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Ascorbic Acid and Cancer: A Review[1]

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Abstract

Host resistance to neoplastic growth and invasiveness is recognized to be an important factor in determining the occurrence, the progress, and the eventual outcome of every cancer illness. The factors involved in host resistance are briefly reviewed, and the relationship between these factors and ascorbic acid metabolism is presented in detail. It is shown that many factors involved in host resistance to neoplasia are significantly dependent upon the availability of ascorbate.

Introduction

Few would now dispute that the behavior of every human cancer is determined to a significant extent by the natural resistance of the patient to his or her disease. As a result, there is now widespread recognition that very substantial benefits in cancer management would be achieved if practical methods could be devised to enhance resistance.

There is a growing body of theoretical and practical evidence suggesting that the availability of ascorbate is the determinant factor in controlling and potentiating many aspects of host resistance to cancer. We have prepared this review as an aid to investigators in this field and as a source of information to others.

History

The history of vitamin C is common knowledge. In the mid-18th century James Lind demonstrated that the juice of fresh citrus fruits cures scurvy (197). The active agent, the enolic form of 3-keto-L-gulofuranlactone, christened ascorbic acid or vitamin C, was isolated in the late 1920's by Albert Szent-Györgyi (317). By the mid-1930's, methods had been devised to synthesize the compound, and it soon became widely available at low cost. It was soon established that the substance was virtually nontoxic at any dosage. The structure of vitamin C is shown in Chart 1.

The basic function of vitamin C is the prevention of scurvy. The current recommended dietary allowance of the Food and Nutrition Board of the United States National Academy of Sciences-National Research Council, 45 mg/day for an adult, is adequate to prevent scurvy in essentially all normal persons. The question of whether or not a larger intake could lead to better health and a greater control of disease was raised almost as soon as the pure compound became freely available, and the debate continues.

Our earlier suggestions that ascorbate might be of some value in the supportive treatment of cancer (54, 55, 60) provoked further controversy, although less than might have been expected. Untreated cancer almost invariably pursues a relentlessly progressive course, at the very least providing some opportunity to measure therapeutic effect. Furthermore, it is well established that cancer patients have a significantly increased requirement for this nutrient, there is persuasive evidence that it can be implicated in many mechanisms concerned with host resistance to malignant invasive growth, and there exist some experimental and pilot clinical reports indicating that administration of ascorbate in amounts substantially in excess of base-line recommended dietary allowance levels does indeed exert some therapeutic benefit.

Our own interest arose from the realization that ascorbic acid, known to be required for collagen synthesis, might be required in increased amounts for the protective encapsulation of tumors (253) and from the independent simultaneous conclusion that the ascorbate molecule (or some residue thereof) must be involved in the feedback inhibition of lysosomal glycosidases (60) responsible for malignant invasiveness (48). From these joint beginnings came the realization that ascorbic acid could be implicated in many other aspects of host resistance and even the tentative proposal that host resistance, no matter how measured, is ultimately dependent upon the availability of ascorbic acid.

We have published a number of clinical reports indicating favorable responses in advanced human cancer to no specific treatment other than regular large doses of ascorbate (51-53, 56-59), but as yet no properly designed controlled trial has been made to confirm or refute these findings and, if confirmed, to determine the most effective dosage. It is our hope that such studies will soon be undertaken, and it is our expectation that, on the basis of sound statistical evidence furnished by such studies, supplemental ascorbate will soon become an essential part of all practical cancer treatment and cancer prevention regimens.

We present below some of the existing evidence in support of our contention. We first draw attention to some of the similarities between scurvy and cancer.

Scurvy and Cancer

Any discussion of the biological function of ascorbic acid has to begin with a consideration of scurvy, the universally accepted "base line," and, if untreated, the invariably fatal illness resulting from 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 substance, the disruption of collagen bundles, and the lysis of the interepithelial and interendothelial cements, leading to ul-

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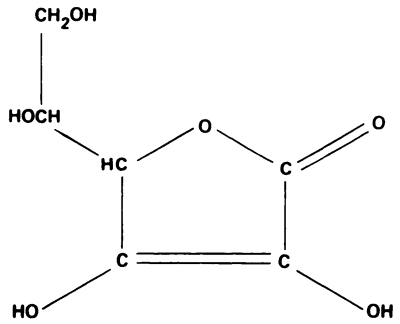

Chart 1. The accepted structural configuration of ascorbic acid (vitamin C), an α-ketolactone with the formula $C_6H_8O_6$, with a molecular weight of 176.13, and containing an acid-ionizing group in water with pK_A 4.19.

ceration with secondary bacterial colonization and to vascular disorganization with edema and interstitial hemorrhage, and also to one feature that is rarely emphasized in the literature, generalized undifferentiated cellular proliferation with specialized cells throughout the tissue reverting to a primitive form (350).

The pathology of scurvy has been summarized as "a generalized structural breakdown of the intercellular matrix associated with undifferentiated cell proliferation, or in evolutionary terms (provided such a disintegrating individual could be kept alive), the gradual reversion from multicellular organization to a primitive unicellular state" (50).

Pursuing this theoretical view even further, one might regard scurvy (generalized undifferentiated cell proliferation with generalized matrix breakdown) as an "omnifocal" variety of neoplastic disease.

The late Dr. William McCormick of Toronto appears to have been the first to recognize that the generalized stromal changes of scurvy are identical with the local stromal changes observed in the immediate vicinity of invading neoplastic cells (211). He surmised that the nutrient (vitamin C) known to be capable of preventing such generalized changes in scurvy might have similar effects in cancer, and the evidence (to be reviewed below) that cancer patients are almost invariably depleted of ascorbate lent support to his view.

There are some other interesting associations between scurvy and cancer. There is some epidemiological evidence (reviewed below) indicating that cancer incidence in large population groups is inversely related to average daily ascorbate intake. There is no real modern evidence that frankly scorbutic patients succumb to cancer, presumably because such patients either die fairly rapidly from their extreme vitamin deficiency state or, more likely, are promptly diagnosed and rapidly cured. However, the historical literature contains many allusions to the increased frequency of "cancers and tumors" in scurvy victims. A typical autopsy report of James Lind (197) contains phrases such as "...all parts were so mixed up and blended together to form one mass or lump that individual organs could not be identified," surely an 18th century morbid anatomist's graphic description of neoplastic infiltration.

Finally, in advanced human cancer, the premortal features of anemia, cachexia, extreme lassitude, hemorrhages, ulceration, susceptibility to infections, and abnormally low tissue, plasma, and leukocyte ascorbate levels, with terminal adrenal failure, are virtually identical with the premortal features of advanced human scurvy.

Host Resistance to Cancer

Many factors are known to be involved in host resistance to malignant invasive growth. These can be conveniently divided into locally acting stromal factors and others acting systemically.

Stromal resistance is primarily exercised by the ability of the host to encapsulate the neoplastic cells in a dense, practically impenetrable barrier of fibrous tissue. The collagenous barrier is scanty and ill defined in highly anaplastic invasive tumors, moderate in amount in tumors of moderate rapidity of growth, and very abundant in slow-growing "contained" atrophic scirrhous tumors. In any individual, the degree of stromal fibrosis is the same around the primary growth and its metastases, indicating a constitutional response. Other important stromal factors in resistance are (*a*) the resistance of the intercellular ground substance to local infiltration and (*b*) the degree of lymphocytic response. Lymphocytes are most numerous in the stroma of slow-growing tumors and scanty or virtually absent around rapidly growing lesions, and again the degree of lymphocytic infiltration is identical around primary growth and its metastases, indicating a constitutional response. A brisk lymphocytic response indicates enhanced host resistance and is associated with a more favorable prognosis.

The systemic factors are less clearly defined. They include such constitutional factors as the relative efficiency of the immune system, feedback mechanisms involved in the restraint of "invasive" enzymes, and, for certain tumors at least, the hormonal status of the individual.

The ability safely to enhance host resistance to maximum levels in every patient would vastly improve cancer treatment. It is our intention to show that ascorbic acid metabolism is implicated in all these resistance mechanisms and that ingestion of this substance in adequate amounts could provide a simple and safe method of achieving this desirable goal.

Chemistry of Ascorbic Acid and the Intercellular Matrix

Ascorbic acid is of enormous biological importance. It is one of the most important reducing substances known to occur naturally in living tissues, and since its discovery it has been the subject of many investigations, theories, and exhaustive reviews (50, 66, 140, 181, 195, 213, 251, 254, 313, 350). It would be inappropriate to review this voluminous literature here. We propose to limit our discussion to areas of special relevance to cancer.

Two points deserve early emphasis: (*a*) although most animals can synthesize ascorbic acid, a human cannot, and he is totally dependent upon dietary intake to satisfy all his requirements; (*b*) 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.

This leads us to a consideration of the intercellular matrix. In the last century the eminent pathologist Rupert

Virchow revolutionized his specialty by focusing attention on the "cellular" nature of disease. It is our impression that we are in the midst of a second revolution in pathology with the recognition that cells do not exist in "empty" space. The cell is still dominant, but it is now increasingly appreciated that for every action, be it physiological or pathological, of the cell, there is an appropriate reaction in the immediate extracellular environment.

All tissue cells in the body are firmly embedded in highly viscous ground substance. This ubiquitous material may be sparse or abundant and be in varying degrees of molecular aggregation, depending upon the particular organ studied, but basically it is of universal distribution, pervading every interspace and isolating every cell from its neighbor. It forms the immediate contact environment of all cells, and it must be traversed by every molecule entering or leaving the cell.

Variations in the physicochemical composition of the extracellular environment (polymerization-depolymerization) exert a profound influence on cell behavior, and, in turn, cells possess a powerful means of modifying their immediate microenvironment. 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 (54).

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 interlacing high-molecular-weight polymers form a structurally stable hydrophilic mesh, which, in turn, is reinforced at the microscopic level by a 3-dimensional network of collagen fibers. This is the true *milieu interieur* of Claude Bernard, within which all cellular activity takes place.

The chemistry of the glycosaminoglycans and of the proteoglycans is the subject of much contemporary study, and some excellent reviews are available (10, 103, 171, 189, 240, 241). The glycosaminoglycans are single-strand long-chain polymers with molecular weights ranging up to 10×10^6. The common varieties are hyaluronic acid (built up from alternating repeating units of *N*-acetylglucosamine and glucuronic acid), chondroitin (built up from *N*-acetylgalactosamine and glucuronic acid), and sulfate esters (the chondroitin sulfates), of which several isomers exist.

The proteoglycans are macromolecules of a more complex multibranched structure. Present evidence indicates that they consist of a primary glycosaminoglycan core, to which is attached, by link proteins, secondary protein cores at spaced intervals, to which are attached tertiary glycosaminoglycan polymers. It should be noted that the proteoglycans of the matrix are not a single molecular species but comprise a polydisperse family of rather similar macromolecules which differ in complexity, molecular weight, hydrodynamic size, chemical composition, and reactivity (132).

In healthy tissues, the intercellular matrix is maintained in a steady-state equilibrium of very slow dynamic change, with the formation of new macromolecules (polymerization) balanced by turnover and decay (depolymerization). 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 in scurvy (48, 50).

Depolymerization of matrix glycosaminoglycans is brought about by the sequential action of "hyaluronidase." This sequence of lysosomal glycosidases involves first an endoglycosidase ("true" hyaluronidase) that cleaves the long-chain polymer into tetrasaccharides; this action is followed by the concerted action of 2 exoglycosidases, β-glucuronidase and β-*N*-acetylglucosaminidase (β-*N*-acetylgalactosaminidase in the case of the chondroitins). The tetrasaccharide is the feedback inhibitor of the endoglycosidase but is also the substrate for the exoglycosidases. Thus, any interference with the latter will inhibit the whole process (152, 309).

Depolymerization of matrix proteoglycans is brought about by the combined action of the glycosidases and neutral proteases, which are believed to be released simultaneously from cellular lysosomes during cell division.

It now seems reasonably certain that the continuous release of these hydrolytic enzymes from neoplastic cells is responsible for their invasive capability, for the selective routing by increased diffusion of nutrients towards the tumor cells, and perhaps even for sustaining the whole momentum of autonomous neoplastic proliferation (48, 54). The effect of these hydrolytic enzymes on the matrix is to release a whole spectrum of glycosaminoglycan and glycoprotein breakdown products into the blood stream, the so-called "acute-phase reactants," the estimation of which forms the basis for the majority of serochemical tests for cancer.

The collagen component of the extracellular matrix is also susceptible to the same hydrolytic enzyme activity. Collagen fibrils are bound together into microscopically visible collagen fibers by matrix cement substance (140). More specifically, electron microscopy show a precise molecular alignment of proteoglycans along collagen chains (191). Ionic interactions between positively charged lysine and arginine residues of polymeric collagen and negatively charged glycosaminoglycan and proteoglycan sulfonic acid groups, together with the formation of hydrogen bonds, offer a probable explanation for this "adhesiveness" (311). The enzymatic degradation of such vital cross-linking bonds in the immediate vicinity of neoplastic cells could result in the dissolution and "microscopic disappearance" of this essential molecular scaffolding. Such structural disintegration is, of course, quite characteristic of the stromal erosion seen in immediate proximity to highly malignant growths.

With regard to collagen fibrillogenesis and its specific relevance to tumor encapsulation, it seems clear that specific interactions with extracellular glycosaminoglycans and proteoglycans play an essential role (132). There is now an overwhelming body of evidence (191, 201, 202, 205, 206, 311) to support the view originally proposed by Meyer (215) that the long-chain matrix polymers with their regular anionic spacings provide the essential molecular template for the precise orderly deposition of collagen precursors and

their regular sequential arrangement into collagen fibrils. Thus, enzymatic dissolution of the matrix would also inhibit new collagen formation.

Any measure that can protect the integrity of the intercellular matrix will (*a*) effectively retard malignant infiltration, (*b*) restrict the selective nutrition of tumors, (*c*) protect preexisting collagen barriers from neoplastic erosion, and (*d*) facilitate protective collagen encapsulation. This is one important function of ascorbic acid.

The Increased Requirement for Ascorbic Acid in Cancer

The contention that ascorbate is involved in resistance to neoplasia gains support from the many studies demonstrating that cancer patients have abnormally low ascorbate reserves. When this was first demonstrated many years ago, there was a tendency to assume that such a finding merely reflected the poor general nutritional status of advanced cancer patients. Such a view is no longer tenable, and it is now generally agreed that low levels in cancer indicate an increased utilization and requirement for the vitamin.

Increased utilization of ascorbate, as measured by depletion of ascorbate reserves, is by no means confined to cancer; it is a characteristic feature of many other "cell-proliferative" disorders, such as inflammation (17, 91), and of the reparative processes after surgical trauma (88, 89), myocardial infarction (160, 161), and thermal burns (16). The last 3 studies demonstrate that ascorbate is removed from the circulating reserves and concentrated at the site of the reparative process.

A similar shift of ascorbate from reserves to the tumor stroma has been demonstrated in cancer. Guinea pig studies have shown that ascorbic acid is selectively concentrated in cancerous tissue and, as a result, normal tissues are depleted (39, 315, 336). Dyer and Ross (109) studied the ascorbate content of tissues of mice bearing a wide variety of tumors, and in all examples the ascorbate concentration was greater in the tumor than in the liver of the respective host. Dodd and Giron-Conland (101), using electron spin resonance, have demonstrated that the ascorbyl radical is present in a wide variety of tumors in higher concentration than in the corresponding normal tissues.

Human cancer tissue also contains elevated levels of ascorbate. Goth and Littman (139) demonstrated this for a variety of human tumors. Chinoy (69) reported that certain human tumors contain far greater ascorbate levels than does their tissue of origin, and using histochemical techniques demonstrated that the greatest concentration of ascorbate was deposited at the periphery of the tumor, against the actively growing invasive margin. In 29 of 30 patients, Moriarty *et al.* (225) found the level in tumors to be higher than that in the surrounding tissues.

Concentration of ascorbate in tumor stroma results in measurable depletion of circulating reserves. Table 1 lists studies that have been carried out, contrasting values in cancer patients with those obtained in normal controls.

The increased requirement for ascorbate in cancer can also be demonstrated by ascorbate loading tests (18, 49, 126, 127, 186, 217). All such investigations have shown an increased utilization of ascorbate by the cancer subjects.

The combined results of all these studies show serious deficiencies of blood and leukocyte ascorbate to be a characteristic feature of cancer. Thus, irrespective of any specific therapeutic effects suggested, replacement of this deficit should be a part of all comprehensive cancer treatment regimens.

Ascorbic Acid and Host Resistance to Neoplasia

Ascorbate metabolism can be implicated in a number of host resistance mechanisms functioning at both stromal and systemic levels.

Ascorbate, Hyaluronidase, and the Intercellular Matrix. Invasiveness is one of the fundamental and distinguishing attributes of malignant tumor cells, conferring upon them their ability not only to infiltrate the local tissue interstices but also to ulcerate through membranous barriers and to penetrate into lymphatics, blood vessels, and other preformed spaces and thus produce remote metastases by passive transfer. The structural integrity of the ground substance matrix is the first barrier to invasiveness.

Depolymerization of adjacent matrix is invariably observed in the immediate vicinity of invading neoplastic cells, and there is now much evidence that the mechanism of malignant invasiveness depends upon the continuous release of lysosomal glycosidases (hyaluronidase) and perhaps other degradative lysosomal enzymes (the neutral proteases and collagenase) from the invading cells; see review by Cameron (48) and publications by Balazs and von Euler (11), Fiszer-Szafarz *et al.* (116–120), Harris *et al.* (152), and Grossfeld (147).

The endoglycosidase has a molecular weight of around 80,000 (6, 7). Other lysosomal enzymes are the exoglycosidases, the proteases, collagenase, nuclease, sulfatases, and phosphatases. The release of hyaluronidase from the cancer cell is usually accompanied by a release of increased amounts of other lysosomal enzymes, as has been demonstrated for a wide variety of experimental and human tumors (93, 110, 120, 172, 190, 282, 292, 316).

The impact of this 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, destroy the structural stability of the molecular micelle, with an abrupt fall in local ground substance viscosity leading to diminished mutual adhesiveness of the tumor cells (76, 212), their increased motility (1), and, most important of all, the erosion of the immediate structural barrier to malignant infiltration (48). These local pericellular changes of matrix depolymerization, affecting all barriers from the cell membrane to stromal capillary interendothelial cement, would result in local zones of increased capillary and tissue diffusion and could account for the so-called selective nutrition of tumors, the all-too-familiar picture of a flourishing tumor growing parasitically in a host steadily dying from cachectic nutritional inanition (48).

Thus, if we could stabilize cell matrix interrelationships in cancer and, more particularly, find a method of safely inhibiting tumor cell glycosidases, we should possess a means not only of restraining malignant invasiveness but also of retarding malignant cell proliferation by depriving

Table 1
Ascorbic acid in the blood of cancer patients

Investigator	Type of cancer studied	No. of cancer patients	Plasma levels (mg/dl)		Leukocyte levels (μg/10⁸ cells)	
			Cancer	Controls	Cancer	Controls
Bodansky *et al.* (38)	Miscellaneous cancers	69	0.48	0.79	27.0[a]	36.1[a]
Waldo and Zipf (333)	Miscellaneous cancers	30	0.10	0.80		35.0
Freeman and Hafkesbring (124)	Miscellaneous cancers	5	0.62[b]	0.88[b]		
Krasner and Dymock (183)	Miscellaneous cancers	50			11.5	29.5
Kakar and Wilson (165)	Advanced breast cancer	1	0.13	0.47	12.5	26.0
Basu *et al.* (18)	Advanced breast cancer	22			12.0	33.0
Cameron (49)	Miscellaneous cancers	24	0.26	0.96	11.2	24.3
Waldo and Zipf (333)	Leukemia	42	0.18	0.80	8.2	35.0
Barkhan and Howard (14)	Leukemia	5	0.21	0.69		
Lloyd *et al.* (198)	Acute leukemia	8	0.3	0.79	12.0[c]	35.0[c]
Lloyd *et al.* (198)	Chronic leukemia	8	0.7	0.79	31.0[c]	35.0[c]
Kakar *et al.* (166)	Acute lymphatic leukemia	10	0.40	0.95	35.9	56.4
Basu *et al.* (18)	Miscellaneous cancers	22			17.0	33.0

[a] Expressed as mg ascorbate per 100 g leukocytes.
[b] Values for whole blood.
[c] Values for platelets.

these cells of their favored nutritional status. This is a highly desirable therapeutic aim, but the benefits could be even greater.

The following working concept was published by Cameron in 1966 (48) and elaborated by Cameron and Pauling in 1973 (54):

"All tissue cells have an inherent tendency to divide but this tendency is normally restrained by the viscous nature of their intimate extracellular 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."

Under this concept, the only difference between "neoplastic" and "normal" cell proliferation is the persistence of hyaluronidase release in the former.

It is a common feature of enzyme-substrate reactions that the build-up of by-products exerts a feedback inhibitory effect. Thus, it is no surprise to find that glycosaminoglycan residues down to D-glucosamine have been shown experimentally to inhibit the growth of Sarcoma 37 (264, 273), Sarcoma 180 (22, 23), Walker carcinoma 256 (24, 224), various ascitic carcinomas (21, 22, 223), and epidermoid carcinomas (121), although *N*-acetylglucosamine and neutral sugars were found to be without effect (23, 223). In passing, it is of interest to note that glucosamine has been reported to inhibit the biosynthesis of protein, RNA, and DNA in label uptake experiments *in vitro* (21, 24). Recently, Schaffrath *et al.* (281) demonstrated inhibition of DNA-dependent and RNA-dependent polymerases as well as DNA-dependent RNA polymerase by a variety of sulfated glycosaminoglycans.

In vitro tests have shown that chondroitin sulfate residues (6, 7) and other polysaccharide polysulfuric acids (8), including heparin, a polysulfated glycosaminoglycan, are hyaluronidase inhibitors (135, 136). Rutin (194), hesperidin and derivatives (20), and polyphloretin phosphate (75) show *in vitro* hyaluronidase inhibition and also stabilize lysozymes (326). Hyaluronidase inhibition by bioflavonoids and related compounds has been reported (125, 207, 239). Stephens *et al.* (309) have recently shown that gold thiomalate is an effective hyaluronidase inhibitor, but we are not aware of any reports of its use in either experimental or human cancer.

Our special interest has focused on the existence in the serum of all species of a specific glycoprotein fraction known as PHI.[2] Serum PHI concentration remains within a remarkably narrow range in health but increases sharply in a variety of disease states, including infections, wound healing, rheumatoid arthritis, and cancer (133, 134, 153, 175). Because of this, serum from cancer patients inhibits hyaluronidase *in vitro* (116, 150). As yet, PHI has not been completely characterized, but it is reported to be a glycoprotein with a molecular weight of about 100,000 (207, 238). Glucosamine and uronic acids are major constituents of PHI (238), and it seems clear that the fraction consists of a polypeptide chain to which is attached a glycosaminoglycan residue which has been modified in some special way to confer upon it its powerful and highly specific inhibitory activity. In 1972, we suggested that the modification is the replacement of one or more than one glucuronic acid residue in the glycosaminoglycan depolymerization by-product by its close chemical relation ascorbic acid (60). It now seems clear that the active component of PHI is a tetrasaccharide with a terminal glucuronic acid unit replaced by an ascorbate residue, resistant to exoglycosidase activity, and therefore capable of blocking the whole process of glycosaminoglycan depolymerization.

The hypothesis that ascorbic acid is required for the synthesis of PHI offers a convincing explanation for its biological function in preventing scurvy, a state of general-

[2] The abbreviations used are: PHI, physiological hyaluronidase inhibitor; UHP, urinary hydroxyproline; 3-HOA, 3-hydroxyanthranilic acid.

ized matrix depolymerization brought about by exposure to uninhibited hyaluronidase evolved during the course of normal cell division and replacement (50). It also explains, in part, why there is an increased requirement for ascorbate in all cell-proliferative states, including cancer. Satisfying this enhanced requirement by increasing intake should enable this restraining mechanism to function at maximum efficiency.

Because everyday clinical experience confirms that ascorbate is necessary for satisfactory wound healing and repair, the natural assumption has been that this vitamin is essential for growth. We offer the alternative hypothesis that the primary function of vitamin C is to restrain excessive growth through its incorporation into the PHI system, directing the inherent proliferative capacity of all cells into a constrained organized differentiated behavior 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 bloodstream, there to mop up ascorbate reserves into an upsurge of PHI levels, all followed fairly rapidly by reversal into a healed phase of resting quiescent cells reembedded in a restabilized matrix, and return of these biochemical indices to a steady state equilibrium. In the absence of ascorbate, *i.e.*, in scurvy, wounds fail to heal, with a destabilized matrix, undifferentiated cell-proliferative "granulomata," and the continuous overspill of matrix by-products into the bloodstream (260, 279).

In vitro, the reaction of ascorbate with hyaluronic acid and with chondroitin sulfate has been reported to yield breakdown products of somewhat lower viscosity than the original preparations (303). Ascorbic acid also inhibits hyaluronidase *in vitro* under conditions that preclude the possibility of PHI production, but it has been shown to have a much greater effect *in vivo*, clearly indicating the existence of some intermediate mechanism (267).

A recent brief report by Shapiro *et al.* (295) indicating that prescorbutic guinea pigs have increased rather than decreased PHI levels is difficult to reconcile with the general pattern. A possible explanation for this apparent contradiction may lie in the finding by Fiszer-Szafarz (117) that serum contains a number of different hyaluronidase inhibitor fractions distinguishable by the use of different laboratory techniques.

The realization that the term "hyaluronidase" embraces a sequence of related degradative enzymes has given rise to some confusion in the literature. Thus, many workers have claimed that neoplastic cells produce significantly increased amounts of hyaluronidase, but other workers have been unable to confirm this; see references in the review by Cameron (48). However, there seems to be no dispute that neoplastic cells release significantly increased amounts of the exoglycosidases β-glucuronidase (63, 74, 115, 152, 243) and β-*N*-acetylglucosaminidase (63, 77, 79, 120, 152). In their recent study of an experimental rat osteosarcoma, Harris *et al.* (152) demonstrated no significant increase in hyaluronidase activity but a significant increase in β-glucuronidase and β-*N*-acetylglucosaminidase activity in osteosarcoma homogenates relative to homogenates of normal bone.

Carr's paper (63) contains an interesting observation. Three different experimental mouse tumors were studied. Inoculated i.p. to form noninvasive ascitic growths, these 3 experimental tumors showed no significant exoglycosidase activity. However, when the same tumors were inoculated s.c. to produce invasive growths in a viscous ground substance matrix, their exoglycosidase activity increased very markedly. Thus, it would appear that neoplastic cells have considerable exoglycosidase potential that is expressed only in the presence of the specific substrate. This observation has important implications for tissue culture studies now in progress.

Saccharo-1,4-lactone is a specific inhibitor of β-glucuronidase (78), and Carr (62) has demonstrated that the administration of this and related compounds results in a marked regression of experimental mouse tumors. Unfortunately, this compound is metabolized to toxic saccharic acid.

The problem is to find some nontoxic exoglycosidase inhibitor, and ascorbic acid, containing the essential lactone ring structure, with close structural similarities to the natural exoglycosidase substrate and possessing this physiological function, would appear to be a favored contender for this role. Furthermore, *in vitro* studies by Kojima and Hess (182) demonstrated that ascorbic acid functions as a noncompetitor inhibitor of *N*-acetylglucosaminidase. Papers on the inhibition of β-glucuronidase have been reviewed by Dutton (108).

To summarize, the dangerous features of neoplastic cell behavior (invasiveness, selective nutrition, and perhaps growth) are caused by microenvironmental depolymerization. In turn, this matrix destabilization is brought about by constant exposure to lysosomal glycosidases continually released by the neoplastic cells. Finally, ascorbate is involved in the natural restraint of this degradative enzymic activity.

Ascorbate, Collagen, and Tumor Encapsulation. The intercellular matrix is reinforced by a 3-dimensional network of interlacing collagen fibers. A generalized increase in the collagen content of the matrix can be induced by certain hormones (estrogens, androgens, corticosteroids, thyroxine) with a gradual shift from an amorphous to a more fibrotic pattern; the same change is observed in the aging process; see references in the review by Cameron (48).

The amount of collagen present determines the strength of the tissue and also its resistance to malignant infiltration. It is common knowledge that invasive tumor cells preferentially spread along "soft" tissue planes and are deflected and constrained by fibrotic tissues such as scars, fascia, capsules, and ligaments. On a generalized scale, the gradual shift to a more fibrotic pattern in the matrix induced by these hormones and the aging process may account for the increased resistance to tumor growth associated with these hormonal and constitutional changes (48).

Thus, if matrix integrity is the first line of defense against invasive growth, this defense is very powerfully reinforced by the next barrier, the collagen network. To appreciate fully the important role played by collagen in stromal resistance, we must first look at the effects of invasive cells on preexisting collagen and then at the variable ability of

individual hosts to encapsulate their tumors within barriers of new fibrous tissue.

Dissolution of preexisting collagen fibers in the immediate proximity of neoplastic cells is a well-recognized feature of active invasive growths. This has led many to postulate that tumor cells release collagenase, a specific proteolytic enzyme found in both mammalian and bacterial cells. Extracts of various animal and human tumors exhibit enhanced collagenase activity (104, 131, 270, 310, 319).

As mentioned earlier, it could well be that neoplastic transformation involves an increased and sustained output of all lysosomal enzymes, including collagenase, but there could be a simpler explanation.

It will be recalled that collagen fibers 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 preexisting collagen barriers ahead of malignant invasive growth.

Basement membrane is ground substance heavily reinforced with collagen. Basement membrane disorganization has been observed during culture of LS402A mouse carcinoma cells in ascorbate-deficient medium, and this effect is reversed by introducing ascorbate (263). Lysis of preexisting collagen has been studied in transplanted rat tumors (262) and during 2-aminoanthracene carcinogenesis (258).

Collagen catabolism results in an increased level of UHP, and, in the human, measurement of UHP is useful to the clinician, rising levels reflecting increasing spread and activity of the disease. It has been shown that UHP levels in patients with breast cancer are directly proportional to the spread of the disease and bear an inverse relationship to leukocyte ascorbate levels and that a loading dose of 1 g of ascorbic acid produces a sharp decrease in UHP excretion (18). Thus, invasive neoplastic cells possess the ability to disrupt preexisting collagen barriers, with increased collagen catabolism and output of resultant hydroxyproline residues, and this disruptive effect can be diminished significantly by increasing ascorbate intake.

Of even greater interest, from the point of view of practical therapy, is the variable ability of individuals to encapsulate their tumors (the so-called scirrhous response). In daily clinical practice, the whole gamut of response can be observed, ranging from the unfortunate individual with a highly anaplastic, remorselessly invasive tumor with quite negligible stromal fibrotic reaction and a very limited life expectancy to that of the more fortunate individual with a very slow-growing, practically noninvasive tumor encased in a dense, almost impermeable barrier of reactive scar tissue (the so-called atrophic scirrhous tumors) and with a clinical prognosis differing little from normal life expectancy. It is true that the former dismal picture tends to occur more frequently in the younger age groups, while the latter clinical presentation is more common in the elderly, but the differentiation by age is by no means clear-cut.

It would be a very considerable advance in cancer treatment if all tumors could be converted to the atrophic scirrhous variety. Even in the present state of our knowledge, this is not an impossible objective. To achieve this aim, we have first to consider the "soil," appreciating that the elderly, presumably because of some hormonal change, seem in general to be more able to elicit this powerful defensive reaction than the young. Thus, some consideration should be given to exploring the possibility that appropriate endocrinal adjustment (not necessarily synonymous with aging) could be used to condition the matrix and render it more adaptable to this encapsulating process (48).

However, irrespective of preconditioning the "soil," encapsulation implies an intense local deposition of fully formed collagen fibers imprisoning the invasive tumor cells. Ascorbic acid is essential for new collagen formation.

The precise role of ascorbate in collagen synthesis has been the subject of much study; see comprehensive reviews by Gould (140) and Barnes (15). During states of ascorbate deficiency, total collagen synthesis as determined by extraction techniques is unaffected (19) even though wound healing by microscopically visible collagen fibers is completely absent (88). Lack of ascorbate sharply reduces hydroxylation of prolyl and lysyl residues into hydroxyproline and hydroxylysine of mature collagen during ribosomal assembly (19, 140, 141, 174, 176), leading to instability of the triple helix of collagen (171). Such instability results in increased collagen catabolism, as has been demonstrated in scurvy (138, 272) and in cancer (18, 258, 262).

Ascorbic acid has been shown to increase collagen synthesis by fibroblasts *in vitro* (99, 280) and to maintain collagen synthesis in nonmitotic fibroblasts for extended periods (99). Prolyl hydroxylase, the enzyme hydroxylating prolyl and lysyl residues of procollagen, requires ascorbate to function *in vitro* (181), and the addition of ascorbic acid to tissue cultures stimulates the prolyl hydroxylase activity of fibroblasts (174).

Thus, there is no doubt that a 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 procollagen.

To summarize, in the cancer situation, 3 factors are involved: (*a*) there is the stimulating effect of neoplastic cells creating a proliferative environment around stromal fibroblasts; (*b*) there is some evidence that the hormonal environment may influence the fibrotic response; (*c*) for an adequate scirrhous response, an abundance of ascorbate is essential, and this is one factor open to therapeutic control.

Ascorbate and Immunocompetence. It is generally accepted that the immune system plays some part in host resistance to cancer, both in the prophylactic sense of an efficient immunosurveillance system destroying neoplastic cells at an early stage in their careers and in the protective sense of retarding the growth of established tumors. Patients maintained on long-term immunosuppressive regimens have an increased incidence of certain forms of cancer, and cancer patients tend to have decreased immunocompetence as measured by standard tests (271). Any practical measure that could enhance immunocompetence could only be beneficial to the cancer patient.

The immunological defense system has the awesome task

of first distinguishing friend from foe by recognizing "self" from "not self" and then acting upon the information by identifying the target so as to permit its elimination through various mechanisms and strategies. Recognition depends upon evaluation of minute differences in molecular structure.

Lewis Thomas (321) pictures the immune system as a police force, constantly patrolling the body cells, keeping an eye open for cells becoming neoplastic and, upon recognition, destroying them. For such a system to work, cancer cells must display a surface antigen for "recognition" different from their nonneoplastic compatriots.

For certain experimental tumors, induced by specific oncogenic viruses and specific chemical carcinogens, there is clear evidence that this is so, and furthermore the antigen is specific for the carcinogenic agent (90, 221, 271). In human cancer, the picture is less clear. Certain tumors derived from the same basic cell type often express a common differentiation antigen, as measured by specific immunoprotein assay (236, 237). These so-called oncofetal antigens are also present on embryonic cells, and indeed there is some evidence that they may be present on the surface of any rapidly dividing but not necessarily neoplastic cell (271). Detection of specific serum oncofetal immunoproteins may reflect the sensitivity of existing techniques. Thus, most states of cell proliferation would be below the level of detection but, in the event of a single cell progressively dividing and subdividing to form an immense clone of neoplastic cells, the clonal antigen would be measurable as a "tumor-specific antigen" (46).

The potentially antigenic cell membrane is a highly complex structure consisting of proteins and lipoproteins, not only quite specific for the individual but also quite specific for the particular cell type, masked in an "exoskeleton" of glycosaminoglycan and collagen macromolecules. Cell division must imply unmasking and dissolution of the cell membrane involving new molecular configurations and thus altering its antigenic response. We advance the proposition that all dividing cells by cell membrane alteration elicit a weak "not self" signal as part of the general homeostatic mechanism of the body. This recognition signal would evoke a weak antigenic response specific to the particular cell type because of its unique protein display, but recognizable only if a very large number of similar clonal cells were dividing, as in the embryo or cancer.

There is some evidence that dissolution of the glycosaminoglycan component of the cell membrane to expose protein and lipoprotein configurations, as would be bound to result from the cellular release of glycosidases, plays an important part in determining antigenicity (43, 44).

Because of protein similarities, the immune response, so devastating in allograft situation, is much less effective against tumor cells indigenous to the host. Nevertheless, it does play some part in determining an individual patient's resistance to his particular tumor.

In our view, ascorbate is essential to ensure the efficient working of the immune system. The immunocompetence mechanisms are a combination of humoral and cell-mediated defensive reactions with ascorbate involved in a number of ways. These mechanisms must now be considered.

The response to the signal "foe" is the rapid mobilization of humoral and cellular agents of high specific activity and the elaboration of recognition units, specific immunoglobulins. The replication of the system is evident in its "search and destroy" function, in which the same complex molecular unit that recognizes a structure also fires a weapon, the complement system, that attacks only the structure identified by the specific antibody, even though it is present in only a single cell.

Although the complement system can be activated by a variety of factors, the specificity of this action results from the fact that it can be focused with the precision of a laser beam to the point of local impact of immunoglobulins with the specific antigen that they have been created to recognize. The cascade of complement factors thus set in motion further spreads the signal by a variety of chemical messengers and the indirect activation, through anaphylotoxin (81, 82), of other cells to induce release of activators to sustain the inflammatory response, including phagocytosis, which will be discussed in a later section.

The precision of recognition and the intensity of the response vary in the same individual from time to time according to endocrine status, reflecting such constitutional factors as age, sex, and hormone environment (90). The influence of endocrinal status on host resistance to cancer will be discussed below. The importance of endocrinal status in preconditioning the "soil" recalls our earlier discussion that similar factors influence the efficiency of the fibrotic response.

The humoral factors involved in immunocompetence are the cell surface-specific immunoglobulins and their ultimate weapon, the complement cascade.

The reticuloendothelial system is concerned with the precise design and production of immunoglobulins, proteins which contain a large number of disulfide 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 S–S bonds has been extensively discussed by Lewin (195), who strongly concludes that ascorbate is essential for immunoglobulin synthesis. A positive correlation between serum ascorbate levels and serum IgG and IgM titers was reported by Vallance (325), studying human subjects isolated from any sources of new infection for nearly 1 year on a remote British Antarctic Research Station.

Regarding the more definitive weapon of the complement cascade, it has recently been demonstrated that the administration of supplemental sodium ascorbate to guinea pigs significantly increases the esteratic power of the first component of complement (C_1 esterase activity, without which the whole complement cascade is inoperable) in animals immunized with antipenicilloyl (guinea pig) γ-globulin.[3]

In cell-mediated immunity, immunocompetence is exercised overwhelmingly by the lymphocytes. In tumors, the degree of stromal lymphocyte infiltration is a measure of the efficiency of host resistance to the neoplastic process. Thus, the degree of lymphocytic infiltration is now accepted as a reliable prognostic indicator.

Relative to other cells, lymphocytes contain substantially

[3] G. Feigen, unpublished observations.

higher concentrations of ascorbate, and there are strong indications that this characteristic feature is "purposeful" and related to their active role in cell-mediated immunocompetence.

It has been neatly demonstrated that guinea pigs maintained on prescorbutic diets have markedly reduced immunocompetence as shown by their prolonged tolerance of allografts and that this change is related to abnormally low lymphocyte ascorbate levels. When ascorbate is administered to restore lymphocyte ascorbate levels to normality, the allografts are promptly and universally rejected (167). This observation led us to suggest that the opposite condition of lymphocyte ascorbate saturation should be associated with enhanced immunocompetence (55). This prediction has subsequently been confirmed. Yonemoto *et al.* (356) demonstrated in healthy young subjects that a high loading dose of ascorbate (5 g/day for 3 days) evokes a significant increase in lymphocyte blastogenesis as measured by response to phytohemagglutinin challenge and, furthermore, that this increase in immunocompetence was further significantly enhanced by larger doses (10 g). A similar effect of increased T-lymphocyte responses to concanavalin 2 with increased intake of ascorbate by mice has been reported by Siegel and Morton (300). These observations support the common sense view that lymphocytes rich in ascorbate should be able to conduct their protective business more efficiently than those that are not, a characteristic feature of established cancer.

To summarize, cancer patients generally exhibit diminished immunocompetence and almost invariably have low lymphocyte ascorbate content. The simplest and safest way to enhance immunocompetence in such patients and to ensure that their humoral and cell-mediated defense systems are working at maximum efficiency is to increase their ascorbate intake. Only when this increased demand and utilization in cancer are fully satisfied can these immune mechanisms afford maximum protection against the wayward cancer cell.

Ascorbate and Hormone Balance. The highest concentrations of ascorbate are found in the adrenal and pituitary glands, and the terminal stages of scurvy are just preceded by complete depletion of adrenal ascorbate, leading, it has been frequently stated, to "scurvy death" from adrenocortical failure. This has caused many to suggest that the ascorbic acid-dehydroascorbic acid system plays an important role in the synthesis and release of hormones of the adrenopituitary axis. The evidence for this is both conflicting and confusing (13, 72, 73, 102, 277, 278).

It has of course been known for many years that changes in the hormonal status of an individual and in particular relative changes in the different components of the adrenopituitary axis can sometimes exert a significant effect on host resistance to neoplasia; see the extensive review by Stoll (312). In 1974, we offered the tentative suggestion that availability of ascorbate by influencing the interrelationship between the members of this hormonal orchestra might determine whether an individual possesses a favorable or an unfavorable steroid environment (55). The proposal has not yet been studied, and the suggestion remains without experimental evidence.

Ascorbate and Phagocytosis. Phagocytosis is believed to play an important part in cell-mediated immune response to tumor cells. In addition, practically all tumors ulcerate through adjacent surfaces (skin, gastrointestinal tract, respiratory tract, etc.) and become subject to secondary bacterial invasion. Thus, the toxemia of secondary infection becomes part of the cancer illness. Efficient phagocytosis offers some protection against this almost inevitable complication.

Numerous studies have demonstrated that ascorbate is required for active phagocytosis both *in vivo* and *in vitro* (71, 87, 128, 137, 210, 240, 270).

Leukocyte motility is dependent upon the activity of the hexose monophosphate shunt, which in turn is activated by ascorbate (84, 94, 173) oxidizing NADPH to $NADP^+$ with release of bacteriocidal peroxide (252). A review of oxygen-dependent phagocytosis has recently been published (9).

The striking effect of increasing p.o. intake of ascorbate on human lymphocyte blastogenesis as measured by response to phytohemagglutinin and [^{3}H]thymidine uptake was noted in the previous section.

Ascorbic Acid and Tumor Prevention

Any measure that will retard the growth of established tumors should also, if applied early enough, effectively suppress "latent" tumors and thus in clinical terms have some prophylactic value. The purpose of this section is to consider whether ascorbate offers any degree of protection against a variety of carcinogenic agents.

The Antiviral Activity of Ascorbic Acid. Some cancers are thought to be initiated by oncogenic viruses [see discussion by Dulbecco (106)], and this whole area is the focus of much current research interest, with proposals that antiviral agents be used in cancer prophylaxis and chemotherapy (208). Against this background the antiviral activity of ascorbic acid assumes fresh importance.

The clinical evidence that supplemental ascorbate offers protection against a broad spectrum of viral disease has been reviewed by Pauling (252, 254) and Stone (313) in their respective books. Particular mention has to be made of the pioneer work of Jungeblut (162, 163), in demonstrating *in vitro* and *in vivo* activity against the poliomyelitis virus, and the clinical work of Klenner (178, 179), who strongly advocates its use against a wide variety of viral disorders. In Japan, Morishige and Murata (227)[4] have used supplemental ascorbate in the successful prevention and treatment of measles, mumps, viral orchitis, viral pneumonia, herpes zoster, and viral encephalitis. The most striking effect recorded by these Japanese workers has been the virtually complete prevention of posttransfusion hepatitis in a country where such a complication is common (227).

In the laboratory, Murata *et al.* (228–230) have investigated the inactivation of bacterial viruses by ascorbate, and they have been able to demonstrate the inactivation of J_1 phage of *Lactobacillus casei* and a variety of viruses of *Escherichia coli* and *Bacillus subtilis*.

The mechanism of *in vitro* viral inactivation is still unclear. It could involve the liberation of peroxide from oxidation of ascorbate, because the addition of catalase provides protection (290, 348). However, Murata *et al.* (231) noted that

[4] F. Morishige and A. Murata, personal communication.

concentrations of peroxide that should have been produced during ascorbate oxidation had no effect on bacterial virus Δ-A. Murata has postulated that free-radical intermediates produced during ascorbate oxidation are the active agents in viral inactivation and has presented sound arguments in support of his view. In this respect, the role of monodehydroascorbate radical in the termination of free radical reactions and its consequent biological function have been reviewed by Bielski *et al.* (28).

Ascorbate has also been shown to enhance interferon production (92, 298–301), as well as to enhance the phagocytic properties of the reticuloendothelial system, both potent *in vivo* defenses against viruses. The interaction of ascorbate with L-lysine in rats (67) may also contribute to the effectiveness of ascorbate.

Whatever the mechanisms may be, there is clinical and experimental evidence that supplemental ascorbate possesses some antiviral activity. Thus, if a viral etiology of human cancer is ever proved, ascorbate might be expected to exert some prophylactic and therapeutic effect.

Ascorbate and the Carcinogenic Hydrocarbons. There is some fragmentary evidence that ascorbate offers some protection against carcinogenic hydrocarbons.

A very marked stimulation of ascorbate synthesis in rats and mice can be evoked by exposure to various noxious compounds including the carcinogenic hydrocarbons, with methylcholanthrene the most powerful (40, 47, 80, 323).

The mixed-function oxidases are a group of closely related microsomal enzymes that metabolize many classes of compounds and are particularly important in the inactivation of chemical carcinogens. Although these microsomal enzymes have been studied most extensively in liver tissue, recent evidence indicates their occurrence in tissues of all major portals of entry, including the gastrointestinal tract, lung, skin, and placenta (337). Thus they represent the initial metabolic barrier to noxious foreign substances. These enzymes are affected by age, species and strain, sex, and nutritional state (358) and require NADPH and molecular oxygen (339–345) for their action. Ascorbic acid is also a requirement of the mixed-function oxidases that hydroxylate tryptophan (83, 233, 307), tyrosine (187, 233, 307, 324), dopamine (193), and phenylalanine (314). Microsomal metabolism of lipid-soluble foreign compounds or carcinogens yields products generally more water soluble, which greatly increases their rate of excretion. It is not surprising therefore that inducers of microsomal mixed-function oxidases (polycyclic hydrocarbons, pentobarbital, phenobarbital, chlordane, β-naphthoflavone, and phenothiazene) provide protection from carcinogens, whereas inhibitors of these enzymes (including carbon tetrachloride, organophosphorus insecticides, ozone, and carbon monoxide) potentiate carcinogenic effects; among the carcinogens studied are benzo(*a*)pyrene, 7,12-dimethylbenzanthracene, *N*-2-fluorenylacetamide, 4-dimethylaminostilbene, urethan, aflatoxin, diethylnitrosamine, aminoazo dyes, 3-methyl-4-dimethylaminoazobenzene, 2-acetylaminofluorene, and bracken fern [see review by Wattenberg (341)].

The direct action of ascorbate on carcinogens has also been reported. Warren (335) demonstrated the oxidation of aromatic hydrocarbons *in vitro* by ascorbate. Floyd *et al.* (122) reported that ascorbate inhibits conversion of *N*-hydroxy-*N*-acetyl-2-aminofluorene into 2 more potent carcinogens, 2-nitrosofluorene and *N*-acetyl-2-aminofluorene. This oxidation-reduction reaction reduces peroxide by the action of peroxidase and an electron donor; under the conditions used, ascorbate was preferentially oxidized. Attack of the carcinogen *N*-acetoxy-2-acetamidofluorene on guanosine is also prevented *in vitro*, probably through the quenching of the triplet nitrenium ion by proton abstraction (291).

Ascorbic acid deficiency in guinea pigs has been shown to decrease microsomal cytochrome P-450 activity to about 50% of its normal value (96, 97, 192, 203, 268) cytochrome b_5 (96, 97, 174, 192, 302, 358), NADPH-linked cytochrome *c* reductase (174, 358), and *O*- and *N*-demethylase activities (174, 185, 192, 358). Conflicting data by Kato *et al.* (170) in which the above-mentioned enzyme activities were not affected by ascorbate deficiency seem to be the result of a shorter depletion period (12 days). Zannoni and Sato (Ref. 174, p. 119) have shown that microsomal enzyme activities are not decreased significantly in a 10-day depletion experiment but are significantly decreased after 21 days. Repletion experiments with scorbutic guinea pigs have shown that supplementation with ascorbic acid returned cytochrome P-450 and demethylation activities to normal within 48 hr (97, 192, 203).

Ascorbate and Nitrosamines. Nitrosamines, products of the reaction of nitrite with a secondary amine, alkylurea, or an *N*-alkylcarbamate group, have been strongly implicated as a major environmental carcinogen (196). Atmospheric nitrosamine pollution could be significant. In some parts of the United States dimethylnitrosamine levels rise to 0.1 μg/cu m of "clear" air, which equals about 1.0 to 1.4 μg/person/day; by comparison, a pack of cigarettes contains around 0.8 μg and 4 slices of nitrite-preserved bacon contain about 5.5 μg dimethylnitrosamine (296). A variety of experimental tumors of the alimentary tract, liver, lung, and urinary bladder can be produced by nitrosamines (218, 219, 234, 275), and a number of nitrosation products are carcinogenic *in vivo*, including those of citrulline, ephedrine, sarcosine, morpholine, methylurea, piperazine, pyrrolidine, aminopyrine, diethylamine, and dimethylamine (347). Nitrosation is usually studied under simulated gastric conditions, but Hill *et al.* (155) have demonstrated that nitrosamines may be produced at any site where bacteria, secondary amine, and nitrite or nitrate are present. Another aspect deserving serious consideration is the transplacental transfer of nitrosamines; respiratory tract tumors were produced at a rate of up to 97% in offspring of mice given diethylnitrosamine during the last 4 days of pregnancy (222).

Ascorbic acid has been shown to exert a protective effect against carcinogenesis by nitrite reacting with aminopyrine (123), morpholine (174), piperazine (Ref. 174, p. 175), and raw fish (204) as well as to reduce the acute hepatotoxicity resulting from feeding nitrite and dimethylamine (61) or nitrite and aminopyrine (Refs. 145, 168, and 169; Ref. 174, p. 160). Studies with preformed nitrosamines have shown that ascorbic acid exerts little or no protective effect (Ref. 174, p. 175). Ascorbic acid does not react with amines, nor does it increase the rate of nitrosamine decomposition; it exerts its protective effect largely by reaction with nitrite

and nitrous acid (Refs. 5 and 220; Ref. 174, p. 181). Under simulated gastric conditions (37°, pH 1.5), an ascorbate/nitrite molar ratio of 1/1 provided 37%, a ratio of 2/1 provided 74%, and a ratio of 4/1 provided 93% protection from *in vitro* nitrosation of methylurea (Ref. 174, p. 181). Because nitrites are found in many foods as normal reduction product of nitrates or by deliberate introduction during processing (Ref. 174, p. 175), it is significant that ascorbic acid added or present in foods offers some protection against dangerous nitrosamine formation (Refs. 5 and 352; Ref. 174, p. 181).

It has also been shown, using the Ames test (148), that ascorbate inhibits bacterial mutagenesis by *N*-methyl-*N*-nitrosoguanidine, and Marquardt, Rugino, and Weisburger, using the same test procedure, have shown mutagenic (and presumably carcinogenic) activity in nitrite-treated foods and have suggested that human stomach cancer might well be related to this dietary factor, which to some extent might be reduced by ascorbate (204).

Ascorbate and Tryptophan Metabolites in Bladder Cancer. Exogenous or endogenously formed chemical carcinogens can often be implicated in the causation of human bladder cancer. The known relationship between occupational exposure to 4-aminophenyl, benzidine, and 2-naphthylamine and bladder cancer has stimulated investigations into the carcinogenicity of *N*-hydroxy aromatic amines (98, 265) and *o*-hydroxy aromatic amines (2). A large number of *o*-hydroxytryptophan metabolites are known carcinogens (357), and efforts have been made to demonstrate increases in tryptophan metabolites in the urine of bladder cancer patients, but the results have been equivocal (2); however, after a loading dose of L-tryptophan, bladder cancer patients excrete a significantly increased amount of the metabolites kynurenine, xanthurenic acid, and *o*-aminohippuric acid, suggesting that such subjects have some abnormality of tryptophan metabolism (357).

Chemiluminescence resulting from the nonenzymatic degradation of the tryptophan metabolite 3-HOA (and believed to reflect degradation from Compound I to Compound IV as shown in Chart 2) is significantly increased in the urine of bladder cancer patients and in the urine of heavy tobacco smokers (285, 286). The administration of ascorbate (1 to 2 g p.o. per day) results in a significant decrease in chemiluminescence and completely prevents the formation of Compound IV even in voided urine to which 3-HOA has then been added (286).

Urine from patients with bladder cancer oxidizes 3-HOA faster than does control urine *in vitro*, and the addition of ascorbate inhibits this reaction (259, 286). With the general working hypothesis that metabolites of 3-HOA are carcinogenic to uroepithelium, the increased amounts of such metabolites, especially Compound IV, found in the urine of heavy tobacco smokers explains their increased susceptibility to bladder cancer. 3-HOA implanted directly into the mouse bladder is carcinogenic, and this effect can be prevented by ascorbate (259, 284). The whole concept that supplemental ascorbate has a protective and inhibitory value in bladder cancer has been developed by Schlegel (284) and his associates in the Department of Urology of Tulane University. The experimental, biochemical, and clinical aspects of their work have been the subject of a recent review (284).

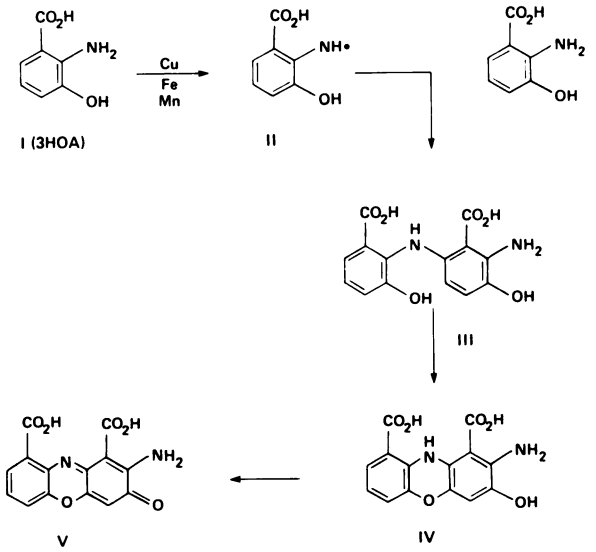

Chart 2. Postulated pathway of nonenzymatic oxidative decomposition of 3-HOA. From Schlegel *et al.* (286).

Ascorbate and Cigarette Smoking. The relationship between cigarette smoking and bladder cancer has been alluded to above. Of far greater importance in terms of numbers is the proved relationship between smoking and lung cancer.

There is considerable evidence that smoking depletes ascorbic acid reserves (4, 142, 156, 255–257, 269), as shown by diminished whole blood, serum, and leukocyte ascorbate levels in smokers relative to nonsmoker controls. It would be presumptuous to say that this difference reflects an increased utilization acting in a protective capacity. Nevertheless, in the present state of our knowledge, it would seem a wise precaution for the compulsive heavy smoker to increase deliberately his ascorbate intake.

Ascorbate and UV Carcinogenesis. Excessive exposure to UV is carcinogenic. In the human, this is evident in the increased incidence of various forms of skin cancer in fair-skinned individuals resident in areas of high solar intensity, such as the southern United States, South Africa, and Australia.

Experimentally, the carcinogenic effects of actinic UV non-ionizing radiation can be duplicated and studied using albino hairless mice (36). Homer Black and his associates have demonstrated that skin exposure in such animals to high-intensity UV results in the formation of the carcinogenic sterol cholesterol-5α,6α-epoxide and in skin cancer and that the process can be suppressed by feeding the animals a number of antioxidants, including ascorbic acid (32–36, 65, 199).

Other Relevant Effects of Ascorbic Acid

Ascorbate and Energy Production. Cytochromes P-450 and b_5 are decreased by ascorbate deprivation in the 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 cancer process (334).

Viral transformation of cell cultures decreases oxygen consumption and increases lactate production (29, 30). An increased rate of gluconeogenesis has been demonstrated in a variety of animal tumors (297) and has been linked to the progressive weight loss syndrome of cancer patients (157), the belief being that actively fermenting neoplastic cells meet their increased glucose requirements at the expense of the host.

All glycolytic enzymes are increased in amount during carcinogenesis (37, 346). Glucokinase is increased to 570% of its control value in the liver of animals exposed to the carcinogen 3′-methyl-4-dimethylaminoazobenzene (12).

These alterations in cellular pathways can to a certain extent be corrected by ascorbate. Takeda and Hara (318) reported decreased oxidation of citrate and lowered activity of aconitase in scorbutic guinea pigs, indicating an impaired tricarboxylic acid cycle. Addition of ascorbate to cultures of embryonic bone tissue (266) or cultures of polymorphonuclear leukocytes (113) results in increased oxygen consumption and a decrease in lactate production. Studies by Benade *et al.* (25, 26) demonstrated 72% inhibition of anaerobic glycolysis in Ehrlich ascites tumor cells by ascorbate; 96% inhibition was achieved when 3-amino-1,2,4-triazole was added with ascorbate, and these additions proved to be highly toxic to the tumor cells. The cytotoxicity was attributed primarily to intracellular H_2O_2 production and the synergistic effect of the aminotriazole to its inhibition of catalase (25).

Ascorbate as an Antioxidant. The antioxidant properties of ascorbic acid have been known since its discovery (151, 308, 328), and because other antioxidants appear to possess some anticancer activity ascorbate might also function in this respect. Thus, Wattenberg has demonstrated the inhibition of tumorigenesis by a number of known carcinogens, such as 7,12-dimethylbenz(*a*)anthracene, benzo(*a*)pyrene, urethan, uracil mustard, dimethylhydrazine, 4-nitroquinoline *N*-oxide, and diethylnitrosamine, by several antioxidants, including butylated hydroxytoluene, butylated hydroxyanisole, ethoxyquin, and some sulfur-containing reactive compounds (337–345). Slaga and Bracken (304) reported that ascorbic acid as well as other antioxidants prevented the initiation of skin tumors following the application of 7,12-dimethylbenz(*a*)anthracene.

As mentioned above, Black has described cholesterol-α-oxide, a precarcinogen formed in skin during exposure to high-intensity UV, and has prevented its formation in hairless mice by addition of ascorbate, α-tocopherol, glutathione, and butylated hydroxytoluene to the diet. Antioxidants may exert their effects by protecting against carcinogen-induced chromosomal breakage (294), by reducing carcinogen-induced peroxidation (293), by altering liver microsomal metabolism (306), or by a combination of these and other actions, as reviewed by Passwater (250) and by Wattenberg (344).

Dehydroascorbic Acid. Oxidation products of ascorbic acid have antitumor activity *in vivo*. Dehydroascorbic acid (150 mg/kg body weight) and 2,3-diketogulonic acid (115 mg/kg body weight) inhibited growth of solid Sarcoma 180 in mice by 88 and 54%, respectively (355). In the same experiments, the antitumor activity of ascorbic acid (46% inhibition) was enhanced by the addition of a compound of copper (to 69% inhibition), indicating that the active agent is an oxidation product. The antitumor activity of dehydroascorbic acid, and to a lesser extent of other metabolites of ascorbic acid, erythorbic acid and dehydroerythorbic acid, was recently confirmed using solid Sarcoma 180 (245, 246). Another metabolite, 5-methyl-3,4-dihydroxytetrone, inhibits growth of solid Sarcoma 180 by 50% (244, 322). The activity of these agents is thought to be mediated by interaction with DNA and decomposition of apurinic acid to deoxycytidylic acid, as well as decomposition of the oligo form of pyrimidine nucleotides, has been demonstrated (235, 244, 247, 355). It has also been suggested that dehydroascorbic acid functions as an electron acceptor in the regulation of mitosis (111, 112).

Ascorbate and Cyclic Nucleotides. Many biological activities are potentiated by hormonal actions that utilize cyclic 3′:5′-AMP and cyclic 3′:5′-GMP as "second messengers." Lewin (195) has reviewed extensive evidence showing that ascorbate potentiates the formation of cyclic 3′:5′-AMP and is concerned in the inhibition of processes that reduce the concentration of both cyclic 3′:5′-AMP and cyclic 3′:5′-GMP by hydrolyzing them to 5′-AMP and 5′-GMP, respectively. The role of these cyclic nucleotides in cancer is uncertain, but diminished adenyl cyclase activity has been noted in polyoma virus-transformed cells (45), and cyclic 3′:5′-AMP has been shown to inhibit cell multiplication *in vitro* (195) and tumor growth *in vivo* (130).

Ascorbate and Erythropoieses. Anemia is a common feature of cancer. Ascorbate is known to promote the absorption and utilization of ingested iron and is necessary for a full erythroblastic response. Thus the anemia of cancer would be helped by increasing ascorbate intake.

Ascorbate and Oxidation-Reduction Potential. The ascorbic acid-dehydroascorbic acid system is believed to play an important part in maintaining optimum oxidation-reduction conditions in the tissues. The oxidation-reduction potential, like acid-base balance and pH, must be balanced within fairly narrow limits for normal health. Any disturbance of oxidation-reduction potential, such as would result from depletion of ascorbate reserves in cancer, could have deleterious systemic effects (3).

***In Vitro* Studies against Cancer Cells.** Because ascorbic acid is nontoxic to normal tissues, few have investigated whether it is equally nontoxic to neoplastic tissues, and what evidence there is tends to be contradictory. Thus, Vogelaar and Erlichman (329) reported that ascorbate enhanced the growth of mouse Sarcoma 180 cells *in vitro*, while Park *et al.* (249) demonstrated stimulation of a mouse plasmacytoma cell line. On the other hand, ascorbate at fairly high concentration is cytotoxic to Ehrlich ascites carcinoma cells (25, 26), and at a much lower "physiological" concentration to 3T3 cells in tissue culture (85). It has been reported that ascorbic acid increases glucose metabolism in neoplastic cells but not in normal cells in identical *in vitro* conditions (257a).

Studies of Experimental Tumors in Laboratory Animals. Omura and his associates in Japan (232, 245, 246, 322, 354, 355) have reported that ascorbic acid and its metabolites

exhibit significant inhibitory effects against the "take" and growth of Sarcoma 180 in mice. Other experiments with mouse and rat tumors have yielded equivocal results (41, 305, 336, 349, 351). Even with the experimental animal of choice, the guinea pig, the reported effects are contradictory. Thus, Russell *et al.* (274) reported that ascorbate deprivation increased guinea pig susceptibility to methylcholanthrene carcinogenesis and promoted tumor growth and spread, whereas Migliozzi (216) found that ascorbate deprivation retarded tumor growth and ascorbate supplementation enhanced it. It seems to us important that these guinea pig experiments be repeated using different levels of ascorbate intake; it is possible that the contradictory results may arise from different points on a dose-response curve.

Epidemiological Studies in Human Cancer. A number of epidemiological studies have demonstrated some relationship between patterns of dietary ascorbate intake in large population groups and their incidence of cancer of various types and cancer in general (27, 31, 70, 86, 107, 143, 144, 149, 154, 158, 180, 353). We propose to review these and similar studies elsewhere.

Clinical Trials in the Management of Human Cancer. Schlegel's use of ascorbate to retard human bladder cancer has already been mentioned (259, 284–287). DeCosse and his associates in Wisconsin (95) reported that ascorbate p.o. induced some regression in familial colorectal polyposis, a well-recognized premalignant condition (42, 105, 200, 209), and they recommend its use as a prophylactic measure. This advice gains support from the demonstration that, in the same clinical condition, ascorbate p.o. reduces the amount of mutagenic (and presumed carcinogenic) fecal steroids (188).

These, however, are specific neoplastic disorders. The scope of this review suggests that supplemental ascorbate may exert a general anticancer effect.

As stressed in the introduction, no properly designed prospective clinical trial has as yet been carried out to assess the value of supplemental ascorbate in general cancer management. However, for those interested in designing such a trial, an encouraging background of publications exists, ranging from individual case reports, through anecdotal accounts, to pilot studies involving large numbers of advanced cancer patients (64, 68, 100, 114, 129, 146, 159, 164, 177, 184, 214, 248, 261, 283, 287, 288, 289, 320, 327, 330, 331, 332), all reporting some degree of clinical benefit conferred by supplemental ascorbate. Our own clinical studies, discussed in several publications (51–53, 56–59, 226), strongly indicate that supplemental ascorbate not only increases well-being but also produces a statistically significant increase in the survival times of advanced cancer patients. Present evidence suggests to us that supplemental ascorbate can offer some degree of benefit to all advanced cancer patients and quite remarkable benefit to a fortunate few and that it has even greater potential value in the supportive treatment of earlier and more favorable patients.

Conclusion

There is evidence from both human and experimental animal studies that the development and progress of cancer evokes an increased requirement for ascorbic acid. 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 his 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 may also offer some protection against a variety of chemical and physical carcinogens and against oncogenic viruses and is also involved in a number of other biological processes believed to be involved in resistance to cancer.

The collective evidence suggests that increasing ascorbate intake could produce measurable benefits in both the prevention and the treatment of cancer, and pilot clinical studies tend to support this view. Ascorbic acid has a unique advantage relative to other remedies for cancer; it is almost completely safe and harmless even when given in sustained high doses for prolonged periods of time. The risks associated with such regimens in cancer have been discussed elsewhere and are thought to be acceptable (52, 54, 55, 252, 254); these are (*a*) a clinical suspicion that, in the very rare patient with a very rapidly growing tumor existing at the very limits of nutritional support through sinusoids and enzyme-assisted diffusion, the sudden exposure to large doses of ascorbate may precipitate widespread tumor hemorrhage and necrosis with real danger to the patient, (*b*) a much stronger suspicion that the sudden discontinuation of such an established regimen produces a rebound effect of a precipitous drop in tissue ascorbate with brisk reactivation of the hitherto controlled neoplastic process, and (*c*) the theoretical but extremely remote risk that a susceptible few of such patients might develop an oxalate urinary tract stone. Earlier concern that high ascorbate intakes might induce vitamin B_{12} deficiency has been shown to be fallacious, and the result of ascorbate interfering with the laboratory assay (Ref. 254, p. 114; Ref. 133). The risks, real or theoretical, are minimal and acceptable to the cancer patient and to any experienced oncologist.

Bearing this in mind, Anderson (3) has recently stated "The risk/benefit ratio relative to the severity of the disease as well as to other available treatments in cancer is so heavily weighted in favor of vitamin C in this situation that validation or refutation by other groups will presumably occur quite quickly."

We agree and believe it to be essential that extensive studies of ascorbic acid in cancer be made without delay.

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