Chapter - 5

SUMMARY AND CONCLUSIONS

Cellular and molecular lesions afflicted by carcinogenic doses of 1,2-dimethylhydrazine (DMH) in microsomal and plasma membranes as well as nuclei and mitochondria have been investigated. Out of these membranes and organelles, microsomes and brush border membranes from small intestine, microsomes, nuclei, mitochondria and particulate fraction (containing all cellular components except those in the cytosol) from liver and microsomes, mitochondria, nuclei and plasma membranes from colon were studied. The results of these studies can be epitomised as follows.

1. DMH has been found to degranulate microsomal membranes in vitro in case of all the three organs studied both on the bases of RNA/Protein and RNA/Phospholipid ratios. However in vivo degranulation of endoplasmic reticulum was only recorded in case of colon and liver but in small intestine, the degranulation was not statistically significant. These experiments demonstrated that microsomal degranulation has validated the carcinogenicity of DMH.

2. Daily oral DMH treatment of the mice for 35 days leads to decrease in the body weight gain, weight and length of the small intestine as well as DNA content of intestinal epithelial tissue. Subcutaneous DMH administration although does not change these parameters in mouse small intestine, yet the weight/unit length of mouse colon as well as DNA content of colonic mucosa increased significantly.

3. Histological studies reveal that weekly s.c. carcinogenic doses of DMH lead to induction of cellular lesions in mouse colon and liver after
35 days of the treatment. Appearance of megalocytes (larger cells) having macronuclei (larger nuclei) in mouse colon and liver and aberrant mitotic figure in liver suggests that premalignant changes are triggered after 35 days. No such changes are however observed in small intestine of subcutaneously treated mouse. Daily oral doses of DMH for 35 days also induce such preneoplastic lesions in mouse colon and liver. However, in case of small intestine, a decrease in the number of duodenal villi is observed. In addition, the villi seem to be broken and shortened. However appearance of few megalocytes having macronuclei in enterocytes suggests that preneoplastic lesions also start developing in small intestine. Hyperplastic nodules appear in mouse colon and liver between 8-10 weeks of the carcinogen treatment of the mice. However, no such nodules could be observed in small intestine after this time interval. It thus seems probable that though oral DMH treatment leads to development of premalignant changes in all the three organs studied, yet due to rapid sloughing off in case of enterocytes, small intestinal tumors appear rarely in 35 days.

4. Oral DMH treatment leads to impairment of the absorptive activities of small intestine as revealed by decreased uptake of some nutrients (glucose and alanine etc.) and inhibition of the activities of some brush border enzymes like sucrase, alkaline phosphatase and leucine aminopeptidase. However in s.c. treated mice, opposite effects on the nutrient uptake and enzyme activities were observed. Contradictory results of the effects of routes of DMH administration on these parameters could result due to the fact that in case of orally treated mice, DMH directly interacts with enterocytes while in case of s.c. treated mice only its metabolites can interact.
The kinetics of glucose uptake reveal that its absorption in orally DMH treated mice decreases (low 'Vmax' value) probably due to a decrease in the number of active carrier molecules as well as increase in the membrane fluidity which might change the affinity constant (km.). In s.c. treated mice, however, glucose uptake is increased (higher 'Vmax' value) possibly due to an increase in the number of active carrier molecules, 'km' value in this case remaining constant.

5. DMH treatment leads to aberrations in the composition of the membranes/organelles in the target tissues. The lipid composition of these membranes/organelles undergoes marked changes due to DMH treatment. Oral DMH treatment leads to decrease in cholesterol content of the small intestinal brush border membranes and increase in their phospholipid content. The increase in total phospholipids is reflected by corresponding increase in sphingomyelin level of these membranes. Although s.c. treatment does not have much effect on small intestine, yet this mode of DMH administration leads to alterations in lipid components of mouse liver and colon subcellular organelles. Cholesterol level declines in colon plasma membranes as well as microsomes of colon and liver. The phospholipid content of colon subcellular organelles as well as liver microsomes increases significantly. Cholesterol/phospholipid ratio shows decrease in these membranes. Phosphatidylethanolamine contents increase in plasma membranes, mitochondria and nuclei whereas phosphatidylcholine level rises in colon mitochondria and liver microsomes. Sphingomyelin content rises in all the membranes/organelles studied due to DMH treatment of the mice. Low cholesterol/phospholipid ratio might
increase the fluidity of these membranes.

6. DMH administration leads to augmentation in the activities of lipid and phospholipid exchange proteins in mouse colon. These proteins are possibly responsible for the observed increases in the levels of different phospholipids in some of the subcellular organelles (nuclei and mitochondria) in DMH treated animals.

7. Studies on the analysis of protein composition reveal that DMH treatment leads to significant alterations in the total protein contents of the various membranes/organelles studied. Their protein profiles suggest that the levels of different protein components of these membranes are affected due to DMH treatment. A number of variations in different protein peaks are observed in different membranes in the three organs studied as discussed in details under 'Results and Discussion'. However one consistent observation noted during present studies is that certain proteins in the molecular weight range of about 50,000-68,000 are enhanced in most of the organelles/membranes of the target cells. Detailed studies are required to understand possible role of these proteins in controlling cell multiplication during malignancy, if any.

8. Glycoproteins and sialic acid levels have been found to significantly increase in colon plasma membranes and microsomes as well as in liver microsomes due to s.c. DMH administration. Sialic acid is known to increase electronegativity of surface membranes and glycoproteins which are known to carry sialic acid at the tips of their oligosaccharide moieties are also known to play very important roles in cell-cell interaction. Enhanced electronegativity of the above mentioned membranes is expected to decrease cell-cell interaction due to
increased sialic acid levels and might help in metastasis.

9. DMH administration to mice also led to enhanced cytochrome P-450, cytochrome b5, aniline hydroxylase and DMH demethylase activities in mouse liver as well as levels of lipid peroxides in liver and colon microsomes.

10. Elevation in diacylglycerol level of liver microsomes as well as enhanced protein kinase C activity of the particulate fraction of the liver are probably being reported for the first time in this thesis. Increased PKC activity has been attributed to perturb cellular regulation leading to malignancy. It is quite possible that DMH acts as a carcinogen through the agency of diacylglycerol as a secondary messenger.