uncompetitive activator of enzyme (Amader and Urban, 1972; McComb and Brown, 1972). The inhibition by levimisol and R 8231 and activation by NEAE takes place at the dephosphorylation step in the enzyme
catalyzed hydrolysis of the substrates. A number of tetramisol derivatives are also reported to inhibit alkaline phosphatase (Bhargava and Kuldeep, 1973).

2.3.4.11. Effect of other compounds: In vivo administration of colchicine induces alkaline phosphatase activity in livers of rats, and glucocorticoids are not required for this inhibition because adrenalectomy does not affect the inhibition pattern (Wilfred, 1977). Also, colchicine when injected along with inhibitors of transcription, had no effect suggesting its action at the transcriptional level. Sanker (1975) found that nicotine caused a significant reduction in rat intestinal total alkaline phosphatase, possibly by lowering the metabolic rates of the intestinal cells. The inhibition of alkaline phosphatase has been correlated with more chances of gastrointestinal ulcers in smokers. Tributyrin and dipeptides have been reported to inhibit intestinal enzyme, allosterically (Ucoler et al., 1975). It has been suggested that the enzyme exists in different conformations which are rearranged by tributyrin and dipeptides.

Messer et al. (1975) reported 100 per cent stimulation of placental alkaline phosphatase by ATP. This effect was abolished in presence of n-butanol.

An elevation in plasma and liver alkaline phosphatase in Beagle dogs on treatment with different
drugs known to induce liver microsomal enzyme (colorimizole, phenobarbital and phenylbutazone) has been reported (Tettenborn., 1973). Hexachlorophene (HCP) is reported to reduce the enzyme levels in plasma (Nakanc et al., 1973). In vitro, thyrocalcitonin strongly stimulated enzyme activity at pharmacological doses and increased Ca$^{2+}$ (Lechi et al., 1974). HgCl$_2$ induced renal tubular lesions in rat; show a transient fall in alkaline phosphatase activity which is restored to normal levels by injections of L-thyroxine (Schulfe-Wissermann et al., 1977).

D-penicillamine (Raab et al., 1974) also causes a fall in mammalian alkaline phosphatase activity. Garbanivarna aushaden (GA), an ayurvedic antifertility agent, causes a rise in uterine alkaline phosphatase activity in female rats (Raman and Rao, 1976).

Atmospheric temperature also affects the alkaline phosphatase activity (Rakhimov and Karotina, 1975); exposure of whole animal to high temperatures decreased the intestinal enzyme activity.

A possible relationship between intestinal alkaline phosphatase; plasma cholesterol, ABO and Lewis (a$^+$) blood groups in mammals has been reported (Benten et al., 1975).