CHAPTER II

REVIEW OF THE LITERATURE
A number of chemical carcinogens including the azo-dyes have so far been known to induce carcinogenic changes in cells and tissues. The azo-dye is one of the common chemical carcinogens which is reported to produce carcinomatous changes in the cyto-morphological and cytochemical level, including a marked qualitative change in nucleic acid content (Miller and Miller, 1953; Rouiller, 1962; Reid, 1962; Daoust, 1963; Bannasch, 1968; Kanasaki, 1969). The induction of carcinogenesis in liver and its different forms was described by Boyd (1951) and Elias (1964). Fitch (1973), Berenblum (1970) and Bannash (1968) described elaborately the development of cancerous growth in general. Wallach (1968) studied the cell membrane in relation to tumour growth and postulated that cancer might be a membrane disease. Ryser (1971) postulated that carcinogen interacted with cellular constituents such as proteins, RNA and DNA. These interactions, particularly the binding to DNA leads to the critical events of initiation, a somatic mutation that confers a slight growth advantage on a target cell. Miller and Miller (1947) investigated the effect of prolonged feeding with azo-dyes to the rats and observed that the azo-dyes were specially bound by the liver cell proteins and after a number of observations a relationship between protein binding and tumour formation was established.

Rouiller (1964) reviewed the preferential action of certain toxins on the liver. (1) Certain drugs, arsenic compounds (Franklin, et al, 1950) or carbontetrachloride (McCollister, et al, 1951) might have a particular affinity for the liver; (2) if the
poison is ingested, it arrives at the liver before reaching any other organ; (3) by virtue of the detoxification activities, the hepatocytes come into contact with the metabolic products of substances which can be more toxic than the latter. The history of the use of azo-dyes has been reviewed by Shear (1937); Kinosita (1940); Miller and Miller (1953); and Badger (1954).

Wagner and Manning (1976) described the susceptibility of guinea-pig to different types of diseases and the change in the blood picture of the same. Until about a decade ago guinea-pigs were considered resistant to chemical carcinogenesis but since 1962 numerous reports indicate that guinea-pigs were susceptible to many different chemical carcinogens (Argus, 1971). Wintröbe (1967) gave a detailed account of the blood picture in the normal and in the toxic state of the body. Agrelo (1978) recorded an increase in the size of the nucleus in carcinogenesis and proposed that changes in nuclear size might be an index of chemical carcinogenicity. Ponder and Ponder (1942) described the cytology of the polymorphonuclear leucocyte in toxic condition and stated that fragmentation occurred in the nuclear lobes in the form of fragmented chromatin material following toxic injury. The enlargement in the size of the neutrophilic cells with the increase in permeability is a result of toximia. Further, he stated that under the influence of carcinogenic leukemic factor the immature leucocytes failed to respond to the forces normally regulating proliferation and maturation.

The liver is an important organ pertaining to a huge number of functions of the body affecting the body physiology. Remarkable functions of the liver are —— the manufacture and
elimination of bile, regulation of protein and fat metabolism, formation of ketone bodies and plasma proteins, detoxication, erythropoiesis, blood coagulation, etc.

Stewart and Snell (1957) reviewed the histopathology of experimental tumours. In 1961, Le Breton and Monle collaborated to write the chapter on the biochemistry and physiology of the cancerous cell in Brachet and Mirsky's treatise "The Cell" whereas Oberling and Bernhard (1961) discussed the whole morphological aspect of the problem in the same treatise. Reid (1962) has made an exhaustive analysis of the biochemical effects of hepatocarcinogens in the rat, and Magee (1962) presented an important synthesis of the research work on the biochemical and pathological mechanisms in experimental liver cancer. Animals fed with carcinogenic amino-azo-dyes showed enlargement of the size and variations in the diameter of the nuclei of the hepatic cells (Miller and Miller, 1953; Christie and Le Page, 1961). Nucleolar hypertrophy was marked in hepatomas (Kleinfeld, et al, 1956; Kleinfeld and Koulish, 1957; Kleinfeld and Von Haam, 1959a, b; Ruttner, et al, 1959; Judah and Rees, 1959; Rouiller and Simon, 1962; Simon and Rouiller, 1962; Salomon, 1962; Salomon, et al, 1962). Rat mitochondria undergo no change after the administration of hepatocarcinogen (Buchner, 1961; Molbert, et al, 1962). But others believe that during months after the administration of carcinogen, even before the appearance of tumour cells, there is a rise in the number of mitochondria (Rouiller and Simon, 1962; Salomon, 1962).
Basslur and Paermentier (1977) studied Ehrlich ascites tumour cells both cytologically and cytochemically and found that the number of nucleoli, DNA, RNA and total protein contents were related to the number of chromosomes. Bhawan, et al. (1975) studied the ultrastructure of the liver cells of rats bearing transplanted mammary carcinomas from 4-12 week after transplantation and found some changes in cytoplasmic structure of the hepatocytes. The significant findings were pleomorphism of mitochondria, presence of large number of myelin figures, marked increase in microbodies, dilatation of rough endoplasmic reticulum, proliferation of smooth endoplasmic reticulum, intracytoplasmic cholesterol clefts and absence of increase in lysosomes. Boquist, et al. (1976) studied cytologically, histopathologically and the ultrastructure of the genuine giant cell tumour of bone. They reported that the tumours were composed of giant cells possessing a great number of mitochondria and stromal cells exhibiting prominent endoplasmic reticulum and golgi complex. Epstein (1977) studied the cytologic appearance of metastatic transitional cell carcinoma and reported three cellular patterns: papillary fragments, cells with a moderate amount of dense cytoplasm and undifferentiated malignant cells. The glycogen content of the liver and the muscle due to the application of dimethylaminoazobenzene and thioacetamide was conspicuously lowered, but this decrease was inhibited by fructose given simultaneously (Fekete and Matolay, 1975). Glucose could not prevent this decrease of the glycogen content. In the course of the treatment of dimethylaminoazobenzene for 8 days the glycogen content did not diminish. Glucose did not
significantly influence the effects of dimethylaminoazobenzene while fructose + dimethylaminoazobenzene enhanced the synthesis of liver glycogen. Flaks and Teh (1976) reported that the fine structure of the hepatic cells of rats, given the combined treatment with cyclohexamide and 3'-methyl-4-dimethylaminoazo-benzene closely resembled that obtained by chronic exposure to carcinogenic azo-dyes. Of the changes produced, the granular endoplasmic reticulum in particular became permanently altered, quantitatively and morphologically. Other persistent changes included mitochondrial abnormalities and glycogen depletion. Cyclohexamide appears to protect the liver cells against the nonspecific acute toxic action of 3'-methyl-4-dimethylaminoazo-benzene, while facilitating the expression of effects that may be associated with the carcinogenic action of this azo-dye. Although the experimental model did not result in the appearance of tumours, it demonstrated that a single exposure to a carcinogen might induce permanent changes that are similar to those observed during carcinogenesis. Gomi, et al. (1976) reported that the ultrastructure of the tumour cell cytoplasm showed many zymozen granules and smaller cored granules along with a large amount of amylase. The work Lombardi, et al. (1978) showed that in malignant histiocytosis some histiocytic cellular proliferation occurred. There was some degree of cohesiveness, in some cases there was capsular invasion or blood vessel invasion by malignant cells which could be seen within the lymph node and in the surrounding tissue. On electron micrograph the tumour cells of malignant histiocytosis appeared to be pleomorphic with three types of cells: undifferentiated cells, histiocytes
with variable degrees of differentiation and cells with intermediate features. The histological and ultrastructural data further support the idea that malignant histiocytosis is a disease that is related to the neoplastic proliferation of moderately differentiated histiocytes. Ohyumi and Takano (1977) found that glycogen appeared in the nucleus as well as in the cytoplasm in the form of a group of particles. Pietruszka, et al. (1978) observed that histologically the tumour was composed predominantly of spindle shaped cells arranged in interlacing bundles with numerous mitosis and much nuclear atypia. Invasion of blood vessels was marked. Ultrastructurally, the main findings included moderate plasma membrane interdigitation, many intracytoplasmic filaments and junctional attachments.

Effects of chemical carcinogen as reported by Reid (1962) suggested an enhanced capacity of DNA synthesis in the liver of rat in the post treatment period. Rouiller (1962) reported a subsequent decline in RNA content in rat liver with progressive carcinogenesis. Workers like Munro, et al. (1953), Munro (1966) reported the RNA content in rat liver in control animal and the effect of proteinfree diet on the RNA content of rat liver. Effects of physical carcinogen like x-radiation showed an enhanced capacity of RNA synthesis in rat liver (Chetty, et al., 1978). Rouiller (1964) reported a quantitative decline in RNA content with the advance in carcinogenesis. Boyland and Green (1960; 1962) suggested that cancer producing polycyclic hydrocarbons interacted with DNA by intercalation between two base pairs in the double helix. The intercalation hypothesis was derived from observations on the intercalation of caridines...
with DNA. The DNA isolated from the nodules of precancerous lesions of the liver differs from that of the neighbouring normal tissue (Farber, 1968). The excised nodule will grow in cultures, form differentiated bile ducts and synthesize serum albumin while comparable pieces of normal liver will not (Slifkin, 1970). Barrett (1978) treated Syrian Hamster embryo cells with 5-bromodeoxyuridine followed by near UV irradiation which resulted in neoplastic transformation of the cells. This demonstrated that a direct perturbation of DNA is sufficient to initiate neoplastic transformation. Iqbal (1978) observed DNA damage in the liver in guinea-pig exposed to chemical carcinogen. Treatments for 1–4 hours with the carcinogen - potassium dichromate a soluble hexavalent chromium salt with a strong oxidizing power markedly reduce DNA and RNA accumulation rates in hamster kidney fibroblasts grown in vitro (Levis, et al., 1978). Dichromate induces a sudden blockage of DNA replication, whereas RNA and protein syntheses are secondarily inhibited. Shakoori, et al. (1977) treated Uromastix hardwickii with thioacetamide at a dose of 100 mg/kg body weight. The liver and kidney were checked for biochemical and cytological changes, until the fifth day after administration of the carcinogen. At the end of five experimental days the body weight decreased by 14%; RNA and DNA content decreased immediately after carcinogen treatment, but in the subsequent experimental days RNA and DNA content recovered.

The cell size increased by 6% only while the nucleus and nucleolus were least effected. Aggarwal (1977) stained ascites
mouse sarcoma cells with Pt.-pyrimidine complexes as the sole electron dense strain and found the presence of distinct dense patches to granular appearance on the surface of the plasma membrane which was suggested to be attributable to DNA. Caputo (1975) studied different types of DNA synthesis and their importance in cancer growth and therapy. He reported three types of DNA replication; spontaneous, stimulated and induced synthesis. The spontaneous synthesis requires, in addition to the building blocks assembled by a series of enzymes, a pre-existing DNA chain which serves as template and primer. This is completed during the S-phase of the cycle and is dependant on the structural integrity of the DNA molecule and the energy level which provokes the signal of initiation. Stimulated synthesis is reported to occur only in G₀ cells after appropriate stimulation. The operative schedule for replication initiates at different times in the G₁ phase and is completed at the end of the S-phase. When chemicals, radiation or viruses modify the helical conformation of DNA, the mechanism which operates for restoration of the function is induced synthesis. The repair observed after induced DNA synthesis has an important general meaning and accounts for the ubiquity of double stranded DNA in living systems. Induced synthesis may spontaneously provoke the initiation process. In cancer transformation an appropriate molecular organization renders the DNA specifically susceptible to the influence of stimulators and inducers. D'souza (1976) studied the effects of the carcinogen-hydrazine sulphate on degradative enzymes of nucleic acids. He reported that hydrazine sulphate stimulated RNA synthesis in the liver and kidney but inhibited
it in the lung tissue of new born Swiss mice. Further, he reported of no significant effect on liver and lung tissue of adult Swiss mice. In tumour bearing mice, RNA biosynthesis is inhibited in lung and tumour tissues. Hydrazine sulphate inhibits DNA biosynthesis in all tissues of new born, lung and kidney tissue in adult male mice and lung and tumour tissues of tumour bearing mice. Hydrazine sulphate also stimulates RNase and DNase activities in all three tissues at acidic or near neutral pH. Evenwel, et al. (1976) reported a marked decrease in DNA synthesis in spleen, femoral bone marrow and occasionally the small intestine when they were exposed to halothane for 24 hours. The effect of adriamycin on DNA synthesis in mouse and rat heart was examined by Formelli, et al. (1978) and found that adriamycin induced an inhibition of DNA synthesis in mouse tissues within 1 hour after treatment. While the effects are short lived in liver and small intestine, DNA synthesis in heart remains below control values for up to 7 days. After this period DNA synthesis in hearts of treated mice is elevated and remains above control values for as long as 4 weeks. Jensen and Reed (1978) reported that there was a positive correlation between the carcinogenic potential of alkylating compounds and their ability to alkylate oxygen sites on DNA.

Cameron and Pauling (1978) studied the effect of supplemental ascorbate in the supportive treatment of cancer and found that ascorbate could sufficiently prolong the survival time of the patients. Roe as early as in 1967 described a process for the determination of vitamin C by 2-4-dinitrophenylhydrazine method. Purr (1933) predicted that by the reversible oxidation and
reduction, ascorbic acid was indirectly related to cell respiration and was also a determining factor in establishing the equilibrium between SH and SS compounds in the organism. He further predicted that the physiological significance of glutathione and ascorbic acid as catalysts of oxidation-reduction process has been considerably increased through the discovery that the activity of certain intercellular enzymes is dependent on the presence of definite oxidation-reduction potentials. And the decomposition of protein is dependent on the presence of vitamin C. Many enzymes have been shown to depend for their activity on the integrity of an -SH group in the molecule and the activating effect of ascorbic acid has been suggested as being due to the protection of such -SH groups from oxidation (Harrer and King, 1941). Woodhouse (1934) suggested a role played by ascorbic acid in the control of malignancy through lactic acid metabolism. Boyland (1936) performed quite a good number of experiments and found out the ascorbic acid content of guinea-pig tumour tissue. A huge number of literatures are available describing the minimum requirement of ascorbic acid by the guinea-pig. Bourne (1942, 1944) concluded that 2 mg. of ascorbic acid was essential for the guinea-pig for optimal wound healing. Workers like Kellie, et al. (1936); Musulin, et al. (1936); Vögtlin, et al. (1937) indicated that the reducing capacity of tumours was largely due to ascorbic acid. The ascorbic acid content of hepatomas induced by feeding γ-dimethylaminoazobenzene and O-aminoazotolune to rats may be somewhat higher and transplanted hepatomas somewhat lower than the normal liver (Fujiwara, et al. 1938; Iki, 1939; Masayama,
et al, 1938; Robertson, 1943). Antes and Molo (1939); Gaehgens (1938); Minor et al. (1942); Spellberg and Keeton (1939); Stepp and Schroeder (1936) reviewed the works indicating that cancer patients may have an abnormal metabolism of ascorbic acid. They further stated that patients with advanced cancer had a greater vitamin C deficit than that of the normal. In cancer patients, given an excess of ascorbic acid daily (500 mg.), there is a longer time interval than normal before the urinary excretion rises to a saturation plateau. Baker (1967) studied the role of ascorbic acid in blood formation and predicated that cytologically vitamin C deficiency caused a reduction in cytoplasm, indistinct cell walls, and loss of hyaluronic acid. Vitamin C is necessary for the protection of intercellular substances having collagen; in its role as an antioxidant it protected hydrogen electron carriers. It participates in deoxyribonucleic acid formation possibly through its involvement with folate metabolism. Chan and Black (1973) studied the effect of a dietary antioxidant mixture on 3-methylcholanthrene mediated carcinogenesis in hairless mice. The antioxidant mixture significantly reduced the frequency of premalignant lesions and their subsequent development into tumours. Avtandilov (1977) reported a decrease in the ascorbic acid content in carcinomas of the uterine cervix. Midden, et al. (1978) reported that suppression of growth of the line - 10 guinea-pig hepatocarcinoma was achieved after the simultaneous injection of line 10 cells and heat killed Staphylococcus aureus. Growth of tumour was also suppressed when line - 10 were injected alone, contralaterally, at the same time as the
vaccine mixture. Immunity developed to subsequent line-10 cell challenges but not to other syngeneic tumours. Mirvish, et al. (1976) studied the effect of sodium ascorbate on tumour induction in rats treated with morpholine, sodium nitrite and nitrosomorpholine. He reported that when ascorbate was given, the period of induction of tumour was longer and metastasis in the lungs was absent. Mottram, et al. (1975) studied the influence of ascorbic acid and pH on the formation of N-nitrosodimethylamine. He reported that ascorbic acid suppressed the formation of the carcinogen N-nitrosodimethylamine. Smith and Jick (1978) studied the consumption of vitamin A in relation to cancer and hoped that vitamin A might have some suppressive effect on cancer. Yagishita, et al. (1976) reported that the prolongation of the survival time was nearly doubled on Ehrlich ascites carcinoma cells when treated with ascorbic acid.

Cyclophosphamide has been reported to inhibit the growth of a variety of rodent neoplasms (Sugiura, 1961) and is widely used in the therapy of human tumours. Marusic, et al. (1978) administered cyclophosphamide to rats 2 or 5 days after an injection of Yoshida ascites sarcoma and the result was the resistance to a subsequent tumour challenge was found in rats treated with the drug 5 days after tumour injection. Murray (1978) studied the cytotoxicity of compounds such as acetic acid for inhibition of tumour growth. He reported an inhibition due to the death of a proportion of initiated cells. Slaga, et al. (1975) demonstrated that application of a higher dose of acetic acid to mouse skin (1000/~ moles) causes superficial ulceration. Although all
promoters induce epidermal hyperplastic, not all hyperplastic agents are promoters (Slaga, et al. 1975, 1976). He emphasized the dangers inherent in interpreting experiments showing the inability of substances like acetic acid to act as efficient promoters. Such experiments leave open the possibility that these hyperplastiogenic substances induce all of the biochemical changes in skin necessary for promotion, but does not assay as promoters because of cytotoxicity. Consequently the observation that not all hyperplastic agents act as tumour promoters (Slaga, et al. 1975, 1976) does not by itself eliminate the possibility that epidermal hyperplasia is a sufficient condition for promotion. Daunorubicin and adreamycin display outstanding efficacy against experimental tumours in mice (Arcamone, et al. 1978). Pessina, et al. (1978) suggested a possible interaction of the carbonyl iron or Fe$^{+++}$ ions with cell surface which decrease the growth rate of tumour inoculum in mice. Angsubhakorn, et al. (1978) induced liver carcinoma in male albino Fisher rats with aflatoxin B$_1$ and then they were subjected to alphabenzene hexachloride. Results showed that alphabenzene hexachloride protects against the development of liver carcinoma in rats.

In the present experiment the effect of ascorbic acid on chemically induced hepatoma has been studied to explore the possible antitumour and anticancerous activity of this vitamin which is well known for its healing and regenerative effect on cells and tissues from early days of biological experimentation.