Introduction

Growth and proliferation of mammalian cells are tightly regulated by a variety of genetic and biochemical factors. Changes in regulation of these processes, resulting in degeneration, regeneration, or hyperplasia, are thought to be important events in the etiology of different pathological conditions ***(Nakatani et al.,1997).***

More and more diseases have been proposed to have a radical or oxidant involvement. Although in most cases it is not easy to determine whether this involvement is a cause or a result of the disease process, it is still valuable to learn about those compounds or enzymes that might block, or prevent radical-initiated reaction ***(Sinclair et al., 1990).***

So the O_2 containing environment would not be possible without the presence of defense system against oxidant induced damage. The studies were showing the importance of antioxidants in our life ***(Kelra et al., 1994).*** Significant evidence has been accumulated indicating involvement of reactive oxygen species (ROS) and antioxidant enzymes in control mechanisms of cellular growth and proliferation in vitro.

Antioxidant supplements have gained the widespread public perception that they are beneficial to good health based

on a belief that certain vitamins can stave-off degenerative diseases such as cancer, heart disease, and other chronic maladies. This perception was taken even more seriously when antioxidant supplementation was reputed to slow the aging process and the progression of age-related pathogenesis ***(Yu et al., 1998).***

Antioxidant vitamins A and C

Antioxidants get their name because they inhibit or combat oxidation. They are substances that protect other chemicals of the body from damaging oxidation reaction by reacting with oxidizing agents within the body. During this process, the antioxidant is oxidized, so it acts in a sacrificial manner ***(Guthrie and Picciano, 1995).*** Biological antioxidants can be defined as compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidations ***(Halliwell and Gutteridge, 1989).***

As regards their function, antioxidants prevent or at least slow down the process of oxidation and as long as their supply lasts in our bodies, these damaging reactions are instantly stopped. However, one antioxidant molecule can stop only one damaging reaction, which necessitates a constant, self-regulating supply ***(Cutler, 1991).*** Thus it becomes increasingly important to understand which compounds can function as antioxidants, where they are located in the body and what their mechanism of action might be. This might give a better chance to propose interventions that might suppress or even reverse some of the dangers of oxidant-based diseases in humans ***(Krinsky, 1992).***

Antioxidants scavenge free-radicals, attracting them and neutralizing their charge before they can do damage to cellular structures. Since the physical basis of life exists on cellular level, antioxidants play a major role in maintaining health and youthful vigor by preserving the integrity of individual cells which make up the whole body ***(Cutler,1991).***

The potential toxicity of "ROS" is counteracted by a large number of cytoprotective enzymes and antioxidants which limit the damage that could be caused by such species ***(Ward and Peters, 1995).***

There are two major sources of antioxidants; those that are supplied in food or food supplements as vitamin C, E, and β-carotenoid, those that are produced inside our body as uric acid and glutathione which scavenge free-radicals directly and antioxidant enzymes which break them down into non-toxic products ***(Krinsky, 1992).***

Classification of Antioxidants.

Antioxidants can be classified into two major categories ***(Halliwell et al., 1992)*** :

1- Endogenous (Biological or Native) antioxidants [Table 1].

2- Exogenous (Pharmacological) antioxidants [Table 2].

Table (1): Endogenous Antioxidants.

Agent	Comments
Enzymatic antioxidants	
Cytochrome oxidase system	Detoxifies 95-99% of O_2 in cell
Superoxide dismutase (SOD)	Detoxifies superoxide anion
Catalase	Detoxifies hydrogen peroxide
Glutathione peroxide	Detoxifies hydrogen peroxide
Peroxidase	Detoxifies hydrogen
Non-enzymatic antioxidants	
Lipid phase	
α-Tocopherol	Vitamin E
β-Carotene	Vitamin A precursor
Aqueous phase	
Glutathione	
Ascorbic acid	Vitamin C
Urate	Scavenges O_2, OH
Cysteine	Scavenges O_2, OH
Albumin	Scavenges LOOH, HOCL
Bilirubin	Scavenges O_2, OH
Ceruloplasmin	Possible mechanism similar to SOD
Transferrin	Binds circulating iron
Lactoferrin	Binds circulating iron
Ferritin	Binds tissue iron

Table (2): Exogenous Antioxidants

Class of agent	Specific agent	Mechanism of action
Xanthin oxidase inhibitors	-Allopurinol -Oxypurinol -Folic acid -Tungsten	Inhibit superoxide generation by xanthine oxidase.
Protease inhibitors	-Soyabean trypsin inhibitor -Other serine protease inhibitors -Phenylmethylsulfonyl fluoride (PMSF)	Block proteolytic activation of xanthine oxidase from xanthine dehydrogenase.
NADPH oxidase inhibitors	-Adenosine -Local anaesthetics -Ca channel blockers -Non steroidal anti-inflammatory agents -Cetiedil -Monoclonal antibodies to NADPH oxidase -Diphenyline iodonium	Inhibit superoxide generation by NADPH oxidase in neutrophils and macrophages
Superoxide dismutases (SOD)	-Native SOD -IgA hinge-linked SOD -Polyethylene glycol-SOD (PEG-SOD) -Liposome-encapsulated SOD -SOD-mimics: Cu-Dips CuTIM, Cu, MeTIM Desferal Mn	Catalyses $O_2 + 2H^+ \rightarrow H_2O_2$

Catalases	-Native catalase -PEG-catalase -Liposome-encapsulated catalase	Catalyses $2H_2O_2 \rightarrow 2H_2O + O_2$
Non-enzymatic free radical Scavengers	-Mannitol -Albumin -Dimethylsulfoxide (DMSO) -Dimethylthiourea (DMTU) -Glutathione -Urate -Spin traps -Bilirubin	-Scavenges OH -Scavenges OH,Fe^{3+}, O_{2-}, F^{2+} Scavenges OH, H_2O_2, HOCL Scavenges LOOH, O_2 Scavenges H_2O_2, OH Scavenges O_2, OH Scavenges free radicals Scavenges free radicals
Inhibitors of iron redoxcycling	-Desfeoxamine -Apotransferrin -Ceruloplasmin	Bind free Fe^{3+}
Substances that augment endogenous antioxidant activity	-Oltipraz -ebselen -Glutathione -Acetylcysteine	Augment endogenous glutathione peroxidase activity
Antineutrophil agents	-Antineutrophil serum -Antiadhesion agents -Monoclonal antibodies to CD11/CD18 -Soluble GMP140 -Platelet-activating factor antagonists	-Depletes endogenous neutrophils -Inhibit neutrophil adhesion to endothelium - Inhibit neutrophil adhesion and extravasation

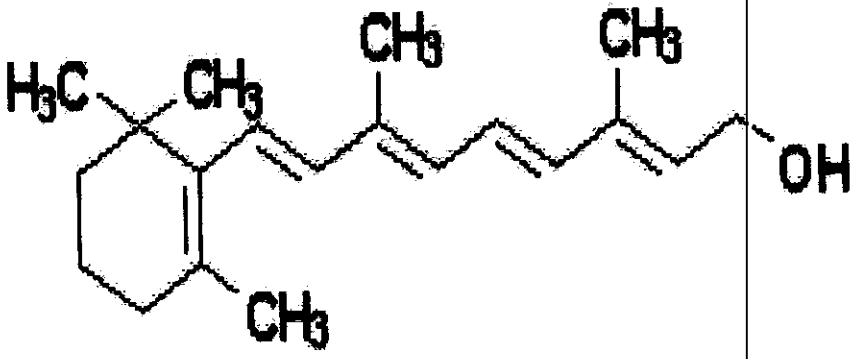
Vitamin A

Vitamin A is necessary for the development and maintenance of human life. It cannot be synthesized in the body and must therefore be taken in with the food as either vitamin A alcohol and its esters or β-carotene ***(Bollag, 1983).***

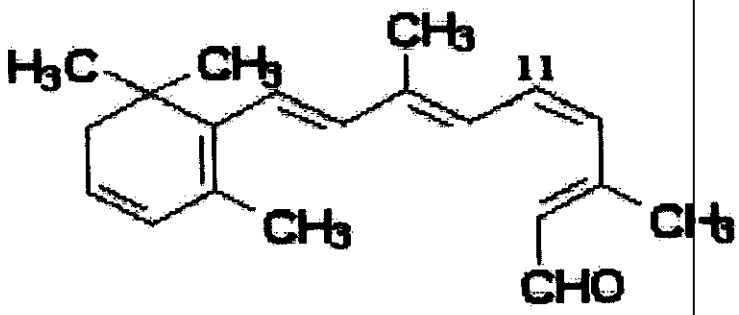
Vitamin A-active substances are compounds other than carotenoids that exhibit qualitatively the biologic activities of retinal. The word "retinoids" is a generic term that includes both naturally occurring compounds with vitamin A activity and synthetic analogues of retinal, with or without biologic activity ***(Goodman, 1984).***

Retinol (vitamin A_1) and 3-dehydroretinol (vitamin A_2) are alcohols with the structures shown in Figure 1. Vitamin A exists naturally in several isomeric forms. This is a cis-trans isomerism resulting from configurational differences at the double bonds in the side chain. The major naturally occurring form of vitamin A is the all-trans isomer ***(Lui and Roels, 1980).***

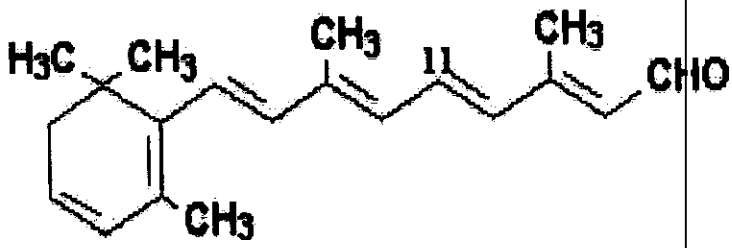
Exclusive of cis-trans isomers, approximately 600 carotenoids have been characterized in nature. Major provitamin A carotenoids in mammals are β-carotene, β-cryptoxanthin and α-carotene. Carotenoids are synthesized in plants and microorganisms from acetyl-coenzyme A by a series of well defined condensation reactions ***(Olson, 1989).*** β-carotene is the most important provitamin A. β-carotene is a



Retinol



11-*cis*-retinal



All-*trans*-retinal

Fig. (1) Different forms of vitamin A ***(after Lui and Roels, 1980)***

symmetrical molecule containing two β-ionone rings connected by a conjugated chain. It has the structure shown in Figure 2 ***(Lui and Roels, 1980).***

Retinol melts at 63°C to 64°C and has an absorption maximum in ethanol at 324 to 325 nm. The vitamin is soluble in fats and in all the usual organic solvents. It is insoluble in water. Retinol and its esters have a yellowish-green fluorescence. It is isomerized by exposure to light. It is extremely sensitive toward acids, which can cause rearrangements of the double bonds and dehydration. Acidic reagents give transient blue color reactions with vitamin A ***(Lui and Roels, 1980).***

Dietary sources of vitamin A include fish, liver, butter, whole milk, cheese, and eggs. Animal products contain the vitamin predominantly as retinyl esters. Of the all carotenoids widely distributed among plants, approximately 50 serve as provitamins or precursors of vitamin A. These yellow-orange pigments are particularly abundant in yellow and dark green leafy vegetables such as carrots, squash, broccoli, and spinach ***(Miller, 1996).***

Vitamin A activity is now conventionally stated in terms of microgram retinal equivalents (RE). One RE equals 1μg of all-trans retinal, 6 μg of all-trans beta carotene, or 12 μg of other provitamin A carotenoids. The recommended daily allowance (RDA) of vitamin A for adult males is 1000 RE.

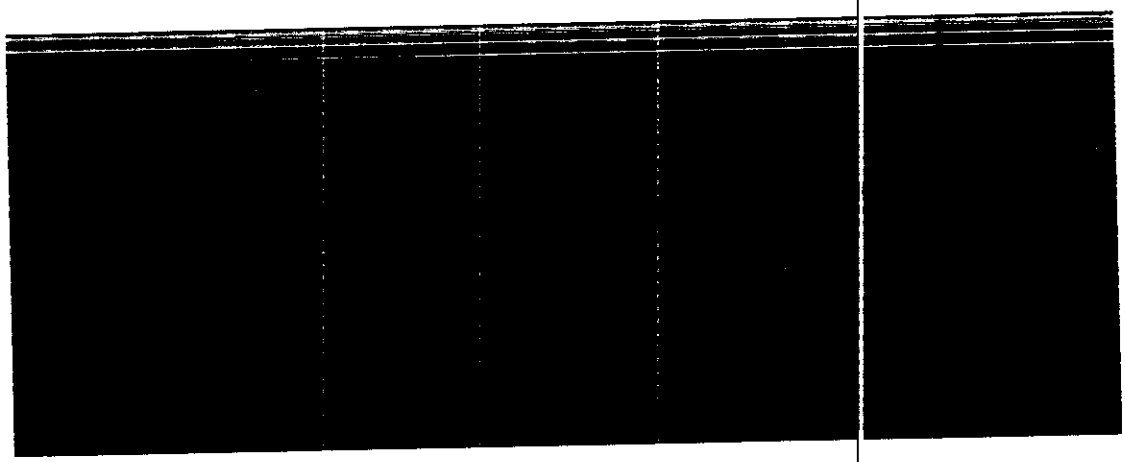


Fig. (2) β- Caroten *(after Lui and Roels, 1980*

The allowance for females is 800 RE and approximately 400 RE for infants and young children. One RE equals 3.33 IU vitamin A activity for retinal. One IU equals the biologic activity of 0.3 μg of all-trans retinal or 0.6 μg of all-trans beta carotene ***(Miller, 1996).***

Man and the bovine can absorb both vitamin A and the carotenoids, and convert carotenoids with provitamin A activity to the vitamin. In contrast, the rat and the pig do not absorb significant amounts of carotenoid pigments. However, they can convert provitamin A to the vitamin in the gut. The small intestine is the most important organ involved in the conversion of provitamin to vitamin, although other tissues are also capable of carrying out this process ***(Lui and Roels, 1980).***

β-carotene is converted to retinol primarily in the intestinal mucosa. The biosynthetic process involves two soluble enzymes: β-carotene-15,15′-dioxygenase and retinaldehyde reductase. Dietary retinyl esters are hydrolyzed in the intestinal lumen, and the resulting retinal is absorbed into the mucosal cell ***(Helgerud et al., 1983).*** Retinol is enzymatically esterified during intestinal absorption. Retinyl esters (primarily retinyl palmitate) are transported in chylomicra via the lymphatics to the general circulation. Plasma contains essentially no retinyl esters except in cases of excessive vitamin A intake. When chylomicron remnants are

cleared from the blood, most of the esterified retinal is taken up by and stored in liver. Typically, 80% to 90% of the body's total retinal is normally present in this organ ***(Miller, 1996).*** Chylomicron remnant uptake by the liver appears to occur mainly by receptor-mediated endocytosis, leading to lysosomal degradation of the remnant constituents. There is evidence that apolipoprotein E plays a part in this uptake process ***(Windler et al., 1980).***

Two liver cell types, parenchymal cells (PC; hepatocytes) and the nonparenchymal perisinusoidal stellate cells (SC; lipocytes; vitamin A-storing cells; Ito cells), play key roles in vitamin A metabolism ***(Vogel et al., 2000).*** Fat-storing cells contain numerous small lipid droplets. When large doses of vitamin A are given, the number and size of these droplets increase considerably, and the fat-storing cells become larger and more prominent histologically. Vitamin A, mainly as retinyl esters, has been localized in the lipid droplets of these cells by a variety of techniques. There are evidences that retinal is transferred from parenchymal to nonparenchymal cells, after its initial hepatic uptake ***(Goodman, 1984).*** Retinol-binding protein (RBP) is the vehicle for transfer of retinol from PC to SC and from SC into the systemic circulation ***(Michael et al., 1993).***

The mammalian liver has a remarkable capacity to store varying amounts of vitamin A over a wide range of dietary

keratinized tissues. Vitamin A prevents or reverses the transformation of pre-malignant cells into malignant cells in vitro ***(Abraham, 1997).*** Vitamin A is also necessary for synthesis of the acid mucopolysaccharide chondroitin sulfate found in bone and cartilage. In its absence, disruption of the intercellular matrix lead to skeletal deformities. In a reproductive role, vitamin A may be linked to modulation of gonadotropin receptors on cell membranes in the testes. Clinical evidence supports a role for the vitamin in maintenance and proper function of the immune system. Vitamin A has been called the "anti-infective" vitamin. Treatment with vitamin A reduces morbidity and mortality in measles, and supplementation for all children seriously ill with measles has been advocated ***(Miller, 1996).*** A number of major diseases, in addition to cancer, are characterized by excessive proliferation of cells, often with excessive accumulation of extracellular matrix material. Such diseases include rheumatoid arthritis, psoriasis, idiopathic pulmonary fibrosis, sclerodermia, and cirrhosis of the liver as well as the disease process atherosclerosis. The possibility exists that retinoids, which can influence cell differentiation and proliferation, may be of therapeutic value in some of these proliferative diseases ***(Goodman, 1984).***

Within the liver, retinol may be conjugated with uridine diphosphoglucuronic acid to form its O-glucosiduronate, or it

may be oxidized to retinal and then to retinoic acid. Retinoic acid also forms a glucuronides in the liver, and these glucuronides, together with a small amount of free retinoic acid, are excreted efficiently into the bile. The oxidation of retinol to retinal and then to retinoic acid also occurs in the kidney and in the intestine. Vitamin A glucuronides in the bile are partially reabsorbed in the gut and transported back to the liver, in an enterohepatic circulation. Most of the biliary glucuronides of vitamin A, however, seem to be hydrolyzed in the gut, apparently by β-glucuronidase of enteric bacteria, then excreted in the faeces ***(Lui and Roels, 1980).***

Signs of vitamin A deficiency include drying (xerosis), degeneration, and accompanying increased risk of infection in the conjunctiva, cornea, skin, and mucous membranes. Severe vitamin A deficiency, with associated xerophthalmia, corneal ulcerations, and keratomalacia, is an important cause of blindness in young children ***(Lockitch, 1993).***

Vitamin A intoxication may arise from therapeutic over-dose or excessive self-medication. Toxicity occurs in adults who ingest more than 15,000 RE (50,000 IU) daily for prolonged periods ***(Miller, 1996).*** A wide variety of toxicity symptoms occur including lethargy, drowsiness, irritability, vomiting, severe headaches, itching peeling skin, brittle nails, hair loss, bone and joint pain, hepatosplenomegaly, and benign intracranial hypertension. Hypercalcemia caused by

chronic hypervitaminosis A has been reported. Psychiatric symptoms may be manifested ***(Silverman et al., 1987).***

Vitamin C

Ascorbic acid (AA), commonly known as an internal ester of a hexonic acid. The double bond between carbons 2 and 3 in the 5-membered lactone ring is readily oxidized. Oxidation at this enediol linkage produces dehydroascorbic acid (DHAA). Ascorbic acid and dehydroascorbic acid (Fig. 3) are the only biologically active forms of vitamin C ***(Miller, 1996).***

Vitamin C is an odorless, white crystalline powder. It is very soluble in water, less soluble in ethyl alcohol and quite insoluble in most lipid solvents. It is easily destroyed by oxidation. Heat, exposure to air, alkaline medium and contact with metallic copper or iron hasten the oxidation of ascorbic acid to dehydro-L-ascorbic acid, then to diketogulonic acid, oxalic acid and other derivatives ***(Hodges, 1980).***

Humans and other primates cannot synthesize vitamin C from glucose and require the vitamin exogenously ***(Carr and Frei, 1999).*** Vitamin C is found in turnip green, green peppers, broccoli, cauliflowers, cabbage, tomatoes, new potatoes, oranges, lemons and other citrus fruits ***(Abraham, 1997).*** The recommended dietary allowances (RDA) for

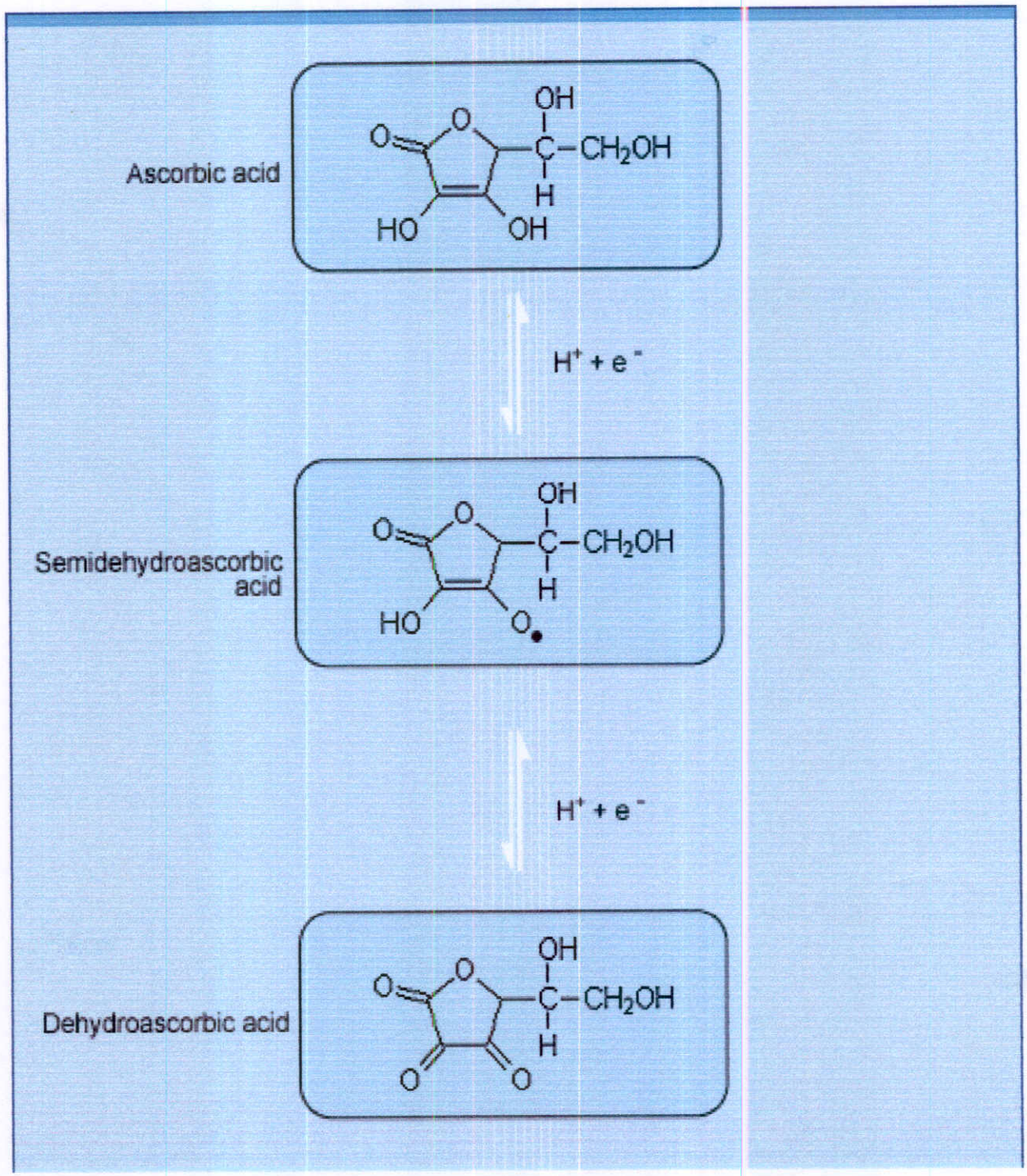


Fig. (3) Ascorbic acid and dehydroascorbic acid ***(after Miller, 1996)***

vitamin C is 60mg/day in adults. However, much larger doses (even 5-10g/day) are well tolerated ***(Shoham, 1992).***

Absorption of vitamin C occurs predominantly in the distal portion of the small intestine. Pectin and zinc may impair its absorption. It is widely distributed among body tissues rather than being stored in one specific organ; leukocytes, in particular, contain high levels of the vitamin when tissue stores are saturated, plasma levels rise, and excess vitamin C is excreted by the kidney ***(Miller, 1996).***

Ascorbic acid (AA) has a wide variety of well-defined biochemical and physiological roles ***(Levine, 1986).*** It is used in the biosynthesis of collagen, hormones, and neurotransmitters, including norepinephrine, epinephrine, and serotonin, and in the regulation of the redox activity of metal ions. It is well known as an important physiologic antioxidant and free radical scavenger, and is thought to protect other antioxidants, such as vitamin E and vitamin A ***(Block and Schwarz, 1994).*** It facilitates gastrointestinal absorption of iron and is believed to play a role in conversion of folic acid to its active form, folinic acid. Ascorbic acid may also play a role in detoxification of poisonous substances by virtue of its cofactorial role in hydroxylation reaction. Apparently, ascorbic acid is essential for normal protein metabolism, both

in experimental animals and in humans ***(Lui and Roels, 1980).*** Ascorbic acid has a well documented role in collagen metabolism as a direct requirement for prolyl and lysyl hydroxylases. It increases procollagen mRNA level in cultured fibroblasts and this effect is the result of the stimulation of procollagen gene transcription and the decreased degradation of the procollagen mRNA (**Chojkier et al., 1989).** Ascorbic acid may also have a role in influencing immune responses to standardized antigens, complement system function, and the cellular production of interferon ***(Beisel, 1982).*** Vitamin C is identified as promoting or facilitating, but not essential for, cholesterol, histamine, and tyrosine metabolism, lymphocytic proliferation and transformation of B and T lymphocytes, and functioning of macrophages and neutrophil chemotaxis ***(Miller, 1996).***

The classic deficiency disease, scurvy, occurs after chronic deprivation of vitamin C. Clinical manifestations of extreme deficiency include profound behavioral changes, severe emotional disturbances, skeletal lesions, necrosis of gums with loss of teeth, ecchymoses, and bleeding into muscles, joints, the kidneys, gasterointestinal tract, and pericardium ***(Miller, 1996).***

Excessive quantities of the vitamin are readily excreted in the urine, and it is generally assumed that adverse effects from mega doses are minimal. However, reports have been published of gasterointestinal disturbances, increased hemolysis in subjects with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, interaction with warfarin anticoagulants, increased destruction of vitamin B_{12}, excessive absorption of dietary iron, uricosuria or oxaluria with increased potential for kidney stone formation ***(Lee et al., 1988).*** Animal studies have demonstrated that large doses of ascorbic acid may cause infertility or abortion or adverse effects on the fetus. In rats, huge doses of an analog of vitamin C, dehydroascorbic acid, can produce permanent diabetes ***(Lui and Roels, 1980).***

Liver and hepatotoxicity

The liver has many metabolic roles and is, accordingly, affected by a very large number of xenobiotics. Xenobiotics are chemicals that are taken up by the body but are not incorporated into the normal metabolic economy of the cell, that is , they are not used for generation of energy, for catalysis or for structure. One of the major roles of the liver is to protect the body against naturally occurring toxins present in. Exposure to small amounts of such materials results in changes in the liver which permit efficient removal of the toxin. These changes are called adaptive because they do not compromise the ability of the liver to perform its other vital functions. As the dose of the compound is increased there may come a point when other functions are compromised and this is defined as the toxic phase of the response ***(Hinton and Grasso, 2000).***

Organization of the liver

The liver is a large organ making up about 3.5% of the body weight of an adult rat or 2% of the body weight of an adult human. The overall shape of the liver differs markedly between species. In rats and mice the liver is divided into several distinct lobes; the human liver, on the other hand, is

divided into two poorly differentiated lobes. The liver lies immediately under the diaphragm, is covered by a thin capsule and is supported mechanically by attachments to the diaphragm and by the blood vessels ***(Jones, 1996).*** The functional requirements of the liver have resulted in it developing a unique dual blood supply, with oxygenated blood entering through the hepatic artery (20%) and blood from the gut, which is rich in nutrients and bacterial endotoxin, entering through the hepatic portal vein (80%). Blood from both of these vessels percolates through the sinusoids, which provide a large vascular bed that maximises exchange of materials prior to exit of blood via the hepatic vein. The portal vein provides a route through which infectious organisms can enter the liver, and mechanisms have evolved to allow rapid and selective immune responses within this tissue. Blood leaves the liver by the very short hepatic veins which join the ascending vena cava ***(Lalor and Adams, 2002).***

The liver acts as an exocrine gland, secreting bile. This bile is conducted down the extra hepatic bile duct into the intestine. In the majority of species, but not in the rat, a portion of the bile secreted from the liver is stored and concentrated in the gall bladder. The portal vein, hepatic artery, extra hepatic bile duct and the major lymphatics all enter the liver substance at a single point, the porta hepatis. Once within the liver the vessels branch to form the portal tree. As the portal tree divides

the vessels gradually become smaller. From the smallest veins, small vessels bud off and pass to the edge of the portal tract, discharge their blood into the capillaries which, in the liver, are termed sinusoids ***(Jones, 1996).***

The bulk of the liver is composed of a single type of cell, the hepatocyte. These are assembled into sheets, each a single cell thick, which bifurcate and fuse to give a most complex network. Between cells within the wall run small branching channels called bile canaliculi. The liver sinusoids are lined by endothelial cells. Within the sinusoids are Kupffer cells ***(Hinton and Grasso, 2000).*** There is no distinct basement membrane in the undamaged liver but some collagen fibers are present and the scant extracellular matrix which is laid down by Ito cells appears to play a role in signaling in the liver and may be rapidly expanded on liver injury ***(Bissell, 1998).***

Parenchymal cells are grouped into concentric zones surrounding the terminal afferent vessels (Fig.4). Zone 1 cells are first to receive blood, the last to undergo necrosis, and the first to regenerate. Those cells in regions more distal to the afferent blood vessels, zones 2 and 3, receive blood of considerably less nutritive value and thus are possibly less resistant to hepatotoxins and other vectors of hepatic injury ***(Jones, 1996).***

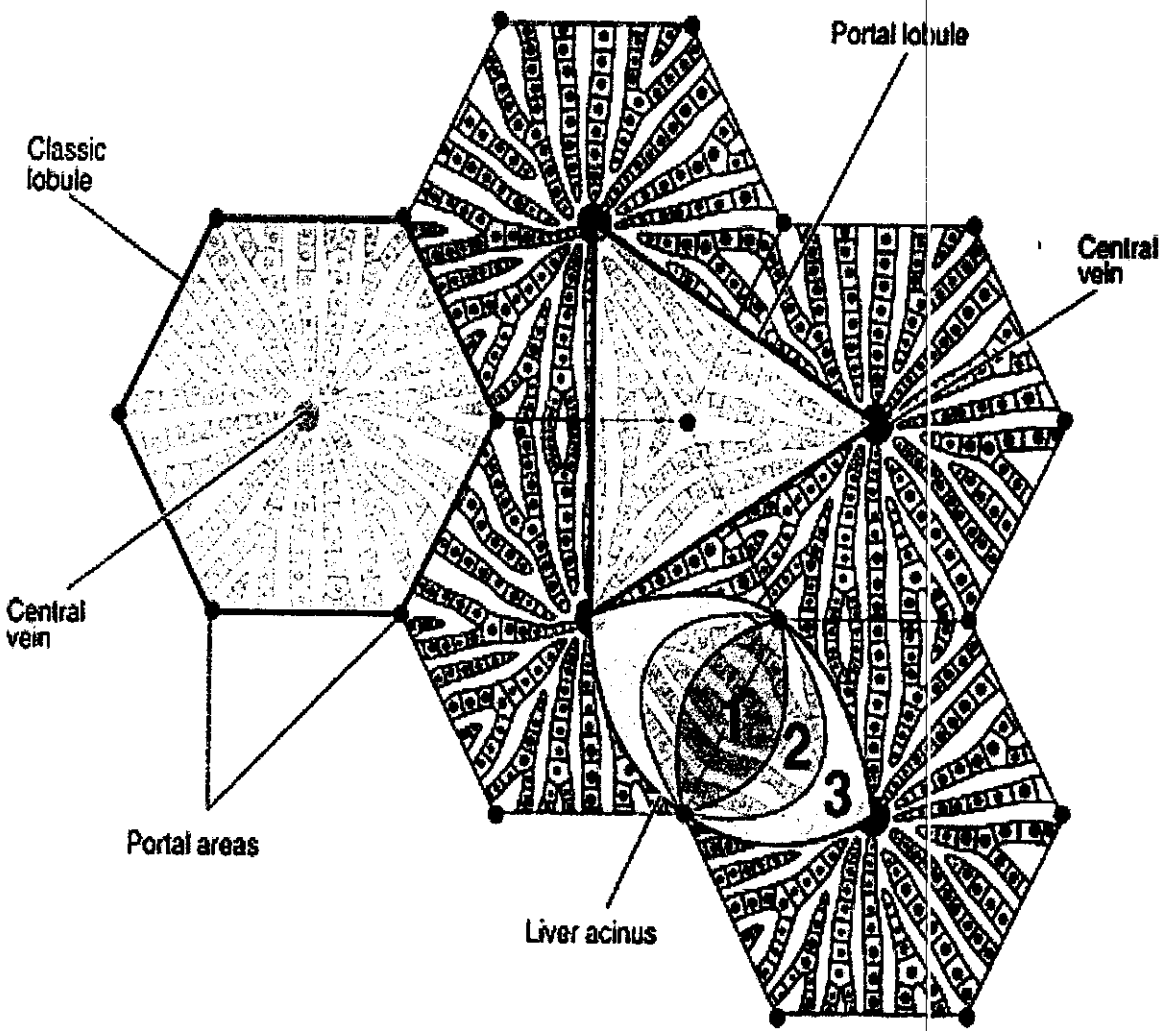


Fig. (4) Concentric zones of hepatic parenchymal cells ***(After Jones, 1996).***

Hepatocytes

Hepatocytes form approximately 90% of the volume of the liver parenchyma but are only 60% of the total cell number. They are large cells approximately cuboidal in shape. The nucleus or nuclei (in rats about half the cells are binucleate diploid cells, the remainder being mostly mononucleate tetraploids) is normally placed centrally; markedly eccentric nuclei are only seen in severely damaged cells ***(Hinton and Grasso, 2000).***

The cytoplasm of the hepatocyte is replete with organelles. It is estimated that the average hepatocyte contains over 2000 mitochondria. Other organelles include microbodies, Golgi apparatus, rough and smooth endoplasmic reticulum (RER & SER), free polysomes, primary and secondary lysosomes, coated and smooth vesicles, multivesicular bodies, microtubules, and microfilaments, which are also abundant. Inclusions in hepatocytes normally include small amounts of lipid, especially triglyceride and lipoproteins. Glycogen in both monoparticulate (beta particles) and rosette forms (alpha particles) is also normal, as are pericanalicular lipofuscin granules, which correspond to large secondary lysosomes or residual bodies ***(Phillips and Latham, 1982).***

At high magnifications it is seen that the ribosomes are arranged with great regularity along most RER elements. Partial loss of ribosomes (RER degranulation), leading to a

'moth-eaten' appearance, is an early sign of hepatotoxicity. Also on treatment with many chemicals the SER proliferates with, in extreme cases, masses accumulating in the center of the cell marginalizing other organelles ***(Hinton and Grasso, 2000).***

The plasma membrane of hepatocytes has four distinct zones: the sinusoidal and biliary regions, which are provided with microvilli; the intercellular region in which the membranes are parallel and devoid of microvilli; and the specialized areas of cell junction. The cell junctions comprise four components: the desmosome (macula adherens), a button like plaque that serves as the site of attachment of tonofilaments to maintain cell shape; the intermediate junction (zonula adherens) into which actin filaments and larger filaments insert that serve as strong adhesive structures between liver cells; the gap junction (macula communicantis, nexus), which allows intimate cell-to-cell communication and which acts as a site of electrical coupling; and the tight junction (zonula occludens), which is composed of rows of contact points between adjacent membranes. The tight junction of hepatocytes are situated at the margins of the bile canaliculi. It may provide a potential passageway for the para cellular movement of fluid between the blood sinusoids and the bile canaliculi ***(Phillips and Latham, 1982).***

Hepatocytes have many functions. The liver serves both as an exocrine and endocrine gland. The exocrine secretion of the liver is bile. In its second role, as an endocrine gland, the liver secretes almost all the major proteins of plasma with the exception of the immunoglobulins. In addition the liver plays a central role in lipid metabolism. It is also responsible for recycling old red blood cells. In addition to these roles, the liver acts as the center of intermediary metabolism in the body. It provides a central store of sugar, in the form of glycogen, and is also a major site for the conversion of sugars to lipids and for conversion of amino acids to sugars and lipids. The final function of the liver is in the modification and excretion of a variety of hydrophobic compounds ***(Hinton and Grasso, 2000).***

Sinusoid endothelial cells

The smallest blood vessels in liver, the sinusoids, are lined by endothelial cells specialized in structure to assist in the liver's major roles of removal of waste materials from the blood and of blood formation. However, in the liver the endothelial cells are pierced by fenestrations about 100 nm in diameter, sometimes called sieve plates which allow all components of the plasma, but not blood cells, to make direct contact with the hepatocytes. The cells contain only small numbers of mitochondria and the endoplasmic reticulum is not well developed. The Golgi apparatus and the lysosomal

system account for a considerably greater proportion of the cell volume than in hepatocytes. It is believed that sinusoid epithelial cells play an important part in cell communication in the liver but the details are not yet known ***(Kawada, 1997).***

Kupffer cells

Kupffer cells are fixed macrophages which are found within the sinusoids. They are attached both to the endothelial cells and, via processes extending through the fenestrations in the endothelial cells, to hepatocytes. Like all macrophages, kupffer cells are actively phagocytic, removing particulate material such as bacteria or bacterial fragments (endotoxins) which may have entered the blood from the intestine. Kupffer cells are also largely responsible for the recycling of old red blood cells and cooperate with hepatocytes in the metabolism of haem. The structure of kupffer cells is similar to that of other macrophages. Phagolysosomes are prominent and numerous filamentous elements are present. There are a fair number of mitochondria but the endoplasmic reticulum is poorly developed and consists mainly of rough-surfaced elements ***(Hinton and Grasso, 2000).***

It is becoming increasingly evident that, in addition to their protective role, kupffer cells are heavily involved in the signaling system of the liver. Kupffer cells and endothelial cells are the major source of the mitotic inhibitory factor;

transforming growth factor-beta (TGF-β); although when stressed, for example in the late stages of liver repair, production is predominantly in hepatocytes. In addition, interchange of signals between kupffer cells and Ito cells is believed to play an important role in liver fibrosis **(Bissell et al., 1995).**

Fat storing cells

Liver fat-storing cells (FSCs) referred to as Ito cells, hepatic stellate cells (HSCs) or lipocytes are nonparenchymal cells of mesenchymal origin situated in the space of Disse, between the sinusoidal endothelium and hepatocytes ***(Pinzani et al., 1989).*** They are modified fibroblasts, one of whose roles in the undamaged liver is to maintain the sparse extracellular matrix of the space of Disse and to store vitamin A. They are small cells compared with hepatocytes. The 'fat' droplets, which are their prominent feature, contain a 40% solution of vitamin A in triglyceride. They have a well developed RER and Golgi apparatus and are the source for hepatic growth-factor (HGF), which is one of the two major mitogens which act on hepatocytes. Their most prominent role, however, is in the liver damage when they differentiate into myofibroblast-like cells and actively form type I collagen and secrete TGF-β. The transition between the two states of the cell is regulated by a network of signals coming from kupffer cells, damaged hepatocytes, endothelial cells, platelets

and inflammatory cells. It is thus clear that Ito cells play an important role in the development of liver diseases, specially those which involve fibrosis ***(Hautekeete and Geerts, 1997).***

Recent evidence has suggested that HSCs are derived from the neural crest since HSCs express glial fibrillary acidic protein (GFAP) and nestin and that neural crest stem cells can differentiate into myofibroblasts expressing - smooth muscle actin which is a marker of activated HSCs ***(Sato et al., 2003).***

Pit cell

Pit cells are rarely observed although there are about 500000 per gram of rat liver. These cells are located on, or embedded in, the endothelial lining and possess number of small granules. Isolated pit cells have natural killer (NK) activity and they morphologically resemble a population of NK cells, the large granular lymphocytes, in the blood although the two populations do not seem to be identical ***(Hinton and Grasso, 2000).***

Hepatotoxicity

The great susceptibility of the liver to damage by chemical agents is presumably a consequence of its primary role in the metabolism of foreign substances. The position of the liver as portal to the tissues for ingested agents and the concentration of xenobiotics in the liver may contribute to its special

vulnerability. The role of the liver in metabolic conversions of foreign compounds is even more important in its susceptibility to chemical injury ***(Zimmerman and Maddrey, 1982).***

Generally, hepatotoxins have their principal effects on a particular portion of the liver lobule. In the majority of cases damage is found principally in the centilobular zone but the effect of certain toxins is on the periportal hepatocytes. There are three reasons for these differences. First, there is the change in oxygen tension across the lobule; second, the enzymes involved in xenobiotic metabolism are not evenly distributed; and finally, when oxygen tension is low the cytochromes P450 may catalyse reductive reaction ***(Hinton and Grasso, 2000).***

Liver injury can be caused by therapeutically useful drugs as well as by foreign chemicals, which are often termed xenobiotic agents. These toxic compounds are classified into two broad categories: agents that cause either a "toxic" or an "intrinsic" toxicity, and those that cause a "drug-induced" or "idiosyncratic" toxicity ***(Dahm and Jones, 1996).***

There appear to be two main types of intrinsic hepatotoxins that we have categorized as direct and indirect. Direct agents, or their metabolic products, injure the hepatocyte and its organelles by a direct physiochemical effect, that is , peroxidation of the membrane lipids, denaturation of proteins,

or other chemical changes that lead to distortion or destruction of the membranes. These changes comprise the first stage in the injury that culminates in necrosis or steatosis. This category includes carbon tetrachloride (CCL_4), other chlorinated aliphatic hydrocarbons, and the yellow allomorph of phosphorus ***(Zimmerman and Maddrey, 1982).***

Indirect agents are antimetabolites and related compounds that produce hepatic injury by interference with a specific metabolic pathway or process. The hepatic damage produced by indirect hepatotoxins may mainly be cytotoxic or cholestatic. This category includes compounds of experimental interest, drugs, and botanical hepatotoxins as ethionine, puromycin, galactosamine, and tetracycline ***(Zimmerman and Maddrey, 1982).***

Acute hepatic toxicity was divided into two groups. Type I lesions are 'predictable, dose and time dependent, occurring in most, if not all, subjects exposed to appropriate doses of the causative chemicals; the lesions are usually readily reproducible in animals. Type II lesions are 'unpredictable, dose and time independent, occurring sporadically and often becoming apparent only after monitoring a large number of exposed individuals; the lesions are not usually reproducible in animals ***(Hinton and Grasso, 2000).***

Chronic lesions can result from the subtle, continued, or repeated injury of prolonged exposure to hepatotoxic agents

or drugs or can be a sequel to an episode of acute drug or other chemical injury. Chronic injury leads to increase in fibrous tissue. The increase may be prominent in the portal-periportal area, the central zone, or both. When the location and degree of fibrosis, accompanied by nodular regeneration and parenchymal collapse, lead to architectural distortion, the result is cirrhosis and portal hypertension ***(Zimmerman and Maddrey, 1982).***

Fibrosis and cirrhosis

Hepatic fibrosis is a common response to chronic liver injury and may result in potentially lethal sequelae ***(Brenner et al., 2000).*** Fibrosis represent the consequences of a sustained wound healing response to chronic liver injury from a variety of causes including viral, autoimmune, drug induced, cholestatic and metabolic diseases ***(Friedman, 2003).***

Cirrhosis represents a late stage of progressive scarring in chronic liver disease. It begins with subendothelial or pericentral fibrosis (hepatic fibrosis) and progresses to panlobular fibrosis with nodule formation (cirrhosis) ***(Bonis et al., 2001).***

Recent developments in the understanding of the process of hepatic fibrogenesis confirm that the process of fibrosis is dynamic with respect to both cell and extracellular matrix turnover and suggest that a capacity for recovery from

advanced cirrhosis and fibrosis is possible ***(Benyon and Iredale, 2000).***

Hepatic fibrosis, regardless of the cause, is characterized by an increase in extracellular matrix (ECM) constituents that collectively form the hepatic scar. This scar consists of fibril-forming collagens (type I and III) as well as matrix glycoconjugates, including proteoglycans, fibronectin, and hyaluronic acid ***(Friedman, 1993).*** Total collagen content increases by three-to tenfold. The perisinusoidal low-density ECM is gradually replaced by a high density ECM ***(Hui and Friedman, 2003).***

The hepatic stellate cells (HSCs) are nonparenchymal, quiescent cells whose main functions is to store vitamin A and probably to maintain the normal basement membrane-type matrix. However, numerous in vivo and in vitro studies indicate that in response to liver injury, HSCs undergo an activation process in which they lose vitamin A, become highly proliferative and synthesize fibrotic matrix rich in type I collagen ***(Safadi and Friedman, 2002).***

The activation of HSCs can be subdivided into two major phases: initiation and perpetuation. Initiation refers to early changes in gene expression and phenotype that render the cells responsive to cytokines and stimuli, whereas perpetuation results from the effects of these stimuli on maintaining the activated phenotype ***(Friedman, 2000).***

The initial activation of HSCs is likely to be a result of stimuli produced by neighbouring cells; namely hepatocytes, Kupffer cells, circulating leukocytes, platelets and sinusoidal endothelial cells; in response to liver injury. Such stimuli include reactive oxygen species(ROS), growth factors and inflammatory cytokines ***(Hui and Friedman, 2003).***

Perpetuation of activated HSCs involves distinct changes in cell biology that result in the accumulation of ECM and fibrosis: proliferation; fibrogenesis; chemotaxis; leukocyte chemoattraction and cytokine release; contractility; retinoid loss; and matrix degradation ***(Pinzani and Marra, 2001).***

Carbon tetrachloride (CCl_4)

Carbon tetrachloride (tetrachloromethane, perchloromethane) is a toxic, colorless, non-flammable liquid, with a distinctive chloroform-like odor and a density 5.3 times that of air ***(Lewis, 1998).***

CCl_4 has been used as a dry cleaning agent and fire extinguishing material. It has also been used as a solvent for rubber cement as well as for cleaning equipment and machinery. Further uses include those of a refrigerant and as a feedstock chemical for fluorocarbon propellants. The largest source of carbon tetrachloride release was fumigation of grains and other substances ***(Fan and Alexeeff, 2000).***

CCl_4 is well absorbed by the lung and gastrointestinal tract. Percutaneous absorption can also occur. CCl_4 is concentrated in fatty tissues. The main excretion route (50-80%) is via the lung as the unchanged compound. Dechlorination occurs in the liver microsomal cytochrome P450 system, and the formation of free radicals may cause lipid peroxidation and subsequent hepatocellular damage. Only a small part of the dose (4%) is excreted as carbon dioxide via the lungs or kidney ***(Ballantyne, 2000).***

CCl_4 causes central nervous system depression and severe damage to the liver and kidneys; it is carcinogenic in experimental animals and has been classified as potential human carcinogen. In animals the primary damage from intoxication is to the liver, but in humans the majority of fatalities have been due to renal injury with secondary cardiac failure. Human autopsy results have included renal tubular necrosis. In humans, liver damage occurs more often after ingestion of the liquid than after inhalation of the vapor ***(Hathaway et al., 1991).***

CCl_4 has been one of the most intensively studied hepatotoxicants to date and provides a relevant model for other halogenated hydrocarbons that are used widely. It causes centrolobular necrosis and associated fatty liver ***(Dahm and Jones, 1996).*** The mechanism of hepatic injury caused by CCl_4 has been extensively studied. It is oxidized by

the cytochrome P-450 2E1 to the CCl_3 and CCl_3OO free radical ***(Anker, 2001)***. Oxygen strongly inhibits the hepatic cytochrome P-450- mediated formation of CCl_3 from CCl_4 and promotes the conversion of CCl_3 to CCl_3OO. Both free radicals injure the hepatocyte by causing lipid peroxidation and binding covalently to cell structures ***(Burk et al., 1984)***.

In contrast, CCl_4-induced free radical processes appear to cause early inactivation of Ca^{2+}-sequestering capacity of endoplasmic reticulum. CCl_4 treatment of hepatocytes causes a sustained elevation of intracellular Ca^{2+} by affecting Ca^{2+} regulation at the level of the endoplasmic reticulum, plasma membrane, and mitochondria. Such sustained elevation of intracellular Ca^{2+} has been associated with mitochondrial dysfunction, endonuclease activation, protease activation, phospholipase activation, and perturbation of cytoskeletal organization, all of which may lead to irreversible cell injury. Other mechanisms have been proposed to explain the hepatotoxicity caused by CCl_4 . Aldehyde products of lipid hydroperoxide degradation, such as 4-hydroxynonenal, are toxic to hepatocytes and have been considered "toxic second messengers". Additionally, following CCl_4 treatment, 4-hydroxyalkenals are generated at levels high enough to account for these toxicities. Another particular novel mechanism to explain CCl_4 hepatotoxicity is that the agent

activates Kupffer cells, which are phagocytic cells lining the hepatic sinusoids, to release products toxic to hepatocytes ***(Dahm and Jones, 1996).***

Factors that impair the capacity of the liver to metabolize CCl_4 diminish its toxicity and vice versa. The observation in animals that an initial sublethal dose of CCl_4 decreases or abolishes the hepatotoxicity of subsequent larger doses support the concept that liver injury from nonlethal doses suppresses the activity of mixed-function oxidase system, preventing subsequent doses of CCl_4 from being metabolized to its hepatotoxic metabolite. Regardless of the mode of administration (oral, inhalation, subcutaneous, per-rectum, or intraperitoneal) the hepatic lesion are similar ***(Gitlin, 1996).***

Substances known to stimulate the hepatic cytochrome P450, such as dichlorodiphenyltrichloroethane (DDT) and barbiturates, potentiate the hepatotoxicity of CCl_4. Likewise, the concomitant ingestion of alcohol enhances the hepatotoxicity of CCl_4, possibly because of enhanced microsomal activation and biotransformation of the CCl_4 ***(Charbonneau et al., 1986).*** Several phenothiazines (chlorpromazine, promazine, and promethazine) and antihistamines have been shown to prevent hepatic necrosis induced by CCl_4. The protection appears to be a manifestation

of the membrane-stabilizing effects of these compounds ***(Gitlin, 1996).***

Accidental or intentional (suicidal) intoxication by carbon tetrachloride is relatively uncommon these days. Industrial or domestic accidents account for most cases. Industrial exposure usually occurs by inhalation of the fumes in a poorly ventilated environment. Domestic exposure occurs following the inhalation of the toxins in a confined unventilated area or more commonly ingestion by alcoholics during a bout of alcoholic intoxication ***(Gitlin, 1996).***

Within hours of inhalation or ingestion, dizziness and headache occur. Visual disturbances and confusion occur and reflect the anesthetic properties of CCl_4. Coma may occur rarely in CCl_4 intoxication, resulting from profound neurologic inhibition. Gastrointestinal symptoms (nausea, vomiting, diarrhea, and abdominal pain) usually occur in the first 24 hours. Hemorrhagic gastritis can also occur. Features of liver disease become manifested usually after 24 to 48 hours, with a rapid increase in jaundice, an enlarged liver; and a striking bleeding diathesis, with spontaneous hemorrhage. In severe cases, ascites and hepatic coma may ensue. Evidence of liver disease may persist for 3 weeks before recovery occurs. Death, if caused by hepatic dysfunction, usually occurs within 10 days of the onset of the symptoms. Renal failure reaches a peak especially during the second or third

week after intoxication. Pulmonary edema occurs, with nonspecific clinical, histologic, and radiographic features similar in type of uremia. Cardiac failure from hypervolemia, which is often iatrogenic because of over-zealous rehydration, renal failure, or sodium retention may occur and aggravate the pulmonary edema ***(Gitlin, 1996).***

Awareness of the toxicity of carbon tetrachloride and the practice of appropriate prophylactic measures are universal in industry, but carelessness still accounts for domestic instances of toxicity. None of the agents used to prevent CCl_4 hepatoxicity in animal research (antihistamines, antioxidants, and beta blockers) is effective clinically. Depending on the severity of the hepatic pathologic condition and the subsequent renal dysfunction, a diet high in calories (mostly carbohydrates) is required. When there is no renal failure or encephalopathy protein may be supplied ***(Williams and Burk, 1990).***

Bone marrow lymphocytes and chromosomes

The primary lymphoid organs are sites where lymphocytes differentiate from stem cells and proliferate and mature into effector cells. These organs are the thymus and the bone marrow *(Lewis and Harriman, 1996).*

Bone marrow is one of the large organs of the body and main site of hematopoiesis. Under normal conditions, the production of blood cells by the marrow is perfectly adjusted to the organism's functions. It can adjust rapidly to the body's need, increasing its activity several folds in a very short time if required *(Junqueira et al., 1998).*

The bone marrow is the primary site of haematopoiesis throughout life, generating over 95 % of the haematopoietic activity in adult mammals *(Mayani et al., 1992).* Essentially all of the medullary space in the bones is occupied by haematopoietic tissue in mice and rats; in contrast, in adult humans, haematopoietic tissue is restricted to the proximal epiphyses of the long bones, the central skeleton, and skull; most bone marrow is gradually replaced by fat cells *(Valli et al., 1990).*

The bone marrow provides an environment for the development of most of the white blood cells of the body. At birth, most bone cavities are filled with actively dividing blood-forming elements known as "red" marrow. As development proceeds, the tibia and femur become filled with fat cells, beginning about the age 3 to 4 years; however, the ribs, sternum, iliac crest, and vertebrae remain 30% to 50% cellular throughout life and continue to produce hematopoietic cells. The main components are found in the bone marrow : the blood vessels, the cells, and the extracellular matrix ***(Lewis and Harriman, 1996).*** The production of cells from the hematopoietic stem cells occurs in areas separated by vascular sinuses. The walls of the surrounding sinus contain a layer of endothelial cells with endocytic and adhesive properties. These specialized endothelial cells of the sinus probably produce collagen type IV and laminin for structural support. These cells also elaborate colony stimulating factors and interlukin-6 (IL-6) ***(Long et al., 1992).*** The outer wall of the sinus is irregularly covered with reticular cells, which branch into areas where cells are developing and provide anchors for those cells by producing reticular fibers. Megakaryocytes lie against this wall, touch the endothelial cells. A functional unit of marrow called a spheroid and contains adipocytes, stromal cell type, and macrophages. These

reticular cell networks compartmentalize the developing progenitor cells into separate microenvironments, called hematons *(Lewis and Harriman, 1996).*

Blood enters the marrow from nutrient arteries at the place where they bifurcate to form the central artery of the medullary canal. Branches of the central artery terminate in capillaries within the medullary space or penetrate the endosteum, where arterial blood from the nutrient artery mixes with blood from muscular arteries in the periosteal capillaries. The blood returns via the vascular sinuses to the central sinus and vein *(Weiss and Geduldig, 1991).*

Cells of the bone marrow are originally characterized by morphology. The predominant cell types are those of the myeloid lineage, which account for about 50% to 70% of the cells. Red blood cell precursors represent from 15% to 40% of the total cells. Other lineages exist in lower proportions (under 5%) *(Lewis and Harriman, 1996).*

Bone marrow lymphocytes

The pluripotential hemopoietic stem cells that generate all blood cell types constitute 0.2% of the total population of nucleated cells in the bone marrow. Development in all myelopoietic cell lineages progress from the pluripotential stem cell to a unipotential progenitor cell and then through a sequence of microscopically identifiable maturation stages

leading to functional blood cells. It is now clear that the lymphopoietic stem cells originate in the bone marrow, the unipotential stem cells destined to form T-lymphocytes leave the marrow to the blood then to the cortex of the thymus to proliferate and differentiate, then they move from cortex to medulla to acquire their characteristic surface marker. The B-lymphocytes acquire their characteristic surface markers and enter the circulation to recirculate continuously through the spleen, lymph nodes, and peripheral blood. The proliferating precursors of small lymphocytes are large cells with a pale staining nucleus, a very thin rim of cytoplasm, and few organelles, the so called transitional cells are actively proliferating and constitute about 20% of all marrow lymphocytes ***(Fawcett, 1994).***

Lymphocytes vary considerably in size, ranging from 6-14 um in diameter. For convenience, they are arbitrarily divided into small cells (6-8um), medium-sized cells (8-10 um), and large cells (10-14 um) in diameter. They are spherical cells with a large, round, dark-staining nucleus surrounded by a halo of sky-blue cytoplasm ***(Telford and Bridgman, 1995).***

Mature lymphocytes that emerge from the thymus or bone marrow are in a quiescent "resting" state they are mitotically inactive (that is, they are in the G_0 phase of the cell cycle) ***(Parslow, 1998).*** The small lymphocyte is

production by differentiating into plasma cells ***(Telford and Bridgman,1995).***

T-lymphocyte activation, by mitogens, results in intracellular changes and subsequent development into lymphoblasts, which are involved in cell- mediated immunity, and the B-lymphocytes become plasma cells, which are involved in humoral antibody synthesis ***(Bryant, 1994).***

Transformed lymphocytes (TL) are cells in an intermediate stage of immunocompetence, the process through which the resting small lymphocyte undergoes blast transformation and ultimately becomes a fully immunocompetent T-lymphocyte or plasma cell. The size of transformed cell (TC) is 12 to 35 μm in diameter and its shape is rounded or irregular, its nucleus has a finely reticulated nuclear chromatin (immature chromatin). These chromatin strands are finely dispersed with loose, indistinct clumping and poorly defined parachromatin. Nucleoli are usually highly visible and elongated or irregular in shape. Its cytoplasm is abundant and may or may not be basophilic. A clear perinuclear area may be seen ***(Anne et al., 1998).***

With the transmission electron microscope, the nucleus of TC becomes larger and clearer and the cytoplasm contains an enlarged Golgi apparatus which increases rapidly and occupies a significant space in the center of the cell.

Ribosomes increase in number and mitochonderia increase in volume. The endoplasmic reticulum develops slightly if it is a T-cell and considerably if it is a B-cell. Electron dense granules increase in number and nucleoli become elongated and enlarged *(Paraskevas and Foerster, 1993).*

Transformation can be induced in vitro by specific and non specific antigens. The most commonly used are phytohemagglutinin (PHA) for T-cell stimulation and pokeweed mitogen (PWM) for both T- and B-cell stimulation leading to mitosis *(Kay and Douglas, 1995).*

The mitosis of T-lymphocytes produces several types of cells, including **(1) T-helper cells,** which release a factor that, when it contacts B-lymphocytes, causes these to be transformed into plasma cells, capable of manufacturing antibodies; **(2) suppressor T-cells** that depress the production of antibodies by suppressing the conversion of B-cells into plasma cells; **(3) cytotoxic or killer T-cells** capable of destroying cells that are carrying an antigen that the T-cell recognizes as foreign; **(4) memory T-cells,** are primarily long-lived T-cells and some B-cells, with a life span of many months or even years, they are programmed to remember an earlier exposure to a foreign antigen *(Telford and Bridgman, 1995).*

Natural killer cells are the third member of the populations (after B and T lymphocytes) typically termed

lymphoid, on the basis of their similar morphology, distribution and function. The name is derived from two features. Unlike B and T lymphocytes, NK cells are able to mediate their effector function (i.e. killing of target cells) spontaneously in the absence of previous known sensitization to that target. Hence the terms ' *killer'* and '*natural'* were coined, without any real understanding of how or why these cells function. In morphological terms, NK cells are larger than typical T and B lymphocytes and have azurophilic cytoplasmic granules, leading to the widespread use of the complementary term large granular lymphocytes or LGLs. In physiological terms, there are two major roles for NK cells: (1) immune surveillance against tumor cells and virus-infected cells; (2) the release of cytokines early in infection to activate phagocytic cells and recruit T lymphocytes ***(Trakman and Vergani, 1997).***

Lymphocytes play a major role in the maintenance of health and in the response to and recovery from disease. Variation of quality or quantity of lymphocytes provide diagnostic data and are indicators of the response to therapy ***(Anne et al., 1998).***

Bone marrow chromosomes

In nondividing cells the chromosomes are not visible, even with the aid of histologic stains for DNA (e.g., Feulgen or Giemsa stains) or electron microscopy. During mitosis and meiosis, however, the chromosomes condense and become visible in the light microscope. Therefore, almost all cytogenetic work (i.e., studies of chromosome morphology) has been done with condensed metaphase chromosomes obtained from dividing cells either somatic cells in mitosis or dividing gametes during meiosis ***(Lodish et al., 2000).***

No tissue or organ in the healthy adult organism contains as many dividing cells directly available for chromosome analysis as does bone marrow. Since the mitotic rate in bone marrow is high, the cells in the metaphase can be prepared without culturing ***(Pfeiffer, 1974).*** The effect of toxic substances on cells and chromosomes in vivo can relatively simply be tested on the bone marrow cell population So, the bone marrow can be considered as a convenient vehicle for studies concerning the induction of genotoxicity by many environmental and microenvironmental agents ***(Ligan and Satga-Parkash, 1985).***

The mitotic index of bone marrow cells could be increased by injecting a mitotic inhibitor such as colchicine or its semisynthetic derivative colcemid prior to the harvest. In mice, the mitotic index increased within one and half

hour after injection of colichicine, the maximum being around 4 hours *(Pfeiffer, 1974).*

Chromosomes are the elements inside the nucleus, which carry the genetic information. They consist of hereditary material, deoxyribonucleic acid (DNA), embedded in a protein matrix (histone in the human). The units of inheritance, the genes, are segments of DNA *(Hafez, 1982).*

Gene was defined simply as a region of DNA capable of being transcribed to produce a functional RNA molecule. To this we can add that not only does the gene need to be capable of being transcribed into the functional RNA (for most genes, an mRNA) but this RNA must also be made at the correct time and in the correct place in the development of the organism. Only then is the gene properly functional. To accomplish this, at one end a gene has a regulatory region, a segment of DNA with a specific nucleotide sequence that enables it to receive and respond to signals from other parts of the genome or from the environment. Ultimately these activation signals are converted into regulatory proteins that bind to the regulatory region of the gene and initiate transcription in the adjacent RNA-encoding region. At the other end of the gene there is a region that contains signals to terminate the transcript *(Griffiths et al., 1999).*

Chromosomes could generally be differentiated on the basis of length, position of the centromere and staining

characteristics. The parts of a chromosome on each side of the centromere are called arms. A chromosome with nearly equal arms is said to be metacentric. When the centromere lies between the centre and one end, the chromosome is acrocentric, but human cytologists restrict this term to chromosomes in which the centromere is very near to one end. Chromosomes with centromeres intermediate between metacentrics and acrocentrics are called submetacentrics. The centromere of a submetacentric is submedian, and of an acrocentric is subterminal. Chromosomes that have short arms so small as to be nearly imperceptible are termed telocentric *(Levitan, 1978).*

Each species has a set of chromosomes characteristic in number and kind. The number of chromosomes in rat (Rattus Norvegicus) has been found to be 42 (20 pairs of autosomes and one pair of sex chromosomes). In female, the sex chromosomes are homologous, consisting of two X chromosomes. In male, the sex chromosomes are not homologous, consisting of an X and a Y chromosomes. Sex chromosomes are acrocentric but the autosome chromosomes consist of 22 metacentric, submetacentric or subtelocentric chromosomes and 18 acrocentric and telocentric chromosomes *(Dowd et al. 1964).*

DNA double-strand breaks (DSB) are considered to be critical primary lesions in the formation of chromosomal

aberrations. DSB may be induced by exogenous agents, such as ionizing radiation, but also occur spontaneously during cellular processes at quite significant frequencies. To repair this potentially lethal damage, eukaryotic cells have evolved a variety of repair pathways related to homologous and illegitimate recombination, also called non-homologous DNA end joining, which may induce small scale mutations and chromosomal aberrations ***(Pfeiffer et al., 2000).***

The genome might show numerical or structural abnormalities in autosomal or sex chromosomes. A given abnormality can affect all body cells or only two or more cell lines; so some cells will be normal while others are not and this is called mosaicism ***(Muench, 1989).***

The numerical anomalies might be polyploidy or aneuploidy. A cell containing the exact multiple haploid number of a species, more than the diploid number, is termed polyploidy. It might be due to multiple fertilization or endoreduplication ***(Carr, 1967).*** A proportion of polyploid cells occurs normally in bone marrow, since megakaryocytes usually have 8 - 16 times the haploid number. Tetraploid cells are also a normal feature of regenerating liver and other tissues. They arise by endomitotic reduplication, in which the chromosomes divide twice and the cell divides only once ***(Connor and Ferguson-Smith, 1993).***

Aneuploidy usually arises from the failure of paired chromosomes or sister chromatids to disjoin at anaphase (non-disjunction). Alternatively, aneuploidy may be due to delayed movement of a chromosome at anaphase (anaphase lag). Thus by either of these mechanisms two cells are produced. One with an extra copy of a chromosome (trisomy) and one with a missing copy of that chromosome (monosomy). Aneuploidy can arise during either meiosis or mitosis ***(Connor and Ferguson-Smith, 1993).***

Structural aberrations are referred to changes in the arrangement of chromosomal breakage followed by recombination in an abnormal order. They include deletion, duplication, inversion, translocation, isochrom-osomes, ring and dicentric chromosomes ***(Levitan, 1988).***

Deletion means loss of any part of a chromosome. It might be terminal with single break or interstitial with multiple breaks. If the deleted portion lacks a centromere (an acentric fragment) it will be lost at a subsequent cell division ***(Connor and Ferguson-Smith, 1993).***

Unequal crossing-over between homologous chromosomes results in duplication of the genes in on chromosome and deletion in the other ***(Gieser et al., 1986).***

Inversions arise from two chromosomal breaks with inversion through 180 degree of the segment between the breaks. Paracentric (excluding the centromere) inversions do not produce clinical abnormalities in the carrier. Pericentric inversion carriers are not clinically abnormal, but there is a risk of producing chromosomally unbalanced offsprings, particularly when the inversion involves a large part of the chromosome ***(Connor and Ferguson-Smith, 1993).***

A translocation is the transfer of chromosomal material between chromosomes. The process requires breakage of both chromosomes with repair in an abnormal arrangement. Three types of translocation are recognized; reciprocal, centric fusion (Robertsonian) and insertional. In a reciprocal translocation chromosomal material distal to breaks in two chromosomes is exchanged. Centric fusion translocations arise from breaks at or near the centromere in two acrocentric chromosomes with cross-fusion of the products. For an insertional translocation, three breaks are required in two chromosomes, one in a chromosome and two in the other ***(Connor and Ferguson-Smith, 1993).***

Transverse misdivision of the centromere, instead of longitudinal, results in the formation of the unequal chromosomes.The first contains two long arms and the other

has two short arms and are called isochromosomes ***(Mets, 1986).***

Ring chromosomes result when two broken ends of a chromosome are joined. If the ring thus formed does not contain a centromere, it will be lost in division. If the ring has a centromere, it could pass through cell division but, they are generally unstable ***(George, 1999).***

Dicentric chromosomes, as their name implies, have two centromeres. These rare structures reflect breakage and rejoining of centromeric fragments from either the same or different parent chromosomes ***(George, 1999).***