Chapter - 1

INTRODUCTION

Although chemical carcinogenesis is initiated and promoted by a variety of chemicals belonging to different groups which are totally unrelated in structural details (azocompounds, alkyl derivatives of different compounds, nitrosamines and polycyclic aromatic hydrocarbons etc.), yet all of them end up in triggering uncontrolled multiplication of cells. The molecular basis of cancer remains to be clearly established although there are strong indications at present that the oncogenes might be involved in controlling cell proliferation. However, this concept is shadowed when one considers the action of a number of mitogens which cannot enter a living cell but still are capable of transducing signals while staying outside the cell at the plasma membrane resulting in directing the nuclei to start multiplying. These observations lead us to think that control of cell proliferation might be influenced by a number of factors, which may initiate this process either by interactions of some of these factors (chemical carcinogens and radiations etc.) at the DNA level in the cell nucleus or at the plasma membrane level through the agency of possibly some secondary messengers. It is therefore desirable that all such factors will have to be thoroughly studied to arrive at meaningful conclusions for understanding the process of carcinogenesis.

In the piece of work presented in this thesis, we endeavoured to use 1,2-dimethylhydradazine (DMH) as a model carcinogen for studying its impacts in causing various cellular and molecular lesions in mice. Although DMH is a very well known carcinogen for causing colon cancer (Druckery, 1970 and Schmahl et al., 1976), yet there are a number of reports attributing this carcinogen to initiate cancer in other organs as well like liver and small intestine. In
In this thesis, results of experiments for studying lesions in all the above three organs in their important organelles have been presented in details. All the experiments presented in this thesis were carried out after the administration of carcinogenic doses of DMH (both orally and subcutaneously) for a period of 35 days, as preneoplastic lesions in the target organs are reported to be present after the administration of its carcinogenic doses for this duration. Neoplastic cells have gross aberrations in biochemical pathways or in the chemical composition of subcellular membranes or organelles, and many of the changes in malignant cells compared with their normal and untransformed counterparts are relatively subtle. This is reflected in the compositional profiles of normal and tumor cells, and although a number of alterations have been noted, few may be important to the continuation of cell proliferation in malignant tumors. Yet it is important to find out such modifications which may be unique to tumor cell or which may be considerably elevated in neoplasia, since they may prove to be reliable markers for identifying cancer cells. In the present studies, molecular aberrations have been studied during DMH carcinogenesis before reaching the tumor development stage. This was deliberately done to determine such molecular and cellular aberrations which might serve as causes leading to malignancy and not its results. To attain some degree of success to determine the triggering aberrations for carcinogenesis, both oral and subcutaneous administration of this carcinogen to mice was adopted. While subcutaneous administration is expected to lead to exposure of the target organs by the metabolites of this carcinogen, oral administration was expected to possibly make direct interactions in the gastrointestinal tract, particularly, in small intestine and colon. Of course, there is no denying the fact that during the daily oral administration of this carcinogen for 35 days, its metabolites might also be poured back into the
duodenum after its absorption from the intestine and metabolism in the liver. So oral administration seems to provide, possibly, the results of the interactions of the carcinogen directly as well as of its metabolites with the brush border membranes in small intestine and plasma membranes of colonocytes. The studies on small intestine were particularly considered important due to the fact that they are always having cell proliferations, even under normal conditions, as the enterocytes are sloughed off constantly and are replaced by crypt cells which mature to villus cells. Our aim was to study the interferences, if any, caused by DMH during this cell maturation process as actively dividing cells are known to be more affected by carcinogens. This type of situation is not available either in colon or liver. As far as chemical composition of brush border membranes is concerned, oral administration modified a number of parameters studied but subcutaneous DMH administration has not much effect. However both these dosage patterns provided contradictory results in respect of the absorptive activities of the enterocytes for some sugars and amino acids. This differential effect might be due to direct interactions of the carcinogen with the brush border membranes on oral administration. DMH carcinogenesis at the preneoplastic stage seems to cause enhanced phospholipid exchange activity permitting the transport of phospholipids from colon microsomes to nuclei and mitochondria. A common feature of plasma membranes of colon and intestine and microsomes of colon and liver is that their cholesterol levels fall upon DMH administration. An increase in sphingomyelin content in all the membranes and organelles (except in microsomes from small intestine) studied was observed. These alterations in lipid composition of the membranes can influence membrane fluidity, which might be important in controlling intracellular molecular traffic. Another important observation with respect to protein composition of all membranes
studied has been that in addition to a number of elevated peaks of proteins of different molecular weights discussed under “Results and discussion” part of this thesis, all the membranes and organelles except colon mitochondria have shown elevated peaks of specific protein(s) in the molecular weight range of 50,000-68,000. Further work is needed to study the effects of these proteins on the functioning of these membranes and in cell proliferation or metastasis, if any. Proteins destined for secretion from the cells are mostly synthesized on membrane bound polyribosomes while soluble proteins are synthesized on free polyribosomes. We have also documented in this thesis that both in vivo and in vitro DMH treatments to mice and microsomes respectively can detach a substantial population of ribosomes from reticular membranes. That alterations can occur in this pattern in transformed cells is exemplified by observed diversion of synthesis of albumin from membrane bound polyribosomes in normal liver to free polyribosomes in cancerous cells (Uenoyama and Ono, 1972). Some of the secretory proteins might be involved in acting as components of gap junctions or plasma membranes or as factors for controlling cell cycle.

A highly interesting observation made during this work is that the level of diacylglycerol (DAG) increases in the microsomal membranes in liver. Diacylglycerol is known to be involved in the activation of protein kinase C (PKC) which can bring about tyrosine phosphorylations in cancerous cells specifically. Assays on the activities of PKC also showed concomitant increases in the activities of this enzyme, which might be responsible in inducing preneoplastic lesions due to DMH administration. This correlation between increased DAG level and protein kinase C activity in mouse liver microsomes and particulate fractions (containing microsomes) is probably being reported for the first time in this thesis.