SUMMARY AND CONCLUSIONS

It is now well established that most of the chemical carcinogens are activated by the microsomal hydroxylase system to ultimate carcinogens which behave as electrophiles and attack the nucleophilic macromolecules like nucleic acids and proteins in a living cell.

Isatins have been commercially employed for a number of purposes in various aspects of human life as detailed in "Review of Literature". A few reports in literature also indicate that isatins might be acting as carcinogens (Jagota and Dani, 1981) and affect the activities of a number of enzymes which might be implicated in the process of carcinogenesis. Studies conducted with cancerous tissues are controversial about the levels of alkaline phosphatase and isatins have been found to be potent inhibitors of this enzyme. Use of microsomal degranulation technique in this laboratory has also shown that isatin detaches ribosomes from reticular membrane under in vitro conditions. This property of environmental chemicals is now being exploited for the short-term detection of carcinogens. However, effects of isatins on ribosome-membrane interaction under in vivo conditions and their effects on some other microsomal parameters, connected with carcinogenesis have not been studied so far. The present work was therefore conducted to study the effects of isatin and 5-bromoisatin on the membrane bound ribosomes of rat liver, kidneys, lungs and brain.
Results presented in this thesis show that both the compounds studied detach ribosomes from endoplasmic reticulum in liver and brain but fail to demonstrate this activity in kidneys. Isatin seems to be a non-carcinogen for lungs but 5-bromo isatin may be a potential carcinogen for this organ as well. One interesting observation during these studies has been that dimethyl sulfoxide, used as a vehicle for carcinogens, has itself carcinogenic potentialities and may be used for such studies with great caution. It must be pointed out here that the phenomenon of \textit{in vivo} degranulation has been quite meaningfully correlated recently with the process of carcinogenesis through a 'deletion-depletion hypothesis' propounded by Rabin et al. (1981). The short-term microsomal degranulation technique for the detection of carcinogens has been found to be about 80 per cent predictive in our laboratory by conducting blind trials.

In addition, experiments were also performed to find out the effects of isatin and 5-bromoisatin on certain microsomal phosphatases like glucose-6-phosphatase, 5-nucleotidase and alkaline phosphatase. Histochemical studies on pre-neoplastic tissues have shown that they have low activities of glucose-6-phosphatase in comparison to their normal counterparts. Our biochemical analysis of microsomes prepared from rat liver, kidneys, lungs and brain showed that activity of glucose-6-phosphatase falls considerably by treating the animals for 20 days with...
isatin and 5-bromoisatin. This observation indicates that lowered glucose-6-phosphatase activity is a general feature of the process of carcinogenesis, even much before the appearance of tumors. 5'-Nucleotidase activity increases in case of liver and kidney microsomes after 20 days of administration of isatin and 5-bromoisatin but surprisingly the activity of this enzyme decreases considerably in case of lung and brain microsomes due to these treatments. Alkaline phosphatase activity was depressed in microsomes prepared from all the four organs studied after 10 days of treatment with isatin and 5-bromoisatin. Only in case of microsomes prepared from liver and kidneys, the enzyme activity increased considerably after 20 days of treatment. These observations suggest that the changes in the enzyme activities studied do not develop at random but in certain patterns which may be correlated with different morphological aspects of the preneoplastic lesions. It is interesting to note that dimethyl sulfoxide also decreases the activity of glucose-6-phosphatase in microsomes prepared from all the four organs. It is possible that this solvent might be converted to some active carcinogenic moieties in the gastrointestinal tract.

In view of the fact that cholesterol levels have been reported to increase in hepatomas (suspected to synthesize more cholesterol) we performed some experiments to examine cholesterol/PL ratios in microsomes from rat
liver, kidneys, lungs and brain. Contrary to the reports in literature, our results show that cholesterol/PL ratios fall down in microsomes of liver, kidneys, and lungs due to isatin and 5-bromoisatin treatments. It has been already reported from this laboratory that cholesterol levels fell down to about 50 per cent in liver and lung microsomes by urethane treatment (Renuka and Dani, 1982).

Detailed studies were further undertaken for elucidating the mechanism of interaction of isatin with rat prostate and liver alkaline phosphatases as well as with purified bovine intestinal enzyme. The purpose of these studies was to elucidate the mode of attachment of isatin to these enzymic molecules which were considered as model proteins. Isatin inhibits prostate alkaline phosphatase uncompetitively. The optimum pH of the enzyme is 9.8, which changes to 10 in the presence of isatin. Maximum per cent inhibition of the enzyme by isatin was found to be at pH 9.0. The optimum temperature for the activity of prostate alkaline phosphatase was found to be 45°C. The values of energy of activation ($E_a$) in the presence and absence of isatin were found to be 1.43 and 1.5 Kcals/mole respectively. The inhibition of the enzyme with isatin was found to be non-allosteric. Isatin seems to interact with this enzyme through $-OH$ groups of the constituent amino acids as well as with $Zn^{++}$ ions for its inhibitory action.
Studies with liver alkaline phosphatase showed that isatin inhibits this enzyme non-competitively, suggesting that its interaction with this enzyme is different in comparison to that with prostate alkaline phosphatase. This observation implies that the binding of isatin may not be only in the active centre of the enzyme but it may also bind at some other sites in the protein molecule. This enzyme was found to be also more labile to heat than its counterpart in prostate glands. Binding of isatin to liver alkaline phosphatase seems to involve in its interaction the -NH₂, -SH and probably -OH groups as well as divalent metal ions. Inhibition of purified bovine intestinal alkaline phosphatase by isatin was also found to be uncompetitive. Further studies with this purified enzyme showed that its interaction with isatin is through -NH₂ and -OH groups and not through -SH groups.

Further in vitro experiments were performed to elucidate the mechanism of interaction of isatin with amino acids having active side chains, which are often expected to be involved in active sites of enzyme molecules. These interactions were studied both by assaying individual amino acids as well as the amounts of isatin reacted. Amino acids-isatin interactions were completed in 6 h at 37°C. In the absence of an appropriate buffer, the order of reactivity for various amino acids was arginine > aspartic acid > serine > histidine > lysine > cysteine > tyrosine. With the exception of aspartic acid, maximum
interaction took place at pH 9.0. Aspartic acid showed maximum interaction at pH 8.0. Maximum interaction with histidine took place at its pK values. The orders of reactivity in phosphate buffer at pH 7.0 and 9.0 were as follows:

- **pH 7**: Aspartic acid $> \text{arginine} > \text{lysine} > \text{cysteine} > \text{serine} > \text{histidine} > \text{tyrosine}.$
- **pH 9**: Arginine $> \text{lysine} > \text{cysteine} > \text{aspartic acid} > \text{serine} > \text{histidine} > \text{tyrosine}.$

The order of reactivity of isatin with amino acids seems to be a function of the ionisation constants of the side chain reactive groups of various amino acids. The theoretical order of reactivity for various amino acids with isatin on the basis of their side groups is arginine $> \text{lysine} > \text{cysteine} > \text{histidine} > \text{aspartic acid} > \text{serine} > \text{tyrosine},$ which is quite similar to that obtained experimentally at pH 9.0. Maximum interaction of all the amino acids was at pH 9.5, which is also the pH used for assays of alkaline phosphatase.

Substitution in isatin ring with such groups which may increase the electrophilicity of 3-oxo groups of isatin might convert this ring system into a more potential carcinogen. On account of this fact 5-bromoisatin seems to be a more potent carcinogen as compared to isatin. Further work is in progress to isolate isatin-protein complexes from various tissues in order to elucidate the mechanism of isatin induced carcinogenesis at molecular
level. We are also trying to detect activated moieties of isatin which may act as ultimate carcinogens.