CHAPTER X

INTRODUCTION

Isatin is an oxidation product of indigo and was discovered by Erdman in 1841. It has now acquired immense importance due to its applications in analytical biochemistry and many other fields of modern biology. It has been used as spraying agent in chromatography for the detection of amino acids like proline and hydroxyproline (Acher et al., 1950; Smith, 1953; Bonomi and Vecchioni, 1959). Its 3-thiosemicarbazone has been used for the detection of metals (Mitra and Guha, 1955). Buscarono and Izuierdo (1964) made an important use of N-ethyl isatin-3-oxime for the gravimetric estimation of uranium and again Divis (1969) employed isatin-3-oxime for the estimation of Cu$^{2+}$. The CNS affecting properties of isatin and its derivatives have been of considerable interest to researchers working for the development of newer drugs against epilepsy. Isatin has been shown to be endowed with the ability to cross blood brain barrier and act as a potent and comparatively non-toxic antiepileptic agent (Muller, 1962; Sareen et al., 1962). Recently several compounds with anticonvulsant activities have been obtained by condensation of acetone and other ketones with substituted isatins, some of which exhibit better potency than the parent compound (Popp et al., 1980). The antiepileptic properties of isatin are attributed to its activated 3-keto group which can possibly bind with ammonia to prevent its excessive accumulation in brain (Sareen et al., 1962).
Isatins have also been implicated both in chemotherapy of cancer as well as carcinogenesis. Thompson et al. (1953) demonstrated that isatin thiosemicarbazone was effective against intracerebral vaccinia infection. In 1975 Yortseva et al. found that thiosemicarbazone of 1-gluco-furanosyl-isatin stimulated the growth of mouse adenocarcinoma. Isatin has been detected in the urine of cancerous patients (Maki, 1959). Isatins have also been reported to possess antineoplastic activities. Lately Lozyuk, (1979) has shown that the antitumor effect of the condensation product of isatin with thiozolinone-4 is possibly due to its ability to act as an antimetabolite of phenyl alanine, the metabolism of which in the presence of melanoma is greatly disturbed.

A review published by Varma and Khan in 1978 provides a good deal of information on isatins as potential biologically active agents with antimicrobial, antineoplastic, antihypotensive, analgesic, anti-inflammatory and cysticidal properties in relation to its molecular structure.

Isatins have also evoked a great interest in protein chemistry for the elucidation of subtle structural differences in these macromolecules. Krawczyk (1962) has shown that peptides from tryptic digest of human and horse hemoglobins could be differentiated on the basis of their interactions with isatin. He observed that while the former were mostly stained pink the latter acquired a dark violet or violet pink color.
on treatment with isatin. Again, sulfonic acid derivative of isatin has been shown to be a specific reagent for the modification of tryptophan residues in peptides and proteins. The yellow color of the derivative has been usefully exploited for the determination of the extent and localization of the modification (Atassi and Zablocki, 1975).

Isatin has been shown to modify the activity of many enzymes both in vivo as well as in vitro. Muller (1962) found that isatin inhibits monoamine oxidase activity in mice liver homogenates and in a subsequent publication (1965) he showed that 5-bromoisatin was more potent inhibitor of this enzyme. Linitskaya et al. (1972) demonstrated that isatin thiosemicarbazone activates viral alkaline DNase but inhibits the acid DNase. Similarly, the reports from this laboratory have shown that isatin activates rat liver acid phosphatase (Singh et al., 1977) but inhibits alkaline phosphatase from kidney (Singh et al., 1978). Isatin has also been shown to be a valuable tool for the screening of species specificity of testicular hyaluronidase (Kumar et al., 1977).

The above information raises the question whether such wide range use of isatin and its derivatives will be safe in the light of scanty evidence available about their carcinogenic potentials. The situation becomes more complicated when one finds reports on anticarcino-
genic activities of isatins (Varma and Khan, 1978; Lozyuk, 1979). Diverse groups of carcinogens have been reported to cause in vivo degranulation of endoplasmic reticulum (Butler, 1966; Williams et al., 1971; Purchase et al., 1978), which is the first morphological lesion seen in the secretory organs of animals treated with carcinogens. We thus conducted experiments to determine the carcinogenicity of isatins by microsomal degranulation technique. Our studies have shown that isatin and its 5-bromo derivative cause the degranulation of not only liver microsomes but also of other tissues in rats when given orally. Thus these drugs, despite their potential chemotherapeutic uses, might not really be safe for human consumption.

The work presented in this thesis also deals with the in vivo effects of isatin and 5-bromo isatin on some microsomal phosphatases and cholesterol.

The interactions of functional groups in the side chains of amino acids constituting the proteins and enzymes with the 3-oxo-group of isatin have been of special interest to protein chemists and enzymologists (Susheela et al., 1969; Singh et al., 1978). There are controversial reports regarding the levels of alkaline phosphatase in various types of cancerous tissues; the levels are reported to be significantly low in leukocytes of patients with malignant disease (Lockich and Zacob, 1971), urinary 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), whereas its level is elevated in non-isophoblastic neoplasma (Huang et al., 1973), cystic fibrosis (Hosh et al., 1976) and osteocarcinoma (Ghanta and Raymond, 1976). Isatins have been found to modify the activities of a large number of enzymes (Bruns, 1954; Muller, 1962; Kumar et al., 1978). Singh et al. (1978) have shown that the increase in alkaline phosphatase activity in rat duodenum and jejunum after isatin administration is possibly due to the induction of the enzyme. In another report on the interaction of rat kidney alkaline phosphatase with isatin they showed that the mode of attachment of the drug with the enzyme protein under in vitro conditions was possibly through essential amino group(s) (Singh et al., 1978).

In view of the above information and the fact that most of the activated carcinogens (electrophiles) attack nucleophiles (proteins & nucleic acids) in living cells.
to induce the phenomenon of carcinogenesis, we conducted detailed trials on the interaction of isatins with alkaline phosphatase, which was thought to be a good model protein molecule for this study. Correlative studies have demonstrated that for some hepatocarcinogens, binding to proteins has a more direct carcinogenic significance than interaction with nucleic acids (Pullman, 1964). Additional experiments were performed to study the interactions between isatin and some selected amino acids having functional groups in their side chains which could possibly interact with isatin. Our *in vitro* studies have shown that isatin is non-competitive inhibitor of rat liver alkaline phosphatase but it inhibits rat prostate alkaline phosphatase uncompetitively.

One interesting observation revealed during these studies has been that isatin can interact with liver alkaline phosphatase through amino, sulphhydryl and possibly also through hydroxyl groups. However, its interaction with prostate alkaline phosphatase is mainly through hydroxyl groups. Our experiments on the *in vitro* interactions of isatin with amino acids, usually having reactive side chains and participating at the active sites of enzymes, have shown that maximum interaction takes place at pH 9.0 in the following order:
The same order of reactivity of these amino acids is also reflected theoretically on the basis of the \( pK \) values of their ionizable groups. Similar studies should be undertaken to work out the mechanism of interactions of other environmental carcinogens with amino acids and proteins to prove Miller's theory of carcinogenesis (1970).