Carcinogenesis is the development of cancer through a multi-step process that is thought to start with neoplasia and progress through many stages to a metastatic cancer. These stages include initiation, promotion, progression, and conversion. Initially, some carcinogenic agent appears to bind DNA, RNA, or proteins resulting in a DNA adduct. If this adduct is not repaired during the replication process then it leads to DNA damage and all future cells arising from replication of this damaged cell will also have this DNA damage present. It is thought that 5-6 of these genetic alterations must occur in order for cancer to develop from these cells. During the promotional phase, a specific long-term environment must be present that will allow for the monoclonal expansion of the cell. This promotion can be in the form of hormones, chemicals, or other substances that promote growth and replication.

Progression is the next event that occurs, but this process may take many years and only a small percentage of the promoted cells will survive into the progression phase. During progression, further genetic changes are happening that lead to a new phenotype. Eventually, a small number of the cells that were able to survive progression will undergo a malignant conversion into what is referred to as cancer.

There are several types of "agents" that are thought capable of causing the initiation event in carcinogenesis. These are known as carcinogenic agents. The carcinogenesis area focus on viruses, chemicals, and hormones as the primary carcinogenic agents. Also within this area is
some information regarding genetic alterations that have known correlation with specific cancer types.

ALOE VERA

There can be few plants whose reputed medicinal properties have aroused so much controversy as those of Aloe vera. Aloe vera has a history of use in folk medicine for skin and other disorders which dates back thousands of years (Crosswhite and Crosswhite, 1984). Today the processing of A. Vera gelm derived from the leaf pulp of the plant, for medicinal and cosmetic use, has become a big industry in the United States, one of the largest based on botanicals. Yet the scientific literature on A. Vera is very confused, with a number of contradictory reports and inconclusive experiments. There are also several properly conducted studies, but these do not always receive the recognition they deserve. While some writers disclaim any of the supposed physiological effect of A. vera gel, others fully endorse them. There is, however, considerable, belief in the beneficial action of the gel among the general public, particularly in the USA, and A.vera is one of the few botanical medications with widespread domestic use in Western Society.

Associated with the promotional activities of the manufacturers of A.vera gel products there has grown up a plethora of legends about its history and properties, for example that it was the secret of Cleopatra's beauty, or even that it is some kind of miraculous gift from the gods, capable of curing virtually any illness (Gjerstad and Riner, 1968). The exaggerated claims made in the past by some companies for amazing, but unsubstantiated cures in a whole variety of conditions have only helped to confuse the issue and have given the whole subject a less than respectable air.

THE USE OF ALOES IN FOLK MEDICINE.

Numerous Aloe species have been used medicinally, but few on a wide spread basis. Many folk uses of aloes in Southern Africa are reported by Watt and Breyer – Brandwijk (1962). Only a few species have had any commercial importance, the main species used being Aloe
ferox Miler (Cape Aloes) Aloe perryi Baker (Socotrine Aloes) and Aloe vera (Barbados or Curacao Aloes). These species are harvested for their bitter leaf exudates or "latex", important in trade as the source of drug aloes or "aloin", used for its purgative effects. Drug aloes is less commonly employed now because it tends to cause griping, and has an effect persisting over several days. Aloe exudates contain a number of pharmaceutically active phenolic compounds, including anthraquinones and their glycosides.

While A.vera has long been grown to supply drug aloes, it has over the last fifty or so years become more widely known for its gel. The plant was widely used by the Egyptians, Assyrians, and Mediterranean civilizations, and in Biblical times. The dried leaf exudate was the main medicinal product, though it seems that the gel was also used. Dioscorides used Aloe as a purge, and to treat wounds, mouth infections, to soothe itching and to cure sores (Gunther, 1934).

Aloe vera still has an important role in the traditional medicine of many contemporary cultures. In India, A vera medications are used for a variety of conditions, particularly for their cathartic, stomachic, emmenogogic and anthelminthic properties (Chopra et al., 1956). Whole leaves, the exudates, and the fresh gel are all used. In China A.vera has been an important medicine for centuries, and it is still a common household remedy (Cole and Chen,1943). In Mexico, the leaves are gathered from plants and growing semi-wild to treat burns, bruises, skin irritations, and even leprosy. A.vera is also widely used as a folk remedy across the rest of Middle America and the West Indies, as reviewed by Morton (1981). In all these countries A.vera is an introduced species, but has been rapidly adopted as an essential part of local material medica.

In present day Western Society, most notably in the USA, A.vera has fairly frequent use in homeopathy and herbalism (Panos and Heimlich, 1980). It is commonly grown in America and the tropics as a pot plant on kitchen windowsills, so the leaves are on hand to treat burns, to soothe the pain and promote healing. The plant has additional use to treat sun burn and various dermatological conditions and taken internally, as a general tonic. Madis Laboratories (1984) list over a
hundred medical disorders that have at some time been treated, such as arthritis, gout, ache, cuts, dermatitis, headache, high blood pressure, indigestion, hair loss, rheumatism, peptic ulcers, mouth diseases, pruritis, psoriasis and, of course, burns. The medicinal use of A. vera is particularly widespread in Florida (Galban, 1952). Some of the more unusual applications include bee stings. A. vera has also had considerable use as a folk remedy for farm animals, as described by Anderson (1983).

There can be no doubt that there is widespread belief in A. vera among the general public, particularly in the USA. According to Anderson (1983) "Judging from the purported sky rocketing sales of aloe vera cosmetics, many people truly believe that they have found the fountain of youth in the aloe vera plant". It does seem to be thought by some that A. vera is some kind of "miracle plant" or "wonder drug". The symbolic association of A. vera with embalming, enduring life and immortality and the boundary between life and death as described by Crosswhite and Crosswhite (1984) has found its way into modern advertising and beliefs about the plant. As Norris (1973) wrote: "If a plant is able to heal its own wounds, to survive without nourishment, even seemingly to return from the dead, might not its power somehow be applicable to man's own maladies?".

BOTANY OF ALOE VERA

Aloes are members of the Liliaceae and are mostly succulents with a whorl of elongated, pointed leaves. Reynolds (1966) described 314 species in his classic monographs; there are now over 360 accepted species. Some species are tree-like with long stems, while others are small, with their leaves at ground level. They occur over most of Africa, Southern Arabia and Madagascar, but not in rain forest regions or dry deserts. A few species have been carried in cultivation around the Mediterranean, and from there have reached as far as Japan in the east and America in the West.
CLINICAL USE OF ALOE VERA GEL IN THE 1930S

Modern clinical use of the gel began in the 1930s, with reports of successful treatment of X-ray and radium burns, which led to further experimental studies using laboratory animals in the following decades. The reports of these experiments and the numerous favorable case histories did not give conclusive evidence, since although positive results were usually described, much of the work suffered from poor experimental design and insufficiently large test samples. In addition some conflicting or inconsistent results were obtained. With the recent resurgence of interest in Aloe vera gel, however, new experimental work has indicated the possibility of distinct physiological effects.

Research into the medicinal effects of A. vera gel began in the 1930s, before then a considerable amount of work had been done on the purgative properties of Aloe exudates, but the gel was virtually ignored. Scientific interest was aroused in 1935 by the paper of Collins and Collins, "Roentgen dermatitis treated with whole leaf of Aloe vera". According to Cutak (1937) Collins and Collins tried A. vera for radiation burns because it was used for severe sunburn in Florida, while Goldberg (1944) describes how they had seen Seminole Indians using the leaves to treat burns.

When X-rays (or "roentgen rays") began to be used therapeutically for cancer, eczema and other related skin conditions, and as depilatory treatment, it was found that accidental overexposure could cause radiation burns or "roentgen dermatitis". The medical workers giving the radiation therapy were particularly at risk, and the principal treatment was usually surgery (Wright, 1936).

Crewe (1937) reported that he had used the pulp of fresh A. vera leaves to treat eczema with some success, and he also obtained promising results treating ulcers on amputation stumps. There had been a cessation of pain, and healing was progressing well before he ran out of leaves. Subsequently he tried using commercial powdered Socotrine aloes in a lanolin base to treat ulcer, pruritis, breast cancer lesions, poison ivy rashes, and burns, and reported variable but generally
encouraging results. Powdered Socotrine aloes is the dried leaf exudates of Aloe perryi, though Crewe thought it was made from Aloe gel, Crewe reported some adverse reactions, as might be expected, including catharsis and skin allergies. However, he concluded that both the fresh leaves and his aloes ointment and powder would relieve pain, had some sort of antiseptic action, and would stimulate granulation and growth of new tissue.

The use of A. vera gel to treat five cases of radiation ulcers, including one of ulceration of mucous membranes of the mouth, was reported by Mandeville (1939).

CLINICAL AND EXPERIMENTAL STUDIES FROM 1940

In Rowe et al. (1941), different parts of the A. vera leaf were tested in a larger-scale experiment using a total of 44 rats. Healing of the radiation burns was apparently estimated by visual examination of the lesions. They found that 64% of the rats treated with gel showed an increased rate of healing, 9.5 times the number in the control group. They also observed that if beneficial results did not occur within 2 weeks of starting application of the gel, then further treatment was not likely to be of benefit. Partially decomposed leaf pulp gave improvements in 87.5% of the rats tested (though only 8 rats were used); as the writers pointed out, this was contrary to Mackee (1938), who was of the opinion that the gel had to be fresh.

Most interestingly, Rowe et al. (1941) found that fresh rind from one of their shipments gave 100% complete healing in 8 rats within 6 days, although the rind form two other shipments gave negative results. Having tried some other commonly used treatments, including an ointment of powdered Curacao aloes as used by Crewe (1937) they reported that “results obtained with aloe ointment and urea ointment show that none of these are effective in promoting healing of acute third-degree X-ray reactions in the skin of white rats”.

Meanwhile, A. vera gel seems to have been widely accepted in the USA in the 1940s for the treatment of radiation ulcers. Demand for the leaves was still high. Lushbaugh and Hale (1953), working for the U.S.
Atomic Energy Commission at the Los Alamos Research Laboratory, produced one of the most convincing studies of the effects of A. vera ointment, application of a dry gauze bandage, and an uncovered, untreated control. Treatment was started immediately after irradiation, and the healing of the lesions followed over a period of 58 days by visual assessment. Histological examinations were carried out on a further 10 rabbits.

Aleshkina and Rostotskill (1957) reported on the use of an Aloe extract to heal lesions caused by radiotherapy treatment for cancer. They used an emulsion from "biostimulated" leaves of an unnamed Aloe species (probably A. arborescens), containing castor oil, eucalyptus oil and an emulsifier. An experiment was described using 12 rabbits exposed to radiation. Six rabbits treated with the emulsion healed in 12 days, while the controls took 20 days. In a second experiment the Aloe emulsion was compared to the emulsion without the Aloe extract as a control. The six rabbits treated with the Aloe emulsion healed in 8 days, with smooth pink skin and some hair regrowth, while the six controls with the emulsion base took 12 days.

Rovatti and Brennan (1959) used albino rabbits to study histological changes in thermal burns over the complete time scale of the healing process. Biopsy samples were taken during the initial stages over the first 48 h, and over a longer period of 5 weeks after application of the burns. Six animals were used for the initial controls to study changes in untreated burns, while 4 batches of 3 animals were used to investigate the effects of various medications. In the latter animals one side was left untreated as a further control. The treatments used were "Aloe Crème Ointment", "Alo-Crème Ointment" with 5% cystine, 1% trinitrophenol butylaminobenzoate ointment and petrolatum with a gauze dressing. Application began immediately after burning, and continued twice a day over the 5-week period of the study.

The two A. veraointments gave similar results, the lesions being more pliable and less inflamed than the untreated controls, with a sloughing of the surface layers. Healing occurred in 2 weeks, without the scaring observed in the control burns, which took 4 weeks to heal. The
animals treated with the trinitrophenol ointment died through hemorrhage, while those treated with the petrolatum and gauze showed swelling, hemorrhaging and the development of abscesses, and healing only occurred after 4 weeks with scarring. The biopsy samples from the A. vera treated groups showed reduced necrosis of the dermis and decreased thrombosis of capillaries compared to the treated burns.

Goff and Levenstein (1964) investigated the effect of various medications on the healing of skin wounds in mice. They used a tensiometric method to measure wound healing by the force needed to separate the edges of a standardized skin incision. This was an improvement over earlier studies which had relied on visual assessment of the wounds to gauge healing. Samples of 6-10 mice were used for each determination to allow some measure of variation, and measurements of wound strength taken at intervals between 6 and 21 days after the wounds were made. The treatments included an ointment containing vitamins a and D, an A. Vera ointment, a Corticosteroid, Artemisia extract and an allantoin-coal tar ointment. There were also untreated controls.

Results were expressed as graphs of tensile strength against time, with an increase in wound strength as healing progressed. They showed that there was "some transitory degree of stimulation of healing by Aloe vera", while the corticosteroid in fact delayed healing. The A. vera preparation gave significantly large tensile strengths at 9 and 15 days, but not at 21 days, when both the control and A. vera curves were leveling off as the wounds healed over. The description of this as a "transitory degree of stimulation of healing' was perhaps an unfortunate choice of words, since once the wounds had healed, one would not expect a difference between the A. vera treatment and the control. What was significant was that A. vera had hastened the healing process. The authors did not say which part of the A. vera leaf was used for their extract, though presumably it was the get.
RECENT MEDICAL, DENTAL AND VETERINARY STUDIES

The use of A. vera gel taken internally to treat peptic ulcers was reported by Blitz et al. (1963). Twelve patients diagnosed as suffering from peptic ulcers, confirmed by X-ray evidence of duodenal lesions, were given an emulsion of A. vera in petrolatum. Complete cures were claimed, even after a period of a year, and they wrote "Usually, such unmistakable lesions are accompanied by exacerbations of distress once and more often twice a year under any form of medical treatment, but no such episodes were experienced in this series of cases". They also reported that X-ray examination showed complete healing. The effect of the A. vera gel was attributed by the writers to cooperation of pepsin, inhibition of hydrochloric acid secretion and a general detoxifying effect. This work has not been followed up by a full clinical trial to the present knowledge of these authors, but Blitz et al. (1963) pointed out that if A. vera was not pharmacologically active "as the indictment of western medicine has intimated" then the observed 100% complete recovery would not be expected, nor would the observed 100% complete recovery would not be expected, nor would the cessation of pain at mealtimes which accompanied the A. vera treatment.

Aloe vera gel has also been used to treat a variety of dental conditions. Bovik (1966), himself a qualified dentist, reported his own personal experiences after he had a complete upper gingivectomy performed. As an experiment he treated one side with A. vera "juice" and found it caused rapid healing and a cessation of pain; in fact he found the Aloe vera treatment preferable to the conventional periodontal pack applied to the other side.

Payne (1970) reported experiments where A. vera gel was used in five patients to reduce pain and accelerate healing after periodontal flap surgery. He treated some quadrants with A. vera so the patients did not know which part had the gel applied, and found that although there was some variability, the patients reported less pain and swelling from the A. vera quadrants. Also in 4 out of the 5 cases the A. vera quadrant was chosen by an "unbiased observer" as being least inflamed after one week's treatment.
A relatively modern description of the clinical use of A. vera is given by El Zawahry et al. (1973) who used A. vera gel on chronic leg ulcers. Three case histories were described, with apparently successful results. They were careful to drain the exudates before extracting the gel from the leaves. The fresh gel was then applied to the ulcers on gauze bandages 3-5 times a day. While there were no controls used, the writers pointed out that there would be inherent variations in healing rate between patients, and suggested it would be useful to carry out trials on patients with bilateral ulcers. In the cases described the ulcers were of long standing, of from 5 to 15 years duration, so the observed improvements would seem to be due to the A. vera treatment. El Zawahry and his colleagues believed that the effects of the A. vera gel were due to increased vascularisation, which was thought to be the cause of a temporary increase in pain observed when treatment was first started. Encouraging results were also reported in treating hair loss due to seborrhea, as well as good control of acne vulgaris in three women patients. However, it must be stressed that these were case reports, and in no way represented conclusive clinical trials.

Ship (1977) writing in reply to a question sent into the Journal of the American Medical Association, was skeptical about the effects of A. vera gel. He suggested that the results obtained in the treatment of burns were due to the "oil content" preventing the wounds drying out and so reducing pain, in a similar way to the application of butter and other home remedies containing oils. Although A. vera was being used in some hospitals to keep burns soft and pliable, ship reported that "patients were more satisfied with a good cream lotion than with aloe vera".

The use of A. vera gel also been described in veterinary medicines. Northway (1975) used a commercial extract to treat a number of external conditions in a total of 76 animals in his practice. He reported "good" (equal to the best of the other drugs on the market) or "excellent" (better than other available drugs) results in ringworm, allergies, abscesses, fungal infections and various types of inflammation. Pain and itching seemed to be relieved very rapidly after application.
Northway wrote: "Although no firm conclusion can be drawn from treatment of 76 patients and a study involving no controls, my observation is that response of fungal infections and local allergic reactions to aloe vera therapy is excellent and that good response is achieved in treatment of mixed bacterial infections caused by susceptible organisms".

Anderson (1983) reviewed the use of A. vera "juice" in veterinary treatment, acknowledging the number of exaggerated, word of mouth claims made for miraculous cures. He felt there was a need for convincing experiments "to satisfy the most discerning observer" but remarked that "If aloe vera juice has even a fraction of the claimed benefits, veterinary professionals need to know about it"

In the last 5 years new work has been done by a team of workers in the USA studying the effects of A. vera in topical applications. Cera et al. (1980) reported in detail two cases where a commercial A. vera cream ("Dermaide Aloe") was successfully used to treat severe accidental thermal burns in dogs. Two of the writers were professors of plastic surgery with particular interest in burns. In a previous paper (Robson et al., 1979) the A. vera product used had been shown to have antibacterial and antiprostaglandin effects, and preserved the vascular supply to the dermis in experimentally burned animals. According to Cera et al. (1980), in dogs the progress is generally poor if more than around 15% of the body surface is badly burned, while euthanasia is recommended if more than 50% is burned.

While the A. vera treatment was being carried out on the dogs, biopsy samples were taken to test for Pseudomonas infection and to determine prostaglandins and thromboxanes by an immuno-histological technique. In the first dog re-epithelisation and superficial healing was complete by 7 days, and hair was beginning to grow by 17 days. In the second dog re-epithelisation had occurred after 17 h and the wound had healed without scarring 10 days after burning, apparently because treatment had been carried out sooner after the burn had occurred. The biopsies before and after treatment showed that the A. vera product had
an apparent antiprostaglandin effect, which prevented dermal ischaemia. Infection by Pseudomonas aeruginosa was also inhibited.

Raine et al. (1980) reported experiments on the treatment of frostbite in the ears of experimental rabbits using antiprostaglandins and antithrom boxanes. Four treatments were used, including an A. vera cream, methylprednisolone, methimazole, and acetylsalicylic acid, as well as a control. Four animals were used for each treatment. All the treatments showed statistically better tissue survival than the control. It was thought that tissue loss was reduced by counteracting the effects of thromboxanes and prostaglandins, which are vasoconstrictors, tissue loss in frostbite being at least partly due to vascular deprivation.

Following up their study in dogs, Cera et al. (1982) reported a course of treatment on a Rhesus monkey which had been brought to them with full-thickness burns over 70% of its body after accidental scalding. The treatment included sedation and an intravenous drip, and topical application of A. vera cream. By 7 days re-epithelisation was extensive, and recovery was complete within 30 days. A number of detailed photographs were included showing the improvement over the period of treatment.

ALOIN

Aloe has been used as a folk remedy to strengthen the stomach and to relieve constipation for 3000 years (John et al. 1990). Today it is widely used as a general health food for some of its acclaimed effects. Aloe is known to suppress stomach acid secretion (Hirata et al. 1977), to cure frostbite, to have an anti-inflammatory action, to cure radiation burns (Lushbaugh et al. 1953) to improve blood sugar levels, to have an antiviral action, and to modulate the immune response (Hart et al. 1990). About 600 species of the aloe genus are known, but Aloe arborescens Miller, A. ferox M., A barpadensis M., A africana M. and A. saponaria Hawavi have been most extensively investigated for their biological activities (Ship 1977). Among these pharmacological effects, the antimeoplastic effect of aloe is of interest since a number of polysaccharides have been studied for antitum origeniaty (Koboyaski et
Thus, aloe is worthy of investigation as a chemopreventive agent. The purgative principle of the present commonly available aloes of commerce, principally cope aloe, has long been recognized as an anthracene glycoside (Fairbairn 1959) and shown to be a C-clygoside of aloe emodin anthrone (Birth et al. 1955). The compound was known as barbaloin and finally characterized as 10 b-D-glucopyromosyl-1, 8-dihydroxy-3-hydroxymethyl-9(10H)-anthracenone. The name aloin a bitter tasting yellow crystal was given to a crude material, amorphous or crystalline according to the degree of purification, from which barbalain could be isolated (Lister et al. 1959).

Possible degradation pathway of aloin

Aloin exist in two diastereomic forms (Wagner et al. 1976) due to opposing configurations of the glucose moiety linked to carbon 10 of the aglycone was recently confirmed by Auter Haff et al. genuine aloin extracted from plant material on chemically synthesized is separated by high performance liquid chromatography on reverse phase columns into aloin A and B which show differences in optical rotation and circular dichroism. The respective diastereomers are inter convertible which might explain their common anthramol form. Aloin is readily oxidized in aqueous solutions.
OCCURRENCE OF ALOIN

The commonest C-glycosylated anthracene derivative is aloin. It is a major constituent of many bitter aloes and represents the active principle of these cathartic drugs (Wade et al. 1977). It has been detected in the leaf juice of more than twenty aloe species (Liliaceae) (McCarthy et al. 1969). Of the more than one hundred species examined, about one-half contain aloin and homonataloin, roughly in equal proportions – outside the Liliaceae, aloin has been identified in only one family, Rhamnaceae (Phamnus purshiana = Cascara sagrada) where it occurs together with cascarosides A, B, C and D and 11-desocylatoin (Baumgartner et al. 1961).

BIOSYNTHESIS OF ALOIN

Recently Grun and Franz studied the biosynthesis of aloin in aloe arbarescens (Liliaceae). Feeding experiments with 14 CO2 and quantitative determinations of both diasteriomeric aloins in leaves of aloe arbarescens at different stages of development demonstrated that only one isomer aloin B, is preferentially synthesized by the plant. The occurrence of the respective isomer, alone A is explained by partial conversion of aloin B (Grum et al. 1982). It was shown that the aloin B/aloin A equilibrium is reached non-enzymatically under physiological conditions.

Biogenesis of aliens in aloe arbarescens seems to be influenced by environmental conditions (Gum et al. 1981). An earlier study on variations in the content of aloin throughout a vegetation period in several aloe species gave similar results. McCarth et al. found that the content of aloin in aloe leaf juices varied considerably with a maximum production during the summer period suggesting that aloin might be metabolized. Feeding experiments with 14C-labeled compounds proved that leaf ---- of aloe arbarescens preferentially is corporate [2-14C] actalo into the aglycone moiety of aloins while [U-14C glucose was incorporated into both the aglycone and sugar moiety (Gum et al. 1982). Further studies with cell free extracts demonstrated that crude enzyme extracts from aloe arbarescens were able to catalyze transglycosylation of [U-

**FLAVONOIDS**

Flavonoids are polyphenolic secondary plant metabolites which commonly exists as multiple O – and C – glycosidic derivatives (Harborne et al 1988 & 1993), but also may be present as aglycones (Wollenweber et al 1981). They are an important part of the human diet (Kuhnau et al 1976 & Hertog et al 1992) – consider the recent discussions on the connection between red wine consumption and reduced risk of heart diseases, the so called “French paradox” or the studies on the proanthocyanidin ingredients in green tea (Imai et al 1995 & Yen et al 1996). They are also considered the active principle in a number of medicinal plants. Owing to these potential benefits to human health, polyphenolic plant metabolites are the major class of the recently popular phytochemicals (Hong et al 1994). Early in the beginning of the research into the structures and functions of flavonoids, their antioxicative capacities, particularly with respect to stabilizing foodstuffs, was recognized (Richardson et al 1947 & Simpson et al 1956). Up to now, this has remained the most important topic of investigation, despite the fact that various other functions have been attributed to them over the years. For instance, they are mutagenic yet they are also anticarcinogens; they exhibit biocidal effects and have antifertility properties, yet express beneficial effects in inflammatory and immunomodulatory systems and interact with singal transduction processes (Table 1).

**Table 1. Flavonoid Functions**

<table>
<thead>
<tr>
<th>Position</th>
<th>In vitro</th>
<th>In plants</th>
<th>In animals</th>
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<tr>
<td>Antioxidant</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Cytotoxic (biocidal)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mutagen</td>
<td>+</td>
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Flavonoids, as ubiquitous plant metabolites, are exclusively catabolized in animal and human organs. While the biochemical and biological functions can, in most cases, be investigated in cell culture systems, any in vivo function of these compounds hinges on their metabolism in the digestive system (Formica et al 1995).

It is so far unknown to what extent an oxidative metabolism, as modeled with peroxidase (Schreier et al 1985 & Oszmianski et al 1990), occurs in vivo. A recently described enzymatic activity, causing sulfation of quercetin and catechin in rat liver (Shali et al 1991), probably effected by sulfotransferases reactive with xenobiotics, awaits further elucidation. It is unclear whether it activates these compounds or facilitates excretion. Gastroprotective/antiulcerogenic effects of flavonoids have been observed after oral (Attaguile et al 1995) or intraperitoneal delivery. The mechanism is still unknown, with the damage to the gastric mucosa being a multicomponent phenomenon, on the one hand, and there being a lack of structure – activating factor (Izzo et al 1994) and of nonprotein sulfhydryl groups (La Casa et al 1995) have been proposed.
unspecifically with proteins (McManus et al 1981 & Hagerman et al 1981)—e.g., the astringency of some flavonoids is explained by this mechanism (Butler et al 1993) — but more specific effects, such as intracellular interaction with calmodulin (Nishino et al 1984), are also known.

EFFECT ON RECEPTOR FUNCTIONS

Receptors as membrane-bound or membrane-penetrating proteins have the important functions of transferring chemical signals between two different organelles. As such they are an important part of the signaling process. Both inhibition (Shisheva et al 1992 & Ramassamy et al 1992) and stimulation (Morita et al 1990) of receptor functions have been observed. The most detailed studies involved ligand affinity with the benzodiazepine (Haberlein et al 1994) and the adenosine (Ji XD et al 1996) receptors of the central nervous system. The structure-activity relationship (SAR) of both studies demonstrated that hydroxyl groups are not essential and methoxylation as opposed to acetylation enhances affinity: criteria quite in contrast to those for antioxidative or radical-scavenging functions.

EFFECT ON ENZYME ACTIVITIES

Most of these studies can be or were undertaken under in vitro conditions, for example, with pure enzyme preparations. Since it can be expected that many of the in vivo effects observed for flavonoids are related to specific enzyme interactions, it is unfortunate that in this research area there are the most glaring deficiencies in the study of flavonoids: less than 20 percent of the studies pertain to SAR comparisons, and most of them are limited to one isoenzyme.

INHIBITION OF ENZYME ACTIVITIES

Lacking a detailed description of the enzymes involved, mitochondrial uncoupling may describe a rather general inhibitory effect on the metabolic pathways in this organelle (Ravanel et al 1982 & Hodnick et al 1988). In a review, Middleton and Kandaswami (Middleton
et al 1986) have specified the crucial metabolic functions of a number of enzymes inhibited by flavonoids. A complete list of the affected enzymes appeared in 1992 (Bors et al 1992) and was later expanded considerably (Bors et al 1986). Studies with SAR or mechanistic implications were preferentially cited, yet, as noted before, most reports are confined to only one enzyme and/or one individual flavonoid. When only single flavonoids were examined, the favored compounds were quercetin, genistein and catechin.

A mechanism common to the most active flavonoids may involve the coplanarity of all three rings—which evidently would not include the various effects reported for the flavanols i.e., catechin and its derivatives—or they may have structures similar to the actual enzyme substrate or cosubstrates. The latter effect seems to be the case for purine-dependent proteases (Ferrell et al 1979). A good example for structural elucidation of inhibiting substrate analogs has been given for the flavonoid-related aurones in the idothyronine deiodinase reaction (Aufmkolk et al 1986), which is also inhibited by flavonoids themselves (Spanka et al 1988).

STIMULATION OF ENZYME ACTIVITIES

General stimulatory effects have been reported in two cases: reductive iron release from ferritin (Boyer et al 1990) and increase of cytoplasmic free calcium (Tomonaga et al 1992). The latter observation is in apparent contradiction to the various reports on the inhibition of Ca\(^{2+}\)-ATPases (Bohmont et al 1987). While the evidence for stimulation of enzyme activities is considerably outpaced by the reports on inhibitory effects of flavonoids. The stimulation of superoxide dismutase (SOD) activity in patients with liver cirrhosis (Feher et al 1988) is an early example of in v ivo activities of flavonoids and may also partially explain their hepatoprotective effects. Anthrones show an inducing effect on the activity of ornithine decarboxylase (DiGiovanni et al 1988) that is in contrast to the inhibitory effect of flavonoids (Gali et al 1991).
BIOLOGY

Considering the human health aspect, the correlation of flavonoid intake with food may be of special importance, as exemplified by the effect of wine (Waterhouse et al. 1995 & Muldoon et al. 1996) or green tea consumption on evaluation of long-term risk of coronary heart disease and cancer. Two independent studies provided evidence, that flavonoids affected rat blood vessels and surrounding tissue.

ANTICARCINOGENICITY

Unlike the antagonistic nudes of antioxidative versus biocidal functions of flavonoids, the dichotomy between mutagenicity versus anticarcinogenicity has only recently been discussed (Sahu et al. 1994 & das et al. 1994). Despite the multiple reports on anticarcinogenic properties of flavonoids (Edwards et al. 1979), it is surprising that these compounds are still considered potential carcinogens in view of their in vitro mutagenicity. A recent SAR study described differential inhibitory effects of polyhydroxylated (quercetin, dihydroquercetin) or polymethoxylated (5,6,7,8,4'-pentamethoxyflavone = tangeretin; 5,6,7,8,3'4'-hexamethoxyflavone = nobiletin) flavonoids on human squamous tumor cells, solid gliosarcoma cells, and fibroblast cells (Kandaswami et al. 1992). Fibroblasts were relatively insensitive toward all flavonoids tested. In contrast, proliferation of both types of tumor cells were effectively inhibited by the polymethoxylated compounds, whereas quercetin and dihydroquercetin were active against the squamous tumor cells only at higher concentrations. In a thorough investigation, the radical intermediates of the potent synthetic antitumor drug flavome-8-acetic acid were investigated by pulse radiolysis (Candelas et al. 1993). Yet, that a radical mechanism is indeed the basis of the antitumor activity of this compound may be doubtful in view of reports claiming its interaction with cytokines (Mace et al. 1990), or with respect to the stimulation of nitric oxide formation by the drug (Thomsen et al. 1991).

Further insight into the anticarcinogenic mechanism of flavonoids might be gained by looking at the individual steps in the multistage
hypothesis of cancer development, which are all affected by these compounds (Rotstein et al 1988), namely, initiation, promotion, and progression/proliferation.

Initiation either by directly acting carcinogens (Edenharder et al 1993) or auler meta activation of procarcinogens can he prevented both by flavonoids and by other polyphenols. Particularly efficient polyphenols are tannic and ellagic acids (Kuo et al 1992). The inhibitory effect on the metabolic activation may be related to the inactivation of drug-metabolizing cytochrome P450 isocnzymes.

Anti-tumor-promoting activity of flavonoids is most often connected with the inhibition of the action of phorbol esters (Ramanathan et al 1994 & Katiyar et al 1992) and/or the inactivation of ornithine decarboxylase (Nakadate et al 1984). The possible correlation with oxidative events, indicated by the proposed involvement of lipoxygenase inhibition (Nakadate et al 1984), has recently been brought into perspective. Further support for a cytotoxic, i.e., DNA-fragmenting effect, of flavonoids on tumor cells comes from a SAR study and another investigation involving the isoflavone genistein (Yamashita et al 1990).

Inhibition of directly acting carcinogens may be rationalized if we take into account the various reports that propose antproliferative effects of flavonoids (Post et al 1972), even though no obvious correlation is evident at present. The observations that the flavonol quercetin, the flavone apigenin (Sato et al 1994), and the isoflavone genistein (Matsukawa et al 1993) all arrest cell cycle progression in late G1 and G2 - M stages are interesting in this context.

These manifold interactions of various flavonoids with tumor cells suggest that different mechanisms may occur, only some of them involving ROS. Compounds such as polymethoxylated flavonoids effectively inhibit tumor cell proliferation. These substances have been shown to exhibit antioxidative capacity, albeit with different SAR than those for polyhydroxylated flavonoids (Mora et al 1990), and probably do not react via radical scavenging.
PHAGOCYTES AND INFLAMMATORY SYSTEMS

The majority of reports again involve individual flavonoids, frequently with quercetin as the favorite compound, and only few SAR studies have attempted to establish some structural perspective. It is also evident that these studies are confined to rather specific aspects of inflammatory systems, for example, effects on luminol-enhanced chemiluminescence of activated neutrophils (T Hart et al 1990 & Krol et al 1992); formation of ROS by stimulated neutrophils; and aggregation and secretion of platelets (Petroni 1995).

IMMUNOMODULATION

Immunomodulation is the major research area of Middleton and coworkers. It is worth noting, however, that most of these studies are confined to histamine release and T cell functions (Lee et al 1995). A more specific effect, e.g., binding of flavonoids to erythrocytes and subsequent induction of antibody formation, has been reported for (+)-cyanidanol (Salama et al 1987). Inhibition of the complement system, preferentially of the classical pathway involving IgG or IgM antibodies, is another example of the immunomodulatory function of flavonoids (Shahat et al 1996).

GENE EXPRESSION AND SIGNAL TRANSDUCTION

The involvement of flavonoids in gene expression is overwhelmingly represented by reports on the regulation of the heat shock response by quercetin (Elia et al 1996)—with one early study involving other flavonoids and induction of apoptosis by quercetin and other flavonoids (Hirano et al 1996). Only two other gene responses have been studied thus far: the multidrug resistance gene and the cell adhesion protein (Gerritsen et al 1995).

Both inhibition and stimulation of intercellular (gap junction) communication by green tea catechins have been reported. Apigenin and tangeretin also show a stimulatory effect (Chaumontet et al 1994). Thus making this the more likely response. The studies concerning signal
transduction and the involvement of cytokines are still too unspecific to allow definite mechanistic conclusions (Gescher et al 1995).

**FLAVONOIDS IN HUMAN DIET INTAKE**

Flavonoids, as a large group of plant polyphenols, are widely distributed in variable amounts in foods consumed by humans. They have little nutritional value, and generally are considered to be inert and nonessential for human health, although they provide certain benefits to plants. Eating a regular Western diet it is practically impossible to avoid consuming flavonoids. Edible plants such as fruits or vegetables contain significant amounts of flavonoids, and their concentrations are consistent and significantly greater in the leaves, skin and peel than in their deeper tissues (Herrmann, 1976). For instance, in the outer skins of certain onions, e.g. *Allium cepa*, the flavonol content may reach up to 6.5% of the fresh weight (Herrmann, 1976). In soft parts of fruits and their juices, the flavonol concentration (usual as the o-glycosides) may be 100 mg/kg fresh weight (Pierpoint, 1986). Apples, prunes, citrus fruits, cabbage, lettuce and potatoes are only a few examples of fruits and vegetables in which flavonoids are found in substantial amounts (LARC, 983). A cup of brewed black indian tea may contain over 40 mg of flavonoids.

Estimates for the average daily intake of flavonoids in the western diet vary from 50 mg (Brown, 1980) to about 1 g/person (Pierpoint, 1986). Quercetin and kaempferol are the two most abundant flavonols in our diet. Pierpoint (1990) suggested that the daily intake of the polymerized flavonoids could be considerably higher. He estimates the daily intake of these polyphenols to be almost 900 mg, from tea alone, for an adult in the UK.

**BIOLOGICAL EFFECTS**

Since the flavonoids were generally considered to be nontoxic, "inert" or "semi-essential" components of the diet (Kuhnau, 1976), until recently little attention had been devoted to their potential nutritional, biochemical, physiological or pharmacological role in human health.
There is well documented experimental evidence (Birt and Bresnick 1991 & Stich, 1991), mainly from the in vitro assays, that many flavonoids may activate or inhibit enzymatic processes of the liver and other organs. For illustration, only a few of the recently reported effects are mentioned here (Newmark, 1987).

Certain flavonoids have been found to inhibit protein phosphorylation by protein kinases in human neutrophils and platelets. Protein phosphorylation is involved in the activation of these cells during inflammation (Wallace, 1990). Similarly, quercetin inhibits protein kinase C, an enzyme involved in the transduction of growth factor signals to the nucleus, an effect which has been suggested as a "novel therapeutic target for an anticancer agents" (Hoffman et al., 1989). Flavonoids have also been shown, by both in vitro and in vivo experiments, to inhibit platelet aggregation (Gryglewski et al., 1987), and to modify the activity of enzymes involved in arachidonic acid metabolism (Alcaraz and Ferrandiz, 1987), thereby acting as anti-inflammatory agents (Alcaraz and Jimenez, 1988). Furthermore, they have been found to inhibit histamine release from basophilic leukocytes and other mediators of allergic hypersensitivity reactions (Middleton et al. 1987). Flavonoids inhibit the activities of hyaluronidases, enzymes that are involved in a number of processes, including allergic reaction, inflammation, migration of cancer cells and malignant cell proliferation (Kuppusamy et al. 1990).

The ability of rutin and quercetin to react with superoxide anion and lipid peroxy radicals and also to form iron complexes that are unable to catalyze the formation of active oxygen radicals makes them useful for possible therapy in combatting cellular damage caused by radicals ("free radical pathologies") (Afanas'ev et al., 1989).

Despite the consensus among most investigators today that quercetin (or other flavonoids) is not a carcinogen (see latter), a controversy surrounds the other biological effects, which could adversely affect health; for instance, the effects of flavonoids on the formation of nitrosamines and oxygen radical species. The majority of N-nitrosamines and N-nitrosamides found in foods, mainly in smoked and pickled products, have been shown to be carcinogenic, producing tumors at
various sites in experimental animals (Druckery et al., 1967). Nitrosamines can also be formed in the stomach under acidic conditions from amines and nitrite which may be available through ingestion of certain foods, drugs and drinking water. It has been observed that vegetables can reduce the formation of N-nitroso compounds. This is presumably due to their content of vitamin C, since vitamin C is a scavenger of nitrite. Other naturally occurring phenolic compounds in vegetables also inhibit the formation of N-nitrosamines (Stich and Rosin, 1984). However, some polyphenols and particularly some flavonoids (quercetin, kaempferol, naringenin) can actually accelerate the process of N-nitrosamine formation (Walker et al., 1982). The precise mechanism of action for the enhancement or inhibition of N-nitrosation by natural polyphenolic compounds is still unknown (Gichner and Veleminsky, 1988).

Both quercetin and myricetin produce reactive oxygen species (superoxide, hydrogen peroxide, and hydroxyl radical) through autoxidation and redox cycling (Hodnick et al., 1986; Canada et al., 1990; Sahu and Washington, 1991). It appears, however, that this reaction is highly pH dependent, and no autoxidation of quercetin can be detected a physiological pH (Canada et al., 1990,) therefore this may not be a significant problem. However, it was also suggested that this autoxidation of flavonoids, in a long exposure, may potentially produce intestinal injury (Canada et al., 1989).

Antibacterial and antifungal activities of flavonoids against plant pathogenic organisms are also effective against human pathogens. Fungi, bacteria and viruses associated with human diseases are frequently susceptible to polyphenols (Mitscher and Gollapudi, 1990).

Recently, it has been found that tea polyphenols were selective growth inhibitors of clostridia. This in turn could indicate an influence of tea polyphenols on the intestinal microflora, and their use for the possible prevention of certain human diseases associated with clostridia (Ahn et al., 1991).

Flavonoids are known to have a number of biological effects which have pharmacological significance. For a long time it has been thought
that flavonoids can maintain or restore the normal integrity of the blood vessel wall. This is the main reason for clinical applications of flavonoids for treatment of decreasing capillary fragility (cataract prevention, recovery from frostbite, myocardial infarction, bruising in contact sports, etc.) (Havsteen, 1983). Quercetin and rutin can reduce the level of serum triglycerides and are antithrombotic (Kota N. et al, 1983), while other flavonoids are effective in the treatment of arteriosclerosis, hyperlipidemia and atherosclerosis (Middleton, 1986; Khushbaktova et al., 1991). Additionally, a number of beneficial pharmacological effects (antiallergic, antiinflammatory) were also found for flavonoids from citrus fruits (Kumamoto et al., 1986; Middleton et al. 1987).

As a result of the above findings, there is considerable interest and effort in research on the therapeutic potential of flavonoids as drugs for the prevention or treatment of certain human diseases (Singleton, 1981; Farkas et al., 1986). In addition, some flavonoids are used as "Model" compounds for new drugs with more efficient pharmacological effects (Middleton, 1990). For instance, flavone acetic acid, a synthetic flavonoid, is currently under clinical evaluation for its antitumor and immune-modulation activities (Ching and Baguley, 1990), while 3-methoxyflavones are tested as antiviral (e.g. against human rhinovirus) compounds (De Meyer et al., 1990).

As mentioned above, some enzymatic effects of flavonoids on cell physiology are being evaluated as novel therapeutic targets as anticancer agents. It has been observed in vitro tests, that the combination of the protein kinase inhibition achieved by quercetin with the activity of busulphan (an antileukaemia chemotherapeutic agent), acts synergistically in inhibiting the proliferation of human leukaemia cells (Hoffman et al., 1989).

The controversy is still unresolved however, whether food flavonoids should be considered to be beneficial or hazardous agents in the human diet (Stich, 1991). The consensus of opinion currently appears to support the view that food flavonoids are more beneficial than hazardous.
GENOTOXIC AND TUMORIGENIC EFFECTS

Interest in research with food flavonoids heated up following two reports published in 1977 that the most abundant food flavonoid, quercetin, had been found to be mutagenic in in vitro tests (Bjeldanes and Chang, 1977; Sugimura et al., 1977). Concern about the potential health hazard from flavonoids in foods increased still further after the publication of results from the first feeding study with rats, when 0.1% of quercetin produced an increased incidence of intestinal and bladder cancers (Pamucku et al. 1980). Other investigations, mainly done in vitro using various microorganisms and mammalian cells in culture (MacGregor, 1984), revealed that some flavonoids possess mutagenic and genotoxic effects. However, the carcinogenic effect of quercetin in rodents, shown previously, could not be confirmed, even when a diet containing 10% of quercetin was used (Stavric, 1984). There is little information on the carcinogenic potential of other flavonoids. Furthermore, it was found in animal tests, that certain flavonoids exhibited some antitumorigenic activity and a number of other potentially beneficial effects for human health (Stich and Rosin, 1984; Birt and Bresnick, 1991).

In mammalian cell cultures, several polyphenols have proven to be potent inducers of chromosome/chromatid aberrations (Stich, 1991). However this strong clastogenic effect in mammalian cells is not matched by a strong mutagenic effect in Salmonella typhimurium (MacGregor and Jurd, 1978) or Saccharomyces cerevisiae (Rosin, 1984) for most of the flavonoids studied. Various modulating factors, some known (e.g. the presence of transition metals Mn$^{2+}$ Cu$^{2+}$ and Fe$^{2+}$; or high pH level) others still unknown, have been shown to influence the extent of genotoxicity of various flavonoids and need to be considered when extrapolating results to human situations.

Recently it has been reported that quercetin induced malignant cell transformation in mammalian cell culture. This finding suggests that daily intake of quercetin (throughout life) may produce genetic effects in somatic cells, "resulting in increased risk of cancer with aging" (Sakai et al., 1990).
On the other hand, the remarkable ability of several flavonoids to suppress the molecular action of a variety of chemical mutagens in \textit{in vitro} experiments is further indication that food flavonoids, under experimental conditions, may not be completely inert, agents. Therefore, it is expected that these \textit{in vitro} observation may reflect on the ability of flavonoids to reduce or inhibit the adverse biological effects of the carcinogens (Bhattacharya, 1990).

Interesting observations have been reported recently for the biological effects of anthraflavic acid, another plant-derived polyphenol. Anthraflavic acid inhibited the mutagenicity of IQ, when incorporated into the Ames Salmonella assay. However, when administered \textit{in vivo} to rats, the opposite effect occurred. Moreover, hepatic preparations from anthraflavic acid-treated rats were more efficient than those from control preparations in converting IQ to mutagenic species \textit{in vitro}. This would suggest that although anthraflavic acid is antimutagenic for IQ in the Ames test, it may potentiate its carcinogenicity in \textit{in vivo} experiments (Ioannides et al., 1991). This is an example that shows that \textit{in vitro} experiments do not always correlate well with \textit{in vivo} results.

Citrus flavonoids were found to possess antimutagenic potential against a number of mutagens using the Salmonella test (Bala and Grover, 1989), and also to have antiproliferative effects on a human squamous cell carcinoma in \textit{in vitro} studies (Kandaswami et al., 1991).

Quercetin has been reported to inhibit proliferation of human ovarian cancer cells \textit{in vitro}, which contain type II oestrogen binding sites. Since the primary ovarian tumors contain the same type of binding sites, there is a possibility that quercetin could also be active in inhibiting ovarian tumors \textit{in vivo} (Scambia et al., 1990).

Nevertheless, the question arises: why are quercetin and other flavonoids genotoxic in \textit{in vitro} tests, but they are not animal carcinogens? Several investigators have suggested that perhaps “these agents are initiators but also possess antipromotional activity, thus negating any potential role as a carcinogen” (Birt and Bresnick, 1991). To understand the complexity of this question, the next two sections, will
provide an overview of animal tests and suggested mechanisms by which flavonoids may act in reducing carcinogenicity.

PROTECTIVE EFFECTS OF FLAVONOIDS IN SUPPRESSING TUMORIGENICITY

This section will summarize results with some polyphenols, which have been found to exhibit certain beneficial or "protective" effects in suppressing tumorigenicity.

There are a large number of regular food ingredients, that have been found to exhibit either antimutagenic or anticarcinogenic properties (Fontham, 1990; Birt and Bresnick, 1991; Stich, 1991). Since all vegetables and fruits contain flavonoids, and no detrimental effects of flavortoids have been observed in humans and in in vivo studies (Wattenberg, 1983; Scambia et al., 1990; Daniel and Stoner, 1991; Kandaswami et al., 1991) there is an increased interest and expectation in possible use of flavonoids as chemopreventive agents in human carcinogenesis (Gabor, 1988; Birt and Bresnick, 1991).

Using experimental animals, flavonoids have been shown to modulate the activity of known carcinogens. Several feeding studies with mice using several polyphenols and known chemical carcinogens, produced encouraging, although in some instances, disappointing results (Nishino, et al., 1984; Smart et al., 1986; Wattenberg et al., 1980; Hirono et al., 1980). For example, quercetin showed an antitumor effect in mouse skin after topical application (Fujiki et al., 1986; Kato R., 1983) but not after oral administration (Horiuchi et al., 1986). Other experiments similarly reported little or no "protective" effect of quercetin when given orally, while the "protective" capacity was observed if quercetin was applied topically. The lack of activity may be due to the fact that quercetin may not have been absorbed in sufficient amounts to exhibit the protective effect. However, other polyphenols, such as ellagic acid, exhibited protective effect when given orally (Nakadate et al, 1984; Mukhtar et al, 1986).

Polyphenols from green tea significantly protected against tumorigenicity of PAHs and 3-methylicholanthrene (3-MC) administered
by various routes in different animal experiments using different protocols (topical administration or oral feeding in drinking water) (Wang et al., 1989).

Acidic components incorporating phenolic groups have been repeatedly implicated as active antioxidants (Larson, 1988). Many foods derived from plants contain such acidic polyphenols, like caffeic acid, chlorogenic acid, ellagic acid, and others. For instance, coffee contains relatively large amounts of chlorogenic acid. In some green coffee, up to 10% of the weight is chlorogenic acid, and substantial amount of it survives the roasting process (IARC, 1991). Chlorogenic acid was found to be the most abundant phenolic acid in plants, and also it has the most active antioxidant property (Larson, 1988). Dietary chlorogenic acid was found to inhibit liver and large intestine tumors in hamsters induced by the chemical carcinogen methylazoxymethanol [MAM] (Mori et al., 1986). Since the carcinogen in these experiments was introduced intravenously, the inhibition may have been due to modification of the activity of detoxification enzymes.

Ellagic acid, a polyphenol present in many fruits and vegetables, has been shown to be a potent antagonist of the carcinogenic effects of several polyaromatic hydrocarbons (PAHS) (Wood et al., 1982; Mukhtar et al., 1984). Ellagic acid was reported to exert a "protective" effect also against other food carcinogens. For instance, 4% of ellagic acid fed to rats receiving subcutaneous injections (for 15 weeks) of N-nitroso-benzylmethylamine (a compound which induces esophageal tumors in 100% of rats) inhibited significantly (60%) the formation of tumors (Daniel and Stoner, 1991). In contrast, in some cases, ellagic acid (and some other polyphenolic acids like chlorogenic and ferulic) failed to produce in vitro assay any inhibitory effect on the mutagenicity of some other food mutagens/carcinogens, like ALAs (amino-imidazoazaraenedes) (Alldrick et al., 1986). It is still unclear why polyphenolic acids in some cases inhibit the mutagenicity of food xenobiotics, while in other instances they do not. It has been postulated that the inhibitory effect of ellagic acid may be due to its interaction with hydroxylated metabolites of the xenobiotics. This reduction in the chemical reactivity would diminish the capacity of
the xenobiotic [e.g. B(a)P] to undergo covalent binding to DNA (Wood et al., 1982). It has been demonstrated, that in addition to the mutagen itself (its chemical structure), the source of the hepatic S9 mixes used in in vitro assays (e.g. obtained either from induced or non-induced mouse or hamster) could play a role in the mutagenic response or inhibitory capacity of the individual flavonoid (Alldrick et al., 1986).

Quercetin, when fed to mice, inhibited B(a)P-induced nuclear-damage in colonic epithelial cells (Wargovich et al., 1985). Similarly, dietary quercetin significantly reduced the number of palpable rat mammary tumors/rat and also the number of rats with tumors, induced by intragastric instillation of 7,12-dimethylbenz(a)anthracene (DMBA) and by i.v. injection of N-nitrosomethylurea, both confirmed chemical carcinogens (Verma et al., 1988). The same xenobiotic, DMBA, induces chromosome aberrations in rat bone marrow. However, fresh or boiled juices from vegetables (e.g. onion, cabbage, egg plant) significantly suppressed the incidence of aberrations (Ito et al., 1986).

THE MECHANISMS OF EFFECT IN REDUCING CARCINOGENICITY

In spite of the progress made by many investigators in the last several years to elucidate the mechanism by which food flavonoids exert their protective effects in reducing carcinogenicity, the precise mechanism of action is still unclear (Stich, 1991). Studies to identify agents (principles) in food with anticarcinogenic potential, have shown that most of these compounds (e.g. vitamins C, E, A beta-carotene, selenium) possess antioxidative potential (Amstad et al., 1990). Since many flavonoids are also antioxidants, it is likely that the antioxidative property of flavonoids is, at least in part, responsible for their reported anticarcinogenic potential. Nevertheless, overwhelming evidence from research conducted in many laboratories in the last several years, indicates that the mode of action of flavonoids extends much wider than their antioxidative properties. This section will review the mechanisms, suggested by different studies, by which food flavonoids may exhibit their "protective" action. They are as follows:
• **By their action as antioxidants.** Flavonoids could protect certain biologically beneficial, but easily oxidizable compounds in foods (i.e. vitamins) by reducing their oxidative degradation (Fraga et al., 1987). Similarly, they exhibit a quality-preserving effect on raw or cooked meat (Herrmann, 1976), and extend the shelf-life of lard, edible oils and fruits (Dick et al., 1985).

• **By their action as scavengers of free radicals** formed either during preparation of food, or as a result of certain metabolic processes in the body (Husain et al., 1987; Robak and Gryglewski, 1988). The deleterious effect of free radicals in cells is assumed to be responsible for many chronic diseases, including aging and cancer. However, there is a controversy concerning the correlation between antioxidative and scavenging activities of flavonoids. Some authors found that the antioxidative properties of flavonoids are mainly due to their free radical scavenging capacity (Cillard et al., 1990), while others could not find that correlation (Yuting et al., 1990).

• **By their action as chelating agents.** Flavonoids can form complexes with transition metals, e.g. with copper, thus preventing destruction of ascorbic acid, or with iron or copper thus preventing the initiation of free radical reaction (Pincemail et al., 1990).

• **By inhibiting lipid peroxidation,** through combining the (above described) chelating and antioxidative properties of flavonoids. Rutin and quercetin were found to be effective inhibitors of iron ion-dependent lipid peroxidation (Afanas'ev et at, 1989).

• **By blocking or trapping ultimate carcinogen** electrophiles by forming innocuous products in a nucleophilic chemical reaction. To carry out this function, flavonoids need to be absorbed and present in the target cell, either unmodified or metabolized. However even if not absorbed, flavonoids could still be useful in blocking chemical carcinogens in the lumen of the gastrointestinal tract (Newmark, 1987).
• By interaction and subsequent binding to the mutagenic/carcinogenic metabolite(s) to render them ineffective to bind covalently with DNA (Wood et al., 1982; Mukhtar et al., 1984; Das et al., 1985).

• By forming adducts with DNA, thus 'masking' binding sites and rendering them unavailable for reaction with mutagens or carcinogens. This mechanism was observed in a series of in vitro tests with ellagic acid and explants of different organs of the rat (Teel, 1986).

• by inhibiting the invasiveness of tumor cells. Using in vitro assay it was found that the flavonoid, (+)catechin, possessed an antiinvasive activity in tumor cells (Bracke et al., 1988). Oral administration of catechin reduced the B(a)P induced forestomach tumors in mice (Nagabhushan, 1990).

• By inhibiting the promotion phase of carcinogenesis. Several investigators have observed that some flavonoids (e.g. quercetin, luteolin, apigenin, aqueous extracts of green tea) when administered topically, act as antipromoters against known promoters of carcinogenesis (Nishino et al., 1984; Wang et al., 1989; Wei et al., 1990). However, only limited antipromoting activity of quercetin was observed in tests with mice receiving a diet with 1-4% quercetin (Fujiki et al., 1986). This low activity could be explained by intensive degradation of quercetin by intestinal flora, which is partly the reason for its limited absorption. There is a good correlation between the ability of some of the flavonoids to inhibit certain promoter-stimulated biochemical processes and their activity as antipromoters (Birt and Bresnick, 1991).

• By decreasing the production of prostaglandin E₂, which in turn may reduce tumorigenicity. After in vitro experiments exposing mouse fibrosarcoma cells to quercetin, diminished tumorigenicity was observed (Okada et al. 1990).
• By inhibiting endogenous nitrosation in the stomach of man. Plant extracts rich in (+)catechin suppressed the endogenous formation of nitrosoproline (Stich and Rosin, 1984).

• By influencing the immune system. Some flavonoids can interfere with tumor development (Wiltrout and Hornung, 1988). It has been shown that some flavonoids can enhance natural killer cell activity and induce interferon production and act synergistically with interleukin-2 (Wiltrout and Hornung, 1988).

• By inhibiting enzymes or blocking biosynthesis of enzymes involved in reaction sequences required for transforming procarcinogenic compounds into ultimate carcinogens, e.g. by inhibiting the arachidonate cascade mechanism or lipoxygenase (Wheeler and Berry, 1986).

• By modulating the balance between activation and inactivation processes of specific enzymes in the liver. It has been found that tangeretin (polymethoxylated flavonoid) inhibits aflatoxin B₁ induced hepatocarcinogenicity in the rat, while quercetin is ineffective (Suschete et al., 1991).

The experimental evidence, as presented above, suggests that there are many plausible mechanisms of action by which flavonoids and other polyphenols can reduce carcinogenicity. It is expected that many of the above mentioned observations, mainly from in vitro experiments, may reflect the ability of flavonoids to counteract the adverse biological effects of the carcinogens (Bhattacharya, 1990). However, not all modes of action are applicable to all flavonoids and it is conceivable that in some cases the same flavonoid may act in more than one way, or may not act at all.

HESPERIDIN

Approximately 4000 different flavonoids have been chemically identified in plant extracts worldwide, making those widespread compounds important constituents of the natural human diet.
Pharmacological activities have been attributed to some flavonoids, particularly those related to their anti-inflammatory and analgesic properties. Hesperidin, a citrus bioflavonoid was shown to decrease the inflammatory reaction when administered subcutaneously to rat and for many years it was included in a general formulation to treat peripheral vascular disease. The mechanism of action of this flavonoid however is still unknown. Hesperidin is an abundant and inexpensive byproduct of citrus cultivation. It is isolated in large amounts from discarded rinds of the ordinary orange citrus curantym L.in view of the potential use of hesperidin. Hesperidin is one of the bioflavonoids, naturally occurring nutrients usually found in association with Vitamin C. These bioflavonoids include Hesperidin, Citrin, Rutin, Flavones, Flavonals, Catechin, and Quercetin. Hesperidin is linked to capillary health as well as healthy circulation. Like other bioflavonoids, hesperidin works best when given with Vitamin C and other bioflavonoids. No signs of toxicity have been observed with normal intake of hesperidin(Ameer et al 1996). Hesperidin is a bioflavonoid. Bioflavonoids are any of a group of colored substances found in many fruits, and enhance the activity of vitamin-C. These substances are not vitamins but were dubbed "vitamin P" by Dr. Albert Szent-Gyorgyi, a famed Hungarian researcher. He is one and the same Gyorgyi who won the Nobel Prize in Medicine for his discovery of vitamin C(Emin et al 1994).

Hesperidin is one of the bioflavonoid naturally occurring nutrients usually found in association with Vitamin C. Some symptoms originally thought to be due to Vitamin C deficiency such as bruising due to capillary fragility were found in early studies to be relieved by crude vitamin C extract but not by purified Vitamin C. The bioflavonoids, sometimes called Vitamin P, were found to be the essential component in correcting this bruising tendency and improving the permeability and integrity of the capillary lining. These bioflavonoids include Hesperidin, Citrin, Rutin, Flavones, Flavonals, Catechin, and Quercetin.(Galati et al 1996).

Hesperidin deficiency has been linked with abnormal capillary leakiness as well as pain in the extremities causing achiness, weakness, and
night leg cramps. Supplemental hesperidin may also help reduce edema or excess swelling in the legs due to fluid accumulation. Like other bioflavonoids, hesperidin works best when given with Vitamin C and other bioflavonoids. No signs of toxicity have been observed with normal intake of hesperidin (Berkarda et al 1998).

Hesperidin, in combination with a flavone glycoside called diosmin, is used in Europe for the treatment of venous insufficiency and hemorrhoids. Hesperidin, rutin and other flavonoids thought to reduce capillary permeability and to have anti-inflammatory action were collectively known as vitamin P. These substances, however, are not vitamins and are no longer referred to, except in older literature, as vitamin P.

Hesperidin is a solid substance with low solubility in water. It is, however, much more soluble in water than its aglycone hesperetin. Hesperidin's molecular formula is C_{28}H_{34}O_{15}, and its molecular weight is 610.57 daltons. The disaccharide of hesperidin, rutinose, is comprised of the sugars rhamnose (6-deoxy-L-mannose) and glucose. Hesperidin is also known as hesperetin 7-rhamnoglucoside, hesperetin-7-rutinoside and (S)-7-[[6-O-(6-deoxy-alpha-L-mannopyranosyl)-beta-D-glucopyranosyl] oxy]-2, 3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-1-benzopyran-4-one. Hesperidin is represented by the following chemical structure:

![Hesperidin](image)

**ACTIONS AND PHARMACOLOGY**

Hesperidin may have antioxidant, anti-inflammatory, anti-allergic,
hypolipidemic, vasoprotective and anticarcinogenic actions (Galati et al. 1994).

**MECHANISM OF ACTION**

Although some studies indicate that hesperidin has antioxidant activity *in vivo*, others do not demonstrate antioxidant activity *in vitro*. The possible anti-inflammatory action of hesperidin is probably due to the possible anti-inflammatory action of its aglycone hesperetin. Hesperetin appears to interfere with the metabolism of arachidonic acid as well as with histamine release. Hesperetin appears to inhibit phospholipase A2, lipoxygenase and cyclo-oxygenase. There is evidence that hesperetin inhibits histamine release from mast cells, which would account for the possible anti-allergic activity of hesperidin (Bok et al. 1999).

Again, the possible hypolipidemic effect of hesperidin is probably due to hesperetin's possible action in lipid lowering. Hesperetin may reduce plasma cholesterol levels by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, as well as acyl coenzyme A: cholesterol acytransferase (ACAT). Inhibition of these enzymes by hesperetin has been demonstrated in rats fed a high cholesterol diet.

The mechanism of hesperidin's possible vasoprotective action is unclear. Animal studies have shown that hesperidin decreases microvascular permeability. Hesperidin, itself or via hesperetin, may protect endothelial cells from hypoxia by stimulating certain mitochondrial enzymes, such as succinate dehydrogenase (Tanaka et al. 1997).

The mechanism of hesperidin's possible anticarcinogenic action is also unclear. One explanation may be the inhibition of polyamine synthesis. Inhibition of lipoxygenase and cyclo-oxygenase is another possibility.

**PHARMACOKINETICS**

There is not much known about the
pharmacokinetics of hesperidin in humans. It is unclear if hesperidin itself is absorbed from the intestine intact as a glycoside. The aglycone hesperetin is detected in the serum following ingestion and may be formed prior to or following absorption. Hesperetin may undergo glucuronidation in the wall of the intestine, as well as in the liver. Hesperetin is detected in the urine within three hours after ingestion of hesperidin. Urinary excretion appears to be the major route of excretion of the aglycone. Not much more is known about the metabolism of hesperidin (Kayumu et al 1999).

INDICATIONS AND USAGE

Hesperidin has demonstrated some ability to favorably affect lipids and to treat some vascular disorders in humans. Other claims made for hesperidin are based on in vitro and animal studies. These include claims that hesperidin is useful in cancer and immune disorders. There are also claims that hesperidin is an anti-allergen and anti-inflammatory agent based on results from animal experiments.

In several animal studies, hesperidin has significantly increased HDL-cholesterol while lowering total lipid and triglyceride plasma levels. A recent clinical trial tested the effects of hesperidin-rich orange juice in 25 subjects with elevated cholesterol levels. Subjects drank one glass of orange juice daily for four weeks, two glasses daily for four weeks and three glasses daily for four weeks. By the third phase of the study, HDL levels in these subjects increased 21% and the LDL/HDL ratio dropped 16%. Folate levels significantly increased. This was interpreted as a positive result, as well, since folate has been shown to cause declines in levels of homocysteine which, at high levels, is believed to increase the risk of heart disease (Montforte et al 1995). These positive effects, attributed by the researchers to the hesperidin content of orange juice, persisted throughout a five-week washout period that followed the
conclusion of testing. During that period, subjects were asked not to drink any juice. Hesperidin has demonstrated antihypertensive and diuretic effects in both normotensive rats and spontaneously hypertensive rats. It has also shown some ability to protect against ischemia-reperfusion tissue damage in some animal models. In combination with micronized diosmin, hesperidin has significantly improved acute internal hemorrhoids of pregnancy in a clinical open trial. Anticancer, antimutagenic and immune-modulating effects have been seen with the use of hesperidin in numerous in vitro and animal studies. Among the cancers investigated in these studies are esophageal, colon, urinary bladder and skin cancers. In one study that compared the cancer-inhibiting effects of a number of dietary flavonoids and bioflavonoids, hesperidin, hesperetin and catechin were said to be the most potent. More research is needed (Miyaku et al 1998). Similarly, more research is warranted to see whether preliminary animal studies suggesting that hesperidin may have significant antiallergenic and antiinflammatory effects will have clinical relevance.

NUTRITIONAL SUPPLEMENTS

Vitamin C: The interaction between flavonoids, such as hesperidin and hesperetin, and vitamin C is unclear. It has been believed for some time that flavonoids work synergistically with vitamin C, enhancing the absorption of the vitamin and preventing its oxidation. However, recent research indicates that flavonoids, such as hesperetin, may actually inhibit the uptake of vitamin C into cells. More research is needed to clarify this issue (Miyaku et al 1998).

DOSAGE AND ADMINISTRATION

Hesperidin is present in such nutritional supplements as vitamin C with bioflavonoids. Typical dose in these products is about 20 mg. Hesperidin is available in hesperidin-complex supplements. Doses for this type of supplement are usually 500
mg to 2 grams daily. In Europe, hesperidin is available for the management of venous insufficiency and hemorrhoids in a combination product with diosmin. A 500-mg dose of this combination product is comprised of 50 mg of hesperidin and 450 mg of diosmin. Dose for this mixed flavonoid product, for the above conditions, is 1 to 3 grams daily. Another flavonoid, hesperidin methyl chalcone, is often marketed in formulations with hesperidin. This is a different flavonoid, and very few studies have been performed using it. A good source of hesperidin is orange juice containing pulp (Emin et al 1994).