CHAPTER I

INTRODUCTION
The sudden transformation of normal cells into malignant types involves a series of dynamic changes in its molecular and structural level. Cancer research or oncology deals with the origin, development and its metabolism of cells including the metastasis of the uncontrolled, unrestricted growth of the cell lines which suddenly loses the controlling machinery of the normal mitotic process. Cancer research is a combination of many independent scientific disciplines as clinical medicine, surgery, pathology, radiation physics, genetics, immunology, endocrinology, biochemistry, cytochemistry and cell biology. The goal of all these scientific disciplines is to find out a common cause of cancer and subsequent cure of the same. Records of the treatment of cancer and its subsequent cure have been lost in the unwritten history of the past. Until the nineteenth century it was entirely under the jurisdiction of the therapeutic measures of the physician and surgeon. But with the advancement of the microscopic techniques, the detailed cellular structure of the tumour was revealed, which became a tool for studying the cytogenesis of the tumour. Biologically, the cancerous growth is a sudden change of the normal rhythm of mitotic process into an uncontrolled one, whereby number of cells formed accumulate in the form of a tumour. The cellular components including the structure and size of the cell get altered and the specific functions assigned to it is also impaired resulting in an imbalance of the physiological state of the body. The chemical interpretation of the cancerous growth is a change in the cellular chemicals and the prevailing environment whereby the
the chemical constituent of the genetic material gets changed. The nitrogenous bases of the deoxyribonucleic acid may be altered qualitatively or quantitatively or both, whereby the protein synthesizing machinery gets changed leading to the synthesis of some different types of proteins. Thus the normal status of the body metabolism gets lost and the cells are transformed into altered forms due to the synthesis of altered proteins. The quantitative evaluation of the nucleic acid content may reveal an indication of the process of cancerous growth. The relation of the physical science with cancer research is found in the induction and possible cure of the same. Radioactive hazards to which the organisms are exposed are the causing agents of many types of cancer. Many forms of radiation, specially those of very short wavelength are capable of inducing the formation of tumours. However, most living organisms are exposed to a form of radiation to a greater or lesser extent and that is sunlight. It appears from statistical observations that there is a high incidence of skin-cancer in man who are continuously exposed to sunlight. The idea that the ultraviolet ray of the sunlight can produce skin-cancer remained largely hypothetical until 1928 when Findlay (1928) succeeded in inducing skin-cancer in mice through the use of radiation from the mercury arc. On the other hand some forms of cancer can be cured by the treatment of the cells with specific doses of radiation. Thus the heat killing of the cancerous cells is an usual procedure in cancer therapy.

The problem of carcinogenesis, even today, remains a challenge to the oncologist. The mechanism of induction of
cancerous growth in cell and their subsequent spreading is not yet clearly known. Cancerous growth can be induced in animals with ionizing radiations, oncogenic viruses or with chemical carcinogens (Kysel 1971). Among these physical, chemical and biological agents some are foreign to the body (extrinsic) and some are produced inside the body or are other living organisms of different species (intrinsic).

Among the chemical hepatocarcinogens used for the induction of carcinogenic transformations in mammalian liver cell, azo-dyes were proved to be a potent one in inducing immediate carcinogenic changes. An intrinsic carcinogenic agent is one of biogenic origin. Hormones and viruses are included under this group. That the oncogenic viruses can induce cancer became a well established fact. It is said to affect the deoxyribonucleic acid molecules (DNA) of the host and then to overpower the same transforming them into altered DNA molecules. The role played by the sex gland in the development of a tumour was illucidated by many classical endocrinological investigators (Atkin, 1967; Cori, 1926; 1927; Murray, 1923 etc.)

The extrinsic carcinogenic agent is a substance which is foreign to the body and which is not of biogenic origin, inducing the cancerous growth. Chemical carcinogens occupy a vast field of research in experimental carcinogenesis. A huge number of chemicals have been identified that can produce cancer. In 1941, Hartwell listed close to 700 chemical substances which had been tested for carcinogenic activity. Some 170 were reported to possess the power to evoke cancer in experimental animals. Ten years later he
studied 600 new compounds of which 150 were found to be carcinogenic. Therefore it is probable that more the chemicals we study more the number of carcinogens will be found. Coaltar, benz(a)pyrine, dibenz(a,i) anthracene, 7,12-dimethylbenz(a)anthracene, 3-methylchloranthrene, azo-dye, aminofluorene, aminodiphenyl, aminostilbene, naphthylamine, urethane, nitrogen mustards etc. are potent carcinogens. Azo-dye is a hepatocarcinogen and acts on the liver cells, transforming them into neoplastic ones. Urethane induces tumors only of the lungs and naphthylamine induces tumors only of the bladder.

The azo-dye is a form of amino compound which differs from the rest by the characteristics like (a) they are almost exclusively active on the liver, (b) they induce tumors of the liver almost exclusively on prolonged feeding, or injection or painting, and (c) their capacity to induce tumors of the liver is frequently dependent upon the nature of the diet simultaneously given to the animals.

Like the azo-dyes, 4-dimethylaminostilbene is more powerfully carcinogenic when administered together with an inadequate diet (Ison, 1952). A low protein diet accelerates the appearance of cholangiomas when the former carcinogen is administered. Azo-dyes as well as 2-acetylamino fluorene produces marked biochemical effects in the liver of the host animal. The concentration of the total nitrogen, riboflavin, and ribonucleic acid (RNA) decreased on feeding 2-acetylamino fluorene to levels of the final tumor, whereas the deoxyribonucleate remained nearly normal (Griffin, 1949). Similar decreases were reported by others (Putman, et al. 1952, 53, 54). They reported a considerable depletion in all of
the intracellular fractions at about 4 weeks, and the tumour cells contained about half as much of each constituent as was found in the liver cells after 4 weeks of treatment. In general the picture of acetaminofluorene carcinogenesis is similar to that shown by active azo-dyes (Laird and Miller 1953).

Ryser (1971) refers to five important points that are basic to the biology of carcinogenesis — the effects of carcinogenesis are dose dependant, additive and irreversible; carcinogenesis requires time; the cellular changes that trigger carcinogenesis are transmitted to daughter cells; carcinogenesis can be influenced by factors that are not truly carcinogenic; and carcinogenesis requires cell proliferation. On the other hand, Greenstein (1954) reported a number of conditions for the carcinogenecity of these chemicals. A chemical which is a carcinogen for a specific individual may not be carcinogenic for another individual or it may be effective in a different tissue. An agent, carcinogenically active in one species of animal may be inactive in another species. An agent may be active under some modes of administration and inactive in others. Thus, it indicates that the carcinogenic potency of an agent does not reside in the nature of the agent alone but is a function of the following factors: the dosage, the nature of the animal, the mode and length of time of administration of the agent; the strain, the species, the sex, and the age of the test animal; the site of application, the presence of concomitant factors such as the level of essential dietary constituents and the number of animals kept in a cage and perhaps still others as yet unknown conditions.
The experimental aspect of tumourgenesis was started at the close of the nineteenth century with the study of animal tumour which later on showed much resemblance with that of the man. The limitations in the study of cancer in man has been crossed to a great extent by the introduction of the animal experimentation. Now-a-days, it is a practical procedure to induce experimental cancer in animal and to study the possible cure of the same with different physico-chemical agents. It may be of great help in the control of carcinogenesis in man. The present experimental project is designed to study a part of the biological and chemical aspect of cancer research in guinea-pig. The carcinogenic transformation in cells and tissues covering the cytomorphological, histological, haematological, cytochemical and biochemical structures have attracted the attention of the workers in the field of cancer research.

Living organisms are boon with a characteristic feature that they can reproduce. The cells of the living organisms can divide in a geometrical progression. Although the animals start their life as a single cell, the zygote, in course of time this single cell gives rise to a huge number of cells leading to the differentiation of the organ systems. This capacity of growth is vigorous in the embryonic tissue while it decreases and ceases in the adult individuals. But not all organs cease this power. The liver and the cells of the skin retain this power and so they can regenerate. Why some cells have retained this power of differentiation and others have lost defied analysis and the cause of the division of the cells is not yet clearly understood.
There are a number of theories accounting for the division of a cell of which the theory of nucleocytoplasmic ratio is of high importance.

The cells of the animal body which are growing in a normal rhythm following a definite regulation of cellular synthetic machinery suddenly switch over to an altered path giving rise to neoplastic growth with a shift in cytochemical, pathological, histological, genetical, biochemical and haematological level. This neoplastic growth is produced by uncontrolled multiplication of body cells which results in the formation of a tumour. The component cells of the tumour are not alien to the body but are actually descendants of normal cells. A tumour cell is a modified normal cell. Structurally, a tumour manifests some degree of exaggerated variability and of abnormalities in the size, shape and staining properties of the cells and their nuclei, and more specially of derangements in the spatial relationship of the cells to one another. The functional peculiarities are more fundamental and refer to a diminuation or even total loss of more specialized functions, rapid proliferation and in case of malignant tumours invasion, i.e., to grow in distant parts of the body after transportation of fragments of the tumour tissue through the blood or lymph stream (Bannasch 1968).

Tumours may be classified on the basis of their etiology or on the basis of the tissue involved in it or on the basis of their effects produced to the host. Accordingly they are divided into two types — (a) Benign tumour and (b) Malignant
tumour. A benign tumour is one that grows in a single place and does not disperse or ramify diffusely and is covered over by a fibrous tissue capsule. They are harmless in comparison to the malignant tumour and so the name "benign". Malignant tumours are usually called cancer, carcinoma or sarcoma. They invade the surrounding tissues and spread from one part of the body to another and it is referred to as metastasis.

Malignant tumours have got the characteristics of invasion, i.e., the ability to infiltrate and actively destroy surrounding tissues; metastasis formation, i.e., the development of secondary centres of tumour growth at a distance from the primary focus; and a structural derangements of the component cells. Cancer comes from a Latin word for crab, because of a fancied resemblance between such spreading neoplasms and a crab with its sprawling legs. Malignant tumour may be of two types — sarcoma and carcinoma. Sarcoma is typically derived from internal connective tissues whereas carcinoma is derived from surface tissues.

Elias (1964) distinguished four kinds of carcinomata in the liver cells. These are —— (a) Hepatocellular carcinoma or hepatoma or hepatocarcinoma which arise in the hepatic cells, (b) Primary carcinoma of the bile ducts, known as cholangioma or cholangiocarcinoma, (c) Carcinoma which originates from liver cells, which have assumed the structure of ductal or ductular epithelium, a kind of tumour which structurally resembles cholangioma. This type is called hepatocholangioma, (d) Metastatic carcinoma of extrahepatic origin lodged in the liver.
The formation of spontaneous tumour is rare in guinea-pig. No neoplasm was observed in 15,000 guinea-pigs of inbred strains born and observed for upto 5 years between 1916 and 1937 (Schmkin and Mider 1940). Papanicolaon and Olcott (1942) reported an incidence of only 1.4% tumours in the guinea-pig population. Rogers and Bluementhal (1960) reported the occurrence of only 0.4% of tumour in the tumour susceptible strain of guinea-pig. Mosinger (1961) reported neoplasms in 5 of 5540 guinea-pigs. Thus there may be some factors for the low susceptibility of guinea-pig to cancer. Kidd (1953) concluded that there was a tumour inhibitory property (TIP) in the guinea-pig serum for the inhibition of cancerous growth.

Dietary factors play an important role in the genesis and growth of cancerous cells. White (1945), and White and Belkin (1945) established tumours in mice fed a diet negligible in protein content, the animals remaining in a state of continuous negative nitrogen balance. A group of mice was fed with an adequate 18% casein diet and the other group received a diet completely lacking casein. Each group received a supplement of 5% of liver extract, which was very low to supply adequate amounts of lysine, histidine, isoleucine, threonine and other amino acids. After seven days, mammary adenocarcinoma was transplanted which although became established uniformly in all mice still the tumours in the mice on the low nitrogen diet growing slightly slower than those on the adequate high nitrogen diet. White concluded that the state of negative nitrogen balance implied that the proteins of the tissues of the host animal were being broken down not only to supply the nitrogenous
factors necessary for its existence but for the growing tumour as well. He further reported that once a tumour had been established it would grow utilising the host's materials. Therefore a tumour can grow even if a minimum quantity of the substances are supplied but the rate of growth may vary.

The rate of growth of a tumour may be decreased in well-fed animals when severe restriction of calories (Bischoff, et al., 1935) of riboflavin (Morris, et al., 1943), or of pantothenic acid (Morris & Lippincott, 1941; Larsen & Heston, 1945) (Montanez, et al., 1951) is imposed but, with the initiation of the restriction, the body weight of the animals also falls off and pathological changes intervene. Morris & Robertson (1943) reported that the riboflavin contents of the tumour under these conditions were not so rapidly depleted as that of liver and muscle. Supplementation of the diet by riboflavin and biotin accelerates the growth of transplanted hepatomas in rats (Voegtlin & Thompson 1949). The autonomy of a tumour as regards to the intake of food is shown by the ability of a tumour to divert a disproportionate allocation of the available foodstuff in the body. A hyperplastic growth and a normal growth reach an equilibrium while the tumour does not. The most destructive feature of a tumour-host relationship is that a tumour may continue to increase in size while the host is suffering from starvation, and that this increase in size involves synthesis of new proteins out of building blocks which if not provided by the intake of food will be derived from the breakdown of proteins of the normal tissues of the host.
Chan & Black (1998) reported the effect of a dietary antioxidant mixture on 3-methylcholanthrene mediated carcino genesis in hairless mice. The antioxidant mixture was reported to significantly reduce the frequency of premalignant lesions and their subsequent development into tumours. The antioxidant mixture was prepared by mixing ascorbic acid, butylated hydroxytoluene, \( \alpha \)-tocopherol, and glutathione. Harman & Gerout (1961) also demonstrated that certain antioxidants produced a marked decrease in incidence of spontaneous development in strains of mice.

With the experimental recognition of various accessory growth factors or vitamins, more and more investigators have attempted to influence and control experimental and clinical cancer by means of vitamin deficiencies or excess. The rationale of many of these efforts has been based on the consideration that malignant tissues which are frequently characterized by a relatively rapid rate of growth, might have some excessive requirements for vitamins; and thus be differently influenced by their omission or supplementation in the diet. But a concrete evidence of relationship between the two is generally lacking. Watson (1936) reported that growing tumours utilized the resources of the host which was based on studies of vitamin C depletion in guinea-pig bearing tumours. Guinea-pigs cannot synthesize vitamin C. If these animals are subjected to a scorbutic diet will result in a loss of vitamin C in both normal and neoplastic tissues. The vitamin reserves were more rapidly exhausted in the animals bearing tumours than in normal animals, and the survival of scorbutic tumour bearing guinea-pigs was considerably
briefer than that of the scorbutic non-tumourous animal. Minor, et al., (1942) made analogous studies on the daily utilization of vitamin C by human beings with extensive neoplastic metastases and revealed that the amount of the vitamin utilized was nearly double than observed in normal individuals.

Baker and Frank (1968) reported the role of ascorbic acid which was clearly established in the formation of blood through the absorption of iron from the intestinal tract and its subsequent incorporation into hemoglobin. On the other hand, cytologically the deficiency of vitamin C causes a reduction in cytoplasm, indistinct cell wall, and loss of hyaluronic acid, the main intercellular cement. He further established that the vitamin C was necessary for the protection of intercellular substances having collagen; in its role as an antioxidant it protects hydrogen electron carriers. It participates in the formation of DNA possibly through its involvement with folate metabolism.

Vitamin C occurs in nature only in the form of L-ascorbic acid. Primates and guinea-pigs require a ready source of vitamin C in their diet because they cannot synthesize the vitamin. Hence the guinea-pig is the standard laboratory animal to conduct experiments on the effect of vitamin C on cancer. On the other hand, guinea-pigs, unlike human beings, do not eliminate ascorbic acid in the urine when the vitamin is consumed orally unless the doses ingested are large (300-500 mg). But the parenterally introduced vitamin is rapidly eliminated by guinea-pig after very much lower doses (25-50 mg) (Penney & Zilva, 1946). Pauling (1976) suggested the probable role of
vitamin C in inhibiting the progress of cancerous growth which according to him, in all probability inhibit the rapid proliferation of the primarily formed malignant tissue and thereby prevent further change in malignant transformation and thereby might be able to prevent malignant changes in cell. The healing property of vitamin C is known to biologists for a long time but there is no direct evidence on the effects of vitamin C in inhibiting malignancy in mammal.

Cameron & Pauling in 1979, proposed that administration of vitamin C has a controlling effect on the malignant cells of the tumour and exert an anticancer effect.

The present experimental project was planned to investigate the effects of the azo-dye-azocarmine, a potent hepatocarcinogen in the liver cells of guinea-pig covering the morphological, haematological, cytochemical, histological, and biochemical levels and their subsequent revival after the administration of vitamin C. It is expected that the present investigation will throw much light on the cytogenesis of cancer in the hepatic cells of guinea-pig. It is also aimed at determining the role of vitamin C in inhibiting the trend of carcinogenic transformation in the hepatic cells after experimental induction of cancer in the morphological, hematological, cytochemical, histological and biochemical levels. It is expected that the findings of the present investigation will definitely indicate the role of vitamin C in inhibiting and curing the disease in structural as well as in molecular levels.