CHAPTER IX

GENERAL DISCUSSION
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The findings of the present experimental project through different experimental procedures have definitely revealed the role of vitamin C as inhibitor of carcinogenic changes in the morphological, histological, haematological, cytochemical and biochemical levels.

The induction of carcinogenic changes in the liver of guinea-pig followed by vitamin C administration revealed a trend of revival which was not only observed in the morphological and histological picture, but in the biochemical level also. In case of DNA and RNA which are believed to be the main target of interaction with carcinogen definitely indicated a revival effect which was found to be highly significant at 1% probability level.

Cameron and Pauling (1979) stated that ascorbic acid was found to increase the life span in case of terminal cancer patients in comparison to control which was probably due to the arrest or inhibition of further progress of carcinogenic changes in the patient.

In the present experimental findings the revival changes after the administration of vitamin C were observed in the histological picture as well as in cytochemical and haematological levels. Revival changes in the biochemical ingredients provided further support to the healing and recovery effect of vitamin C which was more pronounced with the continuation of the treatment.
Biochemical investigation on the nucleic acid and vitamin C content revealed that the different stages of carcinogenic induction was frequently associated with vitamin C deficiency. The administration of vitamin C not only inhibited further changes towards carcinogenic transformation but also recorded an increment in the quantity of vitamin C indicating subsequent revival from vitamin C deficiency which was always associated with carcinogenic transformation which might be regarded as one of the causes for inhibition of further carcinogenic changes. Vitamin C according to Cameron and Pauling (1979) rendered the ground substance — matrix more resistant to the invasive tumour enzymes or increase the production of naturally occurring tissue and serum oligosaccharide known as physiological hyaluronidase inhibitor (PHI) which in all probability increased the natural resistance to cancer and retarded further growth of tumour.

From the results of the experimental findings discussed in the earlier chapters it is evident that vitamin C can be utilized as an antitumor agent.

A close similarity prevails in the changes of scurvy and of invading neoplastic cells. Vitamin C is utilized by the body in the prevention of scurvy. Scurvy results from a severe dietary lack of ascorbate. Scurvy, an illness now rare in its flagrant form, is a syndrome of generalized tissue disintegration at all levels, involving the dissolution of intercellular ground substances, the disruption of collagen bundles, and the
lysis of the interepithelial and interendothelial cements, leading to ulceration, vascular disorganization, generalized undifferentiated cellular proliferation with specialized cells throughout the tissue reverting to a primitive form (Woodruff, 1964). McCormick (1959) recognized the generalized stromal changes of scurvy to be identical with the stromal changes of invading neoplastic cells.

The administration of ascorbic acid had some aspects in host resistance. The encapsulation of the neoplastic cells which is an impenetrable layer of fibrous tissue is a barrier to invasiveness. The reticuloendothelial system is concerned with the precise design and production of immunoglobulins, proteins which contain a large number of disulphide bonds (relative to other proteins). The function of which is to bridge the light and heavy chains. The role of the ascorbic acid dehydroascorbic acid system in the biosynthesis of 3-3 bonds has been extensively discussed by Lewin (1976) who strongly concluded that ascorbate was essential for immunoglobulin synthesis. Ascorbate is essential for active phagocytosis both in vivo and in vitro (Chretien and Gargusi, 1973; Cottingham and Mills, 1943; Ganguli, et al. 1976; Joetzzi, et al. 1974; Robertson and Williams 1969. Pauling (1970, 1976) reviewed the clinical evidence that supplemental ascorbate offered protection against a broad spectrum of viral disease.

Cameron and Pauling (1973, 1974) and Cameron and Rotman (1972) pointed out that the administration of ascorbate had got supportive treatment in cancer. That the cancer patients
had a significantly increased requirement of vitamin C is well known. The direct action of ascorbate on carcinogens is not rare. Warren (1943) reported that ascorbate could oxidise aromatic hydrocarbons in vitro experiments. Cytochrome is decreased by ascorbate deprivation in guinea-pig and because cytochromes are intimately associated with electron transport, and therefore oxidative phosphorylation, it is possible that cell respiratory impairment could result from relative ascorbate deficiency; and of course, an increase in anaerobic glycolysis coupled with a decrease in oxidative respiration is recognized to be a fundamental biochemical change in the carcinogenic transformation (Warburg, 1956).

Ilaga and Bracken (1977) reported that ascorbic acid as other antioxidants prevented the initiation of skin tumors following the application of 7,12-dimethylbenz(a)anthracene. Dehydroascorbic acid also inhibited the growth of sarcoma (Yamafuji, et al. 1971). It is suggested that dehydroascorbic acid could inhibit sarcoma because it worked as an electron acceptor in the regulation of mitosis (Edgar, 1969, 1970).

The behaviour of the cell is influenced by the changes in the physicochemical composition extra cellular environment (polymerization-depolymerization), which in turn becomes a powerful means of modifying their immediate micro-environment. A proliferating cell and its contact environment constitute a balanced system in which each component influences the other. This interdependence is involved in all forms of cell division and is of particular importance in cancer (Cameron and Pauling, 1973).
Cameron (1966) reported that all tissue cells had an inherent tendency to divide but the tendency was normally restrained by the viscous nature of their intimate extra cellular environment of high-molecular-weight ground substance glycosaminoglycans. Proliferation is initiated by the cellular release of hyaluronidase, which permits the cell local freedom to divide and to migrate within the limits of the altered field. Proliferation will continue as long as hyaluronidase is being released; proliferation will cease and normal tissue restraint and organization will be restored when the production of hyaluronidase returns to normal.

The matrix substance is of much importance in the study of cancerous growth and proliferation. The impact of the continuous outflow of lysosomal enzymes on the immediate microenvironment is to bring about profound changes in the physicochemical structure of the matrix. These changes, predominantly dissolution and depolymerization of matrix glycosaminoglycans and proteoglycans destroying the structural stability of the molecular micelle with an abrupt fall in local ground substance viscosity leading to diminished mutual adhesiveness of tumor cell (Coman, 1944; McCutcheon, et al. 1948).

Ascorbic acid, known to be essential for the structural integrity of the intercellular matrix, is closely related to glucuronic acid, an essential building block of the principal matrix structures (Cameron and Pauling, 1979). The ground substance of the intercellular matrix is a complex aqueous gel containing electrolytes, metabolites, dissolved gases, trace elements, vitamins, hormones, enzymes, carbohydrates, fats and
proteins. Its important structural property of extreme viscosity depends upon the abundance of certain long chain mucopolysaccharide polymers - the glycosaminoglycans and related proteoglycans. These interesting high molecular weight polymers form a structurally stable hydrophilic mess, which, in turn, is reinforced at the microscopic level by a 3-dimensional network of collagen fibers. These glycosaminoglycans are single stranded long-chain polymers. The common varieties are hyaluronic acid, chondroitin and sulphate esters, of which several isomers exist (Cameron and Pauling, 1979). To the glycosaminoglycan proteins are attached to which are attached tertiary glycosaminoglycan polymers forming proteoglycans which is present in the matrix and comprise a polydisperse family of similar macromolecules. Locally, in cancer, matrix depolymerization in the immediate vicinity of proliferating invasive cells is a striking feature, this is exactly the change observed to occur on a generalized scale of scurvy (Cameron, 1966, 1976).

The matrix glycosaminoglycans is depolymerized by the action of hyaluronidase (Cameron and Pauling, 1979). Depolymerization of matrix proteoglycans is brought about by the combined action of the glycosidases and neutral proteases. Therefore, it is not impossible that the release of these enzymes from neoplastic cells is responsive for their invasive capacity. The encapsulation of the tumor is helped by the collagen fibrinogenesis, which in turn is aided by extra-cellular glycosaminoglycans and proteoglycans (Ghosh, et al. 1977). Therefore by protecting the integrity of the intercellular
matrix the tumor may be controlled in regards to its malignant infiltration, selective nutrition, protect pre-existing collagen barriers from neoplastic erosion and protective collagen encapsulation. Thus, ascorbic acid helps to maintain these functions by protecting the intercellular matrix.

Cameron (1976) reported that ascorbic acid was required for the synthesis of physiological hyaluronidase inhibitor (PHI). It explains how ascorbic acid may be related with the retention of the matrix substance. In vitro, the reaction of ascorbate with hyaluronic acid and with chondroitin sulphate has been reported to yield breakdown products of somewhat lower viscosity than the original preparation.

Cameron and Pauling (1979) proposed that the primary function of vitamin C was to restrain excessive growth through its incorporation into the PHI system, directing the inherent proliferative capacity of all cells into a constrained organized differentiated behaviour pattern. Thus, in the simple example of wound healing, the initial cell proliferative phase produces depolymerization of the immediate matrix and the release of measurable amounts of glycosaminoglycan residues into the blood stream, there to mop up ascorbate reserves into an upsurge of PHI levels. In the absence of ascorbate, wounds fail to heal, with a destabilized matrix, undifferentiated cell proliferative granulomata, and the continuous over spill of matrix by products into the blood stream (Pirani and Catchpole, 1951; Schack, et al. 1950).
Collagen resists the infiltration of malignant cells and tissues. On a generalized scale the gradual shift to a more fibrotic pattern in the matrix induced by some hormones and ageing process may account for the increased resistance to a tumor growth associated with the hormonal and constitutional changes (Cameron, 1966). Thus, if matrix integrity is the first line of defence against invasive growth, this defence is very powerfully reinforced by the next barrier, the collagen network. Thus, the collagen network may be dissolved by the action of collagenase, a proteolytic enzyme found in both mammalian and bacterial cells. This was proved by the works of Dreseen, et al. (1972); Gersh and Catchpole (1949); Robertson and Williams (1969); Steven and Itzhaki (1977); and Taylor, et al. (1970). The most interesting thing is that collagen fibres consist of innumerable fibrils (themselves optically invisible) glued together into visible fibers by glycosaminoglycan and proteoglycan macromolecules. Exposure to glycosidases would dissolve the cement substance and by converting visible fibers into free floating molecular fibrils in a depolymerized matrix would appear to exert collagenase activity. Either way, the lysosomal overactivity of neoplastic cells is clearly responsible for the disruption of pre-existing collagen barriers ahead of malignant invasive growth.

of collagen (Kennedy, 1976). Such instability results in increased collagen catabolism, as has been demonstrated in scurvy (Gore, et al. 1965; Ross and Benditt, 1964) and in cancer (Basu, et al. 1974; Pinto, et al. 1970, Poole, 1970) Dell'orco and Nash (1973) and Schafer, et al. (1967) reported that ascorbic acid was essential to increase collagen synthesis by fibroblasts in vitro and to maintain collagen synthesis in nonmitotic fibroblasts for extended periods. Propyl hydroxylase, the enzyme hydroxylating propyl and lysyl residues of procollagen, requires ascorbate to function in vitro (Know and Goswami, 1961) and the addition of ascorbic acid to tissue cultures stimulates the prolyl hydroxylase activity of fibroblasts (King and Burns, 1975). Therefore, it is logical to conclude that the sufficiency of ascorbate is essential for collagen fibrillogenesis, both by stabilizing the matrix and protecting it against the erosive effects of lysosomal glycosidases and by facilitating the hydroxylation of prolyl residues in pro-collagen.

The present experimental findings have revealed that administration of ascorbic acid in case of induced carcinogenesis not only inhibited the carcinogenic transformation in the liver of Guinea-pig but also brought about revival in the carcinogenic changes covering the morphological histological and biochemical levels, which definitely suggested the overall recovery effect of vitamin C in inhibiting the malignant transformation in mammal.
Therefore, it may be inferred that ascorbic acid is essential for the integrity of the intercellular matrix and its resistance to malignant infiltrative growth, and there is strong evidence that it is involved in the inhibition of invasive tumor enzymes. It is required for the formation of new collagen, allowing the resistant patient to enmesh the tumor cells in a barrier of new fibrous tissue. There is good evidence that high intakes of ascorbate potentiate the immune system in various ways. Ascorbate is found to offer definite protection against variety of carcinogenic effects physical and chemical carcinogens including the azo-dyes. It also involved in a number of other biological processes believed to be involved in resistance to cancer. It has got the unique advantage in relation to other remedies for cancer because it is completely safe and harmless.

From the facts and findings discussed above it may be concluded that the supplement of vitamin C in case of malignant transformation in mammal has got definite inhibitory effects on its further progress. Moreover it was found to produce revival effects by decreasing the mortality rate of the animals marked by revival effects on cellular and biochemical levels. Therefore it may be inferred that vitamin C played the key role in the process and could produce substantial benefits in the inhibition and prevention of malignancy in mammal.