2 REVIEW OF LITERATURE
Cancer is the generic term used for large number and variety of malignant neoplasm possibly related to as many different causes, that arise from any of the tissues of the human body and result in deleterious effects on the host due to their invasive and metastasizing character. (3)

Cancer is older than the literature of medicine. Its evil signature has been found on palaeopathological remains in distant parts of the globe. Though the erudite German historian of medicine Kari Sudhoff doubted whether the ancient Egyptians were familiar with what we know as cancer certain descriptions in both the Edwin Smith surgical papyrus and in the papyrus Ebers are suggestive of this disease. (12)

Cancer is one of the major causes of death throughout the world. More than half of the cancer risk in men is due to the cancer of skin, prostate, lung, stomach, intestine; whereas more than half of cancer risk in women is due to cancer of breast, intestine, skin cervix uterine. (3)

Cancer is the second most common cause of death in the USA after cardiovascular diseases. Humans in all ages develop cancer and a wide variety of organs are affected. The incidence of many cancers increases with age.

Cancer is the disease caused by unlimited growth of cells. These cancer cells have diminished control of growth. They invade local tissues and spread to other part of the body. In cancer cells the genes which controls growth and interactions with other normal cells are apparently abnormal in structure and regulation.
Cancer can be caused by following three main causes -

A] Radiant energy :-

Damage to DNA is the main mechanism of radiation to cause cancer. Exposure to ultraviolet rays, x-rays and γ rays causes the formation of highly reactive compounds that can interact with DNA and other macromolecules leading to molecular damage and thereby probably contributes to carcinogenic effect of radiant energy.

B] Chemical compounds :-

It has been estimated that chemical compounds can cause cancer upto 80% of human cancers. Person's occupation, diet, life style etc. are the factors of person's exposure to carcinogenic chemical compounds.

Organic carcinogens interact directly with target molecules, while other chemical carcinogen require prior change to become carcinogenic and these chemical carcinogens called as pro carcinogen. The intermediate formed in this process is known as proximate carcinogen and final active carcinogen react with cellular components.

Procarcinogen - Proximate carcinogen - Active carcinogen.

C] Cancer caused by viruses :-

"Oncogens" are genes capable of causing cancer. These were first recognised as unique genes of tumor causing viruses, that are responsible for the process of transformation. Oncogenic viruses contain either DNA or RNA as their genome. Polyomavirus, SV-40 virus, some type of Adenoviruses, Herpes simplex virus are examples of oncogenic viruses.
Bernard Peyrilene (1735-1804) - credit goes to him being first to conduct a systematic experimental investigation of the causation of cancer; in which he attempted to identify its toxin to the spread of disease and to device methods of treatment.\(^{(12)}\)

Xavier Bichat (1801), without the aid of microscope clearly distinguished between the non cancerous stroma and the cancerous parenchyma of a tumor.\(^{(12)}\)

Muller (1938) showed remarkable knowledge far in advance in his time of the various types of tumors, which was distinguished microscopically and the described anaplasia as a frequent feature of malignant cells. His work helped to clear the way for later investigators, who began to appreciate that cancer is fundamentally an abnormal growth of abnormal cells.\(^{(12)}\)

Beryon (1912) produced cancer experimentally by injecting tar into the ears of rabbits. The account of identification of a carcinogenic compound is coal-tar is given by Kennaway (1955). He first attempted to identify the cancer producing compounds in coal-tar.\(^{(12)}\)

Mayneord (1935) discovered a characteristic spectrum of the fluorescent light of various carcinogenic product chiefly derived from coal-tar.\(^{(12)}\)

Goulden (1929), He prepared first synthetic carcinogens 1:2:5:6 dibenzathracene and its 3' methyl derivative.\(^{(12)}\)

**Nature of Cancer** :-

Cancer is a disease of the cell that is transferred to the descendants of the cell. The disease is recognized by the behaviour of population of abnormal cells with in a normal tissue, as manifested by varying degrees of morphologic disorientation, aggressive growth and invasion with ultimate destruction of the normal cell population.\(^{(12)}\)
Neoplasm :-

The neoplastic process in cancer is not a single event that induces cells with immutable, full blown Malignant characters. The basic characteristics of neoplasm are autonomy and anaplasia. Both are relative terms distinguishing cancer quantitatively from normal processes of growth and differentiation.

Autonomy :-

Autonomy is the disregard by cancer for normal limitations of growth. The penetration of normal tissue boundaries by cancer and occurrence of metastases still represent the best evidence of malignancy. Neoplasm show a wide variation in the degree of autonomy.

Anaplasia :-

Anaplasia in tumors, refers to the loss of organization and useful function. The chief activity of neoplasm appear to be self propagation. Departure from normal growth including embryonic growth, may represent either the acquisition of new properties or loss of control mechanism present in normal cells. The rate of growth alone is not a criterion of neoplasia, since many normal growth processes exceed the growth rate of many tumors.

Extensive biochemical studies have been carried out on tumor tissue. The loss of organization and function is demonstrated by reduction or loss of specific enzymatic systems. There is usually deminution in respiratory enzymes such as cytochrome and cytochrome oxidase.
Role of heridity :-

A number of rare cancers are consistent with autosomal dominant inheritance with incomplete expression, including retinoblastoma of the retina, multiple polyposis of colon, Gardner's syndrome and thyroid cancer. Xeroderma pigmentosum is an autosomal recessive defect of skin predisposing to active carcinogenesis. Chromosome breakage disorder such as mangolism increases the risk of myelocytic leukemia.

Role of environment :-

The development of cancer in species is influenced by a wide variety of changes in the internal and external environment of the host. External environmental factors are pollution in air and water, poor hygienic condition of living etc. While aspects of internal environment are hormonal and nutritional status.

Hormonal imbalance in mice leads to the appearance of at least 5 types of tumors in the tissue especially dependent on hormonal secretions in their physiology. The age of animal at the time of exposure profoundly influences the carcinogenic reaction. (120)

Reduction in specific dietary constituents also influences the incidence and time for appearance of certain neoplasm. The addition of selenium or other antioxidants to the diet reduces the occurrences of many tumors in experimental animals. Iodine deficiency may be a factor in the genesis of thyroid cancer. Deficiency of proteins and vitamins may be implicated in the development of pharyngeal cancer.

Cancer is most often treated by surgery, radiation and chemotherapy. Recently immunotherapy was added to the therapeutic armamentarium. New methods for administering radiation therapy under investigation includes in plants high, linear energy transfer (LET) radiation, hypoxic cell sensitizers. (56)
CANCER OF CERVIX

Cancer of the cervix is one of the most important cancer to which womankind is the subject. Approximately 2% of all female may expect to succumb to this legion during their life time.\(^{(118)}\)

'Cervical Cancer ' a major cancer in developing countries, has inverse relationship to socioeconomic status. It appears to be associated with poor sexual hygiene.\(^{(58)}\)

The peak incidence of this cancer occurs between 45-50 yrs. of age average age is 48 yrs. The incidence decreases after the age of 60 yrs.

Cancer of cervix occurs at great frequency and is readily accessible to the application of diagnostic procedures which are simple to execute. Pending the development of reliable chemical screening test the diagnosis of Ca-Cx will be made by the conventional methods of studing the family history, the present history, previous disease, symptoms and general physical examination with the aid of special instruments as well as biopsy or cytologic examination.

Family History :-

This yields a significant information in a few cases because there is a genetic tendency in some cancers, such patient may be more carefully watched than patient having negative family history.

Personal History :-

Significance of personal history has become evident when studing large groups of its with Ca-Cx or of endometrium. There is correlation between early marriage, early coitus, early child birth, multiple marriages with the incidence of cancer of cervix.
Age :

Advancing age predisposes to cancer. The reason for the increased incidence of carcinoma in the older age groups are explained by one possible postulate that with the passage of time, the immune system fails to respond to abnormal stimuli. This results in the failure of normal immunologic surveillance mechanism and allow neoplastically transformed cells to grow and escape the detection mechanism which would normally eliminate them.

Menstrual history :

This may be a sensitive indicator of the presence of an underlying gynecologic neoplasm.

Sexual History :

Because of the apparent relationship of sexual promiscuity to cervical neoplasia, an effort should be made to obtain a reliable sexual history.

Previous Disease :

The history of previous disease, complains, symptoms and duration of the same, plays an important role in diagnosis of Ca-Cx. Repeated and chronic infections of cervix were prone to cancer. Irregular menses and early menopause are the conditions caused by hormonal imbalance. Estrogen is known to be carcinogenic. The injection of Estrogen into mice has led to the appearance of pitutary adenoma, carcinoma of uterine cervix and leukemia.

In (1971) Herbest et. al. reported the occurrence of cancer of the vagina in girls whose mothers had taken diethyl stilbesterol in large doses during their pregnancy.

In 1975 it was shown that exogenous estrogens increase the risk of endometrial carcinoma.
The fact that viruses can cause cancer in animals has been appreciated since the turn of the century. The demonstration by Peyton Rous in 1911 which showed that a filterable agent could cause tumors in chickens sparked off an intense interest in the concept that viruses could cause cancer.

Bosch Fx et al detected Human papilloma virus in 92.9% biopsy specimens collected from 1050 patients with Ca-Cx, these samples were analysed by PCR concluding HPV is the major risk factor for development of invasive cervical lesions.

**Symptoms :-**

Strictly speaking there is no sign specific symptoms for cervical cancer. The patient may notice, the presence of a mass or an area of ulceration with the invasion of the underlying stroma as an interepithelial neoplasm becomes invasive, there may be the presence of an abnormal discharge or possibly bleeding. There are other symptoms found like dysfunctional bleeding, ovulation bleeding, failure of ovulation, traumatic bleeding, ulceration, bleeding in pregnancy, post menopausal bleeding, itching and some times endocrine symptoms.

**Diagnosis :-**

In its early stage cancer of cervix is a highly curable disease. For many years diagnosis staging or confirmation of severity of disease was based on clinical examination only.

**Cacer in situ :-** (Pre invasive cancer)

Term applied to the lesion which has passed from stage hyperplasia or dysplasia to one of the neoplasia; in which loss of normal cell growths are wholly or partly but in which the cell lack the power of invasion or metastasis.
In 1937 the Health Organization of the league nations adopted a gross classification of cervical cancer which has been in general use abroad and in most of clinics in the United States. The classification was covered in four stages: stage I, stage II, stage III and stage IV.

In 1950 this classification was simplified and modified to include preinvasive (in situ) cancer. No alteration of the basic principle of league of nations classification was made but the appearance of preinvasive cancer on clinical horizon made it necessary to add another stage, which was designated as stage 'O'.

New recommendations for the clinical classification of carcinoma of cervix were adopted at the congress of the international federation of Gynaecology and Obstetrics in September 1961 in Vienna and were enacted effective January 1, 1962. This redefined International classification includes following description.

Preinvasive carcinoma of cervix.

Stage 'O': carcinoma in situ, Intra epithelial carcinoma

Invasive carcinoma of cervix.

Stage I: Carcinoma strictly confined to the cervix.
   Stage I is subclassified as -
   Stage IA: cases of early stromal invasion (Preclinical carcinoma)
   Stage IB: All other cases of Stage I with disease confined to the cervix.
Stage II: The carcinoma extends beyond the cervix but has not extended on to the pelvic wall. The carcinoma involves the vagina but not the lower third.

Stage II is subclassified as:
- Stage IIA - No parametrial involvement
- Stage IIB - Parametrial involvement observed.

Stage III: The extension of carcinoma in pelvic and lower vagina.

Stage III is subclassified as:
- Stage IIIA - The tumor involves the lower third of vagina.
- Stage IIIB - The carcinoma has extended on the pelvic wall, on rectal examination, there is no free space between tumor and the pelvic wall.

Stage IV: The carcinoma has extended beyond the true pelvic or has involved the mucosa of the bladder or rectum.

Pap smear, calipid peroxidescopic examination; intravenous pyelogram (IVP), cystoscopy, sigmoidoscope, ultrasound, and biopsy are the investigatory methods which are co-related with clinical examinations for diagnosis and confirmation of staging. Staging provides important information that can affect the choice of treatment, protocol and assist the oncologist in making a correct prognosis. It is also helpful in comparing and assessing the result of different therapies.  

Treatment of Ca-Cx was attempted about century ago with local cauterization and later cauterization by heat.
In (1895) J.G. Clark and Eries designed and carried out a radical procedure of block dissection that excised the uterus, the surrounding tissue and ligaments including the regional lymphnodes taking special care to preserve the uterus. (30)

In (1898) Pierre and Marie Curie discovered radium and its tissue effect soon becomes apparent for by 1903 Margareta Cleaves applied it in to the treatment of cancer of cervix. (58)

In 1915 principles and techniques of treatment were established by Bailey C. Regaud, G. Forssell and hyman opening an era of co-operation between surgeons and radiotherapists for the treatment of tumor. However surgery gradually gave away to the predominance of radiotherapy for the latter seemed to offer a better result with less injury and less mortality. (58)

The advanced cervical cancer is highly metastatic and it requires line of therapy of high dose radiation and if necessary in combination with surgery.

**Radiation Oncology** :-

The administration of ionizing radiations to patients is only a part of the speciality of radiation oncology. The pretreatment definition of the extent of the cancer evaluation of patients for irradiation, care during irradiation and post treatment care and follow up examinations are vital to good radiotherapy which of course means good patient care. Such a speciality demands a thorough knowledge of the origin and clinical evaluation of the disease and the efficiency of alternative methods of treatment, an appreciation of the clinical aspects radiophysiology and radio biology and a knowledge of the pertinent physical characteristics of the radiations used. (106)
Mode of Action of Radiation on Cancer Cells:

The use of radiation therapy is dependent on this concept. It must be emphasized that radiation has no specification for cancer cells or for any other differentiated cells. It is not selective in any way. Its primary action is always destructive. Since it interferes with the biochemical processes in operation and so disrupts the biological action, its value in radiotherapy is therefore primarily dependent on the fact that which in the field of irradiation those cells more sensitive to these changes will be affected more readily and by a smaller amount of irradiation. The cancer cells by virtue of their marked biochemical activity will be more readily interfered with, then their neighbouring normal cells which are more likely to be in a resting phase and not involved in active growth. \(^{(76)}\)

Ionising radiation induces cell death by two distinct mechanisms, Necrosis and Apoptosis. Necrosis is a form of cell death associated with massive tissue damage by which in individual cell frequently preceded by increased plasma membrane permeability, swelling of matrix of mitochondria and irreversible cell swelling and lysis. \(^{(96)}\)

An alternative form of cell death termed Apoptosis results from programmed sequence of events. This form of death is characterized by condensation of chromatin, alterations in membrane permeability and degradation of nuclear DNA into oligonucleosomal fragments. \(^{(135)}\)
When considering the injurious effects of radiations, cells are pictured as containing certain critical structures that play essential roles in their reproductive capacities. These structures are called targets and are generally accepted as being DNA molecules in genes. Radiation induced damage to a DNA molecule (target) may trigger a sequence of serious cellular changes. However, if this type of DNA damage occurs singularly, it is rapidly repaired. Mammalian cells are said to have several such targets which must be hit within the same short period to end cellular reproduction.

**Radiosensitivity**:

It can be defined as the ability of radiations to biologically change (biologic change may mean cell killing or destruction of reproductive integrity) cells comprising a tumor or other tissue. These changes will ultimately manifest themselves clinically as an alteration in structure or function.

**Radioresponsiveness**:

It refers to the time required for these changes to occur and can be measured in terms of the rate at which clinical manifestations of radiation induced biological change take place.

Cancerous mass shrinks when cell death, cell loss and absorption exceeds new cell production. The changes necessary for shrinkage are:

i) Mitosis must be slowed or arrested.

ii) Existing non-mitotic cells may die at increased rate.

iii) Dead cells must be absorbed either by autolysis or phagocytosis.
Relationship of Dose and Volume:

The larger cancer requires a higher dose for several reasons. If a certain minimum number of cancer cells is necessary to produce regrowth of a cancer a larger number of cancer cells is present initially, then a higher dose of radiations is necessary to reduce the total number of cells below the critical number.

Fig. 2.1 - Relationship of size of Cancer to the dose in Rad required

High dose radiation therapy of large volume is associated with rather well defined risks of damage to normal tissue. Reduction of these risks by improving dose-time relationships, reducing irradiated volume whenever possible improving beam quality and the use of precision techniques have been major steps leading to reduced damage of normal tissue. However the fear of serious normal tissue damage is the most common cause of under treatment.
In addition to its curative usefulness, radiotherapy is one of the most valuable palliative tools available. Many cancerous masses that are not curable by any means are made to regress or are held in check by irradiation. Irradiation can make infected bleeding cutaneous or mucosal ulcers heal, obstructing pressure producing masses shrink and painful bone destroying metastasis regress. In fact the great palliative value and radiotherapy sometimes masks its curative usefulness.

There is nothing worse in cancer therapy than being only half certain of the anatomic extent of the cancer and of the histologic diagnosis and proceeding with treatment. This not only delays adequate treatment but also prejudices future adequate irradiation. The histologic diagnosis must be established if at all possible and the clinical extent of the cancer must be determined with as much precision as is practical full treatment is then undertaken with an acceptable risk.

David L. showed usefulness of radiation during his work, he observed group I consisted of 68 patients who had endometrial cancer were treated by hysterectomy followed by 3000 rad pop radium. There has not been single case of recurrence. While group II consists of 19 patients treated with only hysterectomy and of 19 patients 47.4 % patients died due to recurrent disease. (35)
FREE RADICALS

The astonishing advances in molecular biology in past two decades some times referred to in the lay press as the 'Biologic' revaluation and the developing bridge from the laboratory to the bed side have forced gynaecologists to consider increasing need for scientists who have the capacity to scan the scientific horizon as it broadens and deepens. They must seek advances in accelerating knowledge of cell growth and cell death that can be translated in to therapeutic gain in patient care.

The living systems are mainly built of nucleic acid and proteins. Nucleic acids are the guardians of the basic blue prints while the business of life is carried out by protein. The proteins thus have to share the stable reactivity of living systems. A close shell protein molecule however has no electronic mobility and has low chemical reactivity. Its orbitals are occupied by electron pairs which are held firmly. This stable firm situation can be changed by taking single electrons out of the system. This is achieved by electromagnetic radiation in which electron is raised from its original orbit to a higher energy level. This unpairs the electron and makes the molecule as a highly reactive paramagnetic free radical.⁶¹

Free radical is an atom, molecule, molecular fragment that has one or more unpaired electrons. Its consequence tendency to acquire an electron from other substances makes it highly reactive.

Free radicals can be formed by two ways -

1) Homolytic bond fission - A:B → A⁺ + B⁻
2) Electron transfer reaction - A:B → A⁺ + B⁻

These reactions can take place either by irradiation or redox reactions. Metal enzymes catalyse the electron transfer.

The individual molecules with univalent reduction are highly reactive and potentially damaging to tissues.
In humans the importance of sub cellular compartments in the maintainance of cellular integrity is wellknown. A physical damage to a lysosomal membrane can result in the release of hydrolytic enzymes into the main body of the cell with terrible results. The importance of such compartmentalization can also be extended to molecular level. Sub cellular structures themselves contain many chemicals which if able to interact directly could cause extensive damage.

The most important free radicals in biological systems are radical derivatives of oxygen. Reduction of oxygen by the transfer to it of a singal electron will produce the superoxide free radical anion (superoxide).

\[ \text{O}_2 + \text{e}^- \rightarrow \text{O}_2^{--} \]

A two electron reduction of oxygen would yield hydrogen peroxide.

\[ \text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2. \]

Hydrogen peroxide is often generated in biological systems via the production of superoxide: two superoxide molecule can react together to form hydrogen peroxide and oxygen.

\[ 2\text{O}_2^{--} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

The increasing interest in the role of free radicals in the pathogenesis of human disease has led to an increased need for techniques to measure free radicals and their reactions in vivo and most importantly in clinical situation. Free radicals are extremely reactive and thus short lived. Consequently, free radicals are not a menable to direct assay and free radical activity is usually assessed by indirect methods such as measurement of various end products of reactions with lipids, proteins and DNA. Lipid peroxidation is the most intensively studied process and provides a number of possibilities for assays.\(^{(68)}\)
Lipid Peroxidation :-

One major feature of oxygen metabolism is its interaction either molecular oxygen or as derived free radical with (PUFA) in the process described as Lipid peroxidation.

When lipid peroxidation occurs in biological membranes (e.g. endoplasmic reticulum, mitochondrial membrane or plasma membrane) there may be gross disturbance in structural organization and in associated enzyme function.

This peroxidation of lipids exposed to oxidation i.e. auto-oxidation is also responsible for cancer, inflammatory disease, atherosclerosis, aging etc. This is also responsible for the rancidity of food i.e. deterioration.

The membrane contains a relative high content of PUFA residues and often had heme or flavin as part of the basic structures. The media on both sides of the membrane contains both oxygen and trace elements. Under these circumstances the membrane is potentially more susceptible to free radical mediated lipid peroxidation damage than cytoplasmic compounds.

The process of lipid peroxidation takes place in three steps - 1) Initiation 2) Chain propagation 3) Quenching.

Initiation Step :-

Initiation step in lipid peroxidation involves interaction of PUFA with a reactive oxidizing radical \( R^\nearrow \) which abstracts a proton to form the fatty acid radical

\[
\text{PUFAH} + R^\nearrow \rightarrow \text{PUFA}^\text{\nearrow} + \text{RH}
\]
**Chain Propagation**

This is followed by chain propagation steps in which the first is the formation of fatty acid peroxyradical. This is the step in which rearrangement of double bond and acceptance of oxygen by the free radical takes place

\[ \text{PUFA}^* + O_2 \rightarrow \text{PUFAO}_2^* \text{ i.e. PUFAOO}^*. \]

The peroxy radical then interacts with another molecule of PUFA to form a semistable unsaturated hydroperoxide (ROOH) which also regenerates a molecule of free radical.

\[ \text{PUFAO}_2^* + \text{PUFAH} \rightarrow \text{PUFAO}_2^*\text{H} + \text{PUFA}^* \]

Hydroperoxide may undergo homolytic fission of covalent bonds to form free radical

\[ \text{PUFAO}_2^*\text{H} \rightarrow \text{PUFAO}^* + \text{HO}^* \]

\[ 2\text{PUFAO}_2^*\text{H} \rightarrow \text{PUFAO}_2^* + \text{PUFAO}^* + 2\text{H}_2\text{O} \]

Thus upon homolytic fission of hydroperoxide a free radical reaction chain may again be initiated. This reaction can be catalyzed in the presence of heme or certain heavy metal ions such as Cu or Fe.

**Quenching**

The chain of lipid peroxidation can be stopped by quenching.

\[ \text{ROO}^* + \text{ROO}^* \rightarrow \text{ROOR} + \text{O}_2 \]

\[ \text{ROO}^* + R^* \rightarrow \text{ROOR} \]

\[ R^* + R^* \rightarrow \text{RR} \]
The lipid peroxidation can result in a wide range of toxic effects including some at a considerable distance from the initial site of peroxidation. This is because the secondarily derived long chain peroxyl-radicals and lipid hydroperoxides can diffuse in the plane of membrane, whereas short chain degradation products including aldehydes can escape from the membrane and can cause general cellular disturbances and even escaped short chain aldehydes abduct with amino acid and polyamines. Malonaldehyde causes crosslinkage of protein chains and thus reactivity of malonaldehyde suggests that these agents can modify the fluidity of membrane that are exposed to them.

DNA is readily attacked by oxidising radicals if they are formed in its vicinity as has been clearly demonstrated by radiation biologists. It must be therefore considered a vulnerable and important target. As with proteins there appears little possibility of rapid chain reactions occurring and again we must consider that for damage to be significant it must be either 'site specific' such that damage is focused and of high density, leading to strand breaks or must elude the repair system before replication occurs, leading to mutations. The detection of oxidised nucleobase in human urine has been taken as evidence for a continual oxidative attack on DNA, even with a very high level of efficiency of repair, sufficient damage may accumulate over a lifetime to lead to mutations and hence cancer. (22)

Lipid peroxidation can be of major significance to carcinogenesis produced by free radical mechanism but it is often very difficult to primary if the contribution of peroxidation is at a primary stage leading to disease or at a later stage resulting from cancer.
Antioxidant systems :-

Free radical production in animal cells is inevitable and because they can be very damaging, defences against the deleterious actions of free radical have evolved. These are known as antioxidant defences.

Fig. 2.2 - Pro-oxidants and Antioxidants balance

In normal cells there is an appropriate pro-oxidant anti-oxidant balance. However this balance can be shifted towards the pro-oxidants when production of oxygen species is increased greatly in some pathogenic conditions. This state is called as oxidative stress and can result in serious cell damage if this stress is massive and prolonged. To overcome with these consequences of oxidative stress generating free radicals, cells has antioxidant defence systems. They exist in both intracellular and extra cellular compartments of cells and can be enzymes or non enzymes.

Intracellular Anti-oxidant Defence System :-

It involves scavanging of free radicals and reduction of detoxification of hydroperoxides with enzymes like superoxide dismutase, Glutathione peroxidase, catalase etc.
Extracellular Antioxidant Defence System:

Enzymes like Superoxide dismutase (SOD), Glutathione peroxidase (GPx), catalase gives the intracellular antioxidant protection. However in extracellular, there is very little SOD, GPx and no Catalase and under normal conditions there are no iron catalysts of radical reactions available. Hence if 
\[ \text{O}_2 \text{ and } \text{H}_2\text{O}_2 \] can be generated in reasonable quantities they will not do a great deal of damage. In ECF there is excess transferrin, much more than necessary to carry out the amount of iron that is normally carried. Uric acid has been proposed as an antioxidant recently. It acts by binding the metal ions. Extra cellular antioxidant protection is not directed towards removing \[ \text{O}_2 \] and \[ \text{H}_2\text{O}_2 \] but is directed to strapping them reacting together and making the real damaging species of \[ \text{OH}^- \].

Scavanging and antioxidant function both reduces the severity of radical reactions. Scavanging is done by enzymes SOD, GPx, catalase while antioxidant function is carried out by vitamin A, vitamin C, Beta carotene etc.

The preventive defences have been alluded above and efficiency of electron transfer and sequestration of transition metal ions. For example iron is held tightly bound to special proteins such as transferrin and ferritin. Some iron however, is postulated to exist in more reactive low molecular weight pool and moreover, the generation of free radicals may actually release free transition metal ions. An another form of preventive antioxidant defence is the removal of peroxides that react with transition metal ions to produce reactive free radicals. This includes both hydrogen peroxide and also the lipid hydroperoxides that are produced during lipid peroxidation. Catalase and Glutathion peroxidase are enzymes whose role is to safely decompose peroxides.
Other defences exist to intercept or 'Scavange' free radicals; one of these is the only known enzyme whose substrate is a free radical scavengers are not enzyme however in cell membrane the best characterised and possible the most important is alpha - tocopherol; the major member of the vitamin E family. This molecule is known as 'chain breaking antioxidant', because it functions to intercept lipid peroxyl radicals (LOO') and so terminate peroxidation chain reactions.

A third category of natural antioxidant defences are repair processes, which remove damaged biomolecules before they can accumulate and before their presence results in altered cell metabolism or viability oxidatively damaged nucleic acids are removed by proteolytic systems and oxidised membrane lipids acted upon by lipases, peroxidases and acyl transferases.

One of the principle problems associated with attempts at antioxidant intervention regime is that reactive free radicals cannot easily and specifically targetted and scavanged in biological systems. The hydroxyl radical for example will react readily with practically any molecule so the concept of a specific OH' scavanger in a biological system is nonsense. To compete with the cell constituents for reaction with the OH' radical the scavanger would have to be present at concentrations much higher than the biomolecules, which is not feasible.

**Superoxide dismutase (SOD)**

Superoxide dismutase (EC 1:15:1:1) plays major role in antioxidant defence system was first identified discovered its enzymatic activity and purified by McCord and Fridovich in 1969 from bovine erythrocytes.
Superoxide dismutase is ubiquitous among aerobic and aerotolerant organisms and is essential for defence against oxygen toxicity. SOD is metalloenzyme that catalyses the dismutation of the superoxide radical ($O_2^-$). The enzyme catalyses the reaction:

$$O_2^- + O_2^- + 2H^+ \xrightarrow{\text{SUPEROXIDE DISMUTASE}} H_2O_2 + O_2$$

This reaction can proceed spontaneously but enzyme superoxide dismutase increases the rate of reaction by more than 10,000 fold.

Transition metal cations can also catalyse the above reaction but they are neither as effective nor as plentiful inside the living cells as superoxide dismutase. There are three distinct types of superoxide dismutase depending upon presence of metal ion; Manganese containing (MnSOD), Iron containing FeSOD and both Copper and Zinc containing (Cu-ZnSOD). MnSOD and FeSOD are characteristic of prokaryotes and are closely related to each other (Steinman and Hill 1973). Cu-Zn SOD is characteristic of eukaryotes (McCord and Fridovich 1969), with the exception of the bacterium Photobacterium leiognathi, which contains Cu-ZnSOD and is usually found in symbiotic association with the Pony Fish (Puget and Michelson 1974).

No sequence homology is found between Cu-Zn SOD and MnSOD or FeSOD which suggests that Cu-ZnSOD evolved from a different origin but under the same selective pressure.

**Copper Zinc Superoxide Dismutase Cu-ZnSOD:** (Mol. wt 32000)

This was originally known as Cupreins (Mann and Keilin 1938) because their catalytic activity had not been demonstrated at that time. McCord and Fridovich 1969 reported the catalytic function of this enzyme. Cu-ZnSOD has been isolated from a wide range of eukaryotes including Neurospora and yeasts.
The Cu-ZnSOD from vertebrates, fungi and plants have very similar amino acid composition, with high degree of structural homology. It contains one copper and one zinc atom per subunit with weight of 16000 daltons. Copper plays a catalytic role whereas zinc plays a structural role.

The mechanism of enzymic dismutation of superoxide Cu-Zn SOD indicates that the copper undergoes by cycle of reduction and reoxidation during successive encounters with superoxide radical can be represented as:

\[
\begin{align*}
E-Cu^{++} + O_2^{-} & \rightarrow E-Cu^{+} + O_2 \\
E-Cu^{+} + O_2^{-} & \rightarrow E-Cu^{++} + H_2O_2
\end{align*}
\]

Over all effect \(O_2^{-} + O_2^{-} + 2H \rightarrow O_2 + H_2O_2\)

The list of effective inhibitors of Cu-ZnSOD is limited, cyanide causes reversible inhibition by the binding its carbon portion to the copper (Haffnov and Coleman 1973).

Azides also binds reversibly on to the copper of Cu-Zn SOD (Been et al 1977) but it is much less effective.

*Manganese Superoxide Dismutase MnSOD : (mol. wt. 40,000)*

MnSOD is prokaryotic enzyme characteristic but is also found in the mitochondria of eukaryotes and is extensively homologous with prokaryotic MnSOD. It is made up of two identical subunits containing one manganese atom per subunit.
The MnSOD oscillates between Mn +++ and Mn ++ during its catalytic action dismutation with superoxide radical. The MnSOD from E.Coli and chicken liver mitochondria showed similarities in gross properties suggesting their relationship might be very close. Aminoacid sequences obtained demonstrate that the mitochondrial and the bacterial superoxide dismutase are closely related where as the mitochondrial and the cytosol enzymes are unrelated.

The Manganese containing superoxide dismutase has recently been isolated from human liver mitochondria.

**Iron Superoxide Dismutase FeSOD :-**

E. Coli B actually contains two superoxide dismutases one of these is MnSOD already discussed while other is a Ferri superoxide dismutase (FeSOD) localized in the periplasmic space which can be selectively removed from these cells by osmotic shock. (49)

FeSOD shows close relationship with MnSOD from the Matrix of E coli and from chicken liver mitochondria. The increase in superoxide dismutase content of `E Coli` in response to oxygenation is actually due to an increase in MnSOD. Since this was correlated with increases tolerance for hyperbaric oxygen (Gregory Em. Fridovich I 1974). We may conclude that the matrix enzyme serves to scavenge endogenous O₂. The levels of the periplasmic FeSOD could be modified by nutritional means and this demonstrates that role and FeSOD appears to that of providing a defense against exogenous O₂. The valance of the iron in this FeSOD has been established as Fe+++.
Glutathione Peroxidase: \textit{GPx}

Glutathione peroxidase (Glutathione \( \text{H}_2\text{O}_2 \) oxidoreductase, EC 1.11.1.9) was discovered in 1957 by Mills. It is selenium dependent enzyme. It consists four identical subunits each containing one atom of selenium. It has molecular Wt 85000.

Glutathione (GSH) peroxidase is capable of reducing hydrogen peroxide as well as lipid hydroperoxides (68) and therefore may be the major mechanism for protection of cellular components from the deleterious effects of hydroperoxides. The nontoxic products formed from the enzymatic reduction of free fatty acid hydroperoxides mediated by GSH and GSH Peroxidase were identified as their corresponding monohydroxy polynoic fatty acids.\(^{27,28}\) The hydroxy acids formed can also be metabolized via the \( \beta \) oxidation pathway.

The action of GSH peroxidase in reducing hydroperoxides is dependent on the availability of GSH. The level of GSH is maintained by the reduction of oxidized glutathione (GSSG) by NADPH via GSH reductase and by de novo synthesis from glutamic acid, cysteine and glycine. The NADPH level in turn is maintained by the reduction of NADP by pentose shunt enzymes, Glucose-6 phosphpate (G-6-P) dehydrogenase and 6-phosphogluconate dehydrogenase (6-PG).

Fig. 2.3 - The action of GSH peroxidase in reducing hydroperoxides.
The activities of GSH-Peroxidase, GSH-reductase and G-6-P dehydrogenase, have been shown to increase in certain tissue after animals were exposed to ozone \(^{(26,24)}\) fed lipid hydroperoxides for prolonged periods \(^{(107)}\) or made Vit. E deficient. The ability of animals tolerant to ozone, to maintain higher activities of the GSH-Peroxidase enzyme system than the non tolerant group has been suggested as partly responsible for their greater resistance. Thus the ability of an organ or organ system to maintain the activities of this protective enzyme system appears to be an important feature in determining its relative susceptibility to oxidative damage.

The GSH Peroxidase system also has an important function in protecting erythrocytes from oxidative damage. When person because of genetic defect lack one or more of these enzymes, they become more susceptible to intravascular hemolysis which may be accelerated by drugs that promote oxidation. The ability of GSH - Peroxidase to utilize various types of hydroperoxides as substrates suggest that this very active and widely distributed enzyme system may also protect against other types of oxidative stress.

The activity of GSH Peroxidase in an animal tissue has been shown to be directly related to the availability of dietary selenium \(^{(25,123,64)}\) and selenium is an integral part of one of the GSH Peroxidase enzyme. \(^{(108,48)}\)

In addition to the selenium dependent enzyme, the presence of a non-selenium dependant GSH Peroxidase has been demonstrated in tissues of several species of animals \(^{(82)}\). While the selenium GSH - Peroxidases reacts with a wide variety of hydroperoxides including \(\text{H}_2\text{O}_2\) and organic peroxides, non-selenium GSH - Peroxidase does not utilize \(\text{H}_2\text{O}_2\) as substrate.
The presence of widely distributed GSH-Px system appears to explain nicely the lack of lipid hydroperoxides accumulating in vivo.\textsuperscript{(28)} The failure to detect significant amounts of hydroxyl fatty acid after adding GSH-Px in a nonenzymatic (mitochondria + ascorbate) and in an enzymatic (microsomal NADPH oxidase) lipid peroxidation system led McLay et al. to propose that GSH-Px may exert its antioxidant protective effect by preventing free radical attack on the polyunsaturated membrane lipids rather than the reduction of lipid hydroperoxide as in the case of free fatty acid hydroperoxides.

**Lipid peroxides, SOD and GPx in cancer**

With the increasing acceptance of free radicals as commonplace and important biochemical intermediates these have been implicated in a very large number of human diseases. It is extremely difficult to measure free radical as they have life time in microsecond and in this situation the investigator must generally rely on the measurement of products of free radical reactions referred to as their ‘Foot prints’ like Lipid peroxides.

It is essential that the role of free radicals in causation of disorder and their production as a consequence of disorders be clearly distinguished but it is very difficult as there is no border line for separation of facts and we can study these as involvement in disease:

In recent years damaging effects of lipid peroxidation, its involvement in cancer and protective role of superoxide dismutase and Glutathione peroxidase is widely studied.

Dormandy T L 1983 documented the close relationship between free radical activity and malignancy.
Fischer et al. have reported that free radicals particularly oxygen radicals play an important role in the complex course of multistep carcinogenesis.

Oberley L W, Oberley T D 1984 expressed the role of SOD and gene amplification in carcinogenesis: chemical carcinogenesis is hypothesized to be caused by destruction of DNA that enables the cells to induce MnSOD. Tumor promotion then causes amplification of MnSOD gene and the cell proliferation gene because of selective pressure exerted by the promoter. Because promoter causes cell division and chromosomal rearrangements, unequal segregation of the amplified gene results. Because cells which have high amounts of oncogene and low amount of MnSOD gene grow faster, these cells become dominant and a tumor forms.*94*

Bannister W H et. al. (1986) studied the levels of antioxidant enzymes, ferritin and total iron in human hepatoma cell line and observed over 80% decrease in Cu-ZnSOD, over 90% decrease in MnSOD over 70% decrease in catalase activity and absence of Glutathione peroxidase and Glutathione transferase activity. (10).

Bartoliam et. al. (1988) observed similar alterations in superoxide dismutase and lipid peroxidation those found in microsomal membrane from fast growing hepatomas which exhibit pronounced saturation and fatty acid pattern and lack superoxide dismutase. These observations support the hypothesis that during hepatocarcinogenesis the loss of superoxide dismutase causes an oxidative stress that increases cellular membrane lipid peroxidation as a consequence of which cell responds by synthesizing more saturated fatty acids that permanently modify cell membrane structure and properties. (8)
Yang K C et al. (1989) proposed a new parameter the ratio of Lipid peroxide and vitamin C and vitamin E \( [\text{LPO} / \text{VE+VC}] \) and used to reflect the balance between Lipid peroxide and antioxidant capability of cancer patients and controls. Results showed that average LPO / VE + VC ratio in cancer (135 cases) was significantly higher than control (222 cases). The author suggest that this ratio might be used as one of the parameters for early diagnosis and prognosis of disease. \(^{(132)}\)

Dai R. 1989 determined superoxide dismutase activity in blood of cancer patients by microchemical luminescence method. 151 cancer patients were divided in five groups; Lung cancer, Digestive tract cancer, Breast cancer, Gynaecological cancer and other cancers. The result showed that superoxide dismutase activity was closely related to the development of tumor. The level of superoxide dismutase in each group was higher than that in healthy controls. \(^{(34)}\)

Benitez - Bribiesca I et al. 1989 studied 50 patients with invasive carcinoma of cervix and 33 healthy controls. Patients were treated with radiation therapy. Serum plasminogen activators (PA), cathespin B (CB), antiprotecnase \( \alpha \) antitrypsin (AIAT), Trypsin inhibitory capacity (TIC) and antiproteolytic activity ratio (AAR) determined before and after therapy. Serum proteolytic activity was elevated many fold in all patients. The antiproteolytic activity was significantly reduced to about 50% of its normal. In patients with good response to radiotherapy a great reduction of proteolytic activity as well as a recovery to normal of AAR was observed. \(^{(11)}\)

St. Clair D R (1990) examined activity of enzyme MnSOD in normal and tumor cell models. He suggests with the results of experiment, that the lower level of MnSOD activity in tumor cell is likely to result from a reduction of enzyme concentration and in the reduction in reduced expression of the fully processed form of MnSOD transcript rather than defect in the structure of MnSOD gene in tumor cells. \(^{(129)}\)
Kumar K et. al. (1991) from experimental studies and epidemiological data inferred that serum Lipid peroxide level is increased in cancer patients, cases of post menopausal, untreated women with benign and malignant breast tumor when compared with their age matched controls. (80)

Punnonen R et. al. (1993) analysed antioxidant enzyme activities and Lipid peroxide in normal endometrium and endometrial cancer tissues from Finnish and Japanese patients. The catalase and GPx activities were significantly lower in Finnish than in Japanese. Lipid peroxide was significantly higher in endometrial cancer as compared with normal endometrium. This study suggests that endometrial cancer tissue is associated with an impaired enzymatic antioxidant defence system. (105)

Monte M et. al. (1994) states that increased oxygen free radical levels due to an over production or to a failure in the control system of antioxidants induces cellular and tissue injuries that could lead to disease. In this article they consider the use of superoxide dismutase as therapeutic agent both in human and experimental models and also refer to the administration of superoxide dismutase as a protective factor against secondary injuries during radiotherapy and to the determination of superoxide dismutase as a tumor marker. (90)

Shukla V K et. al. (1994) done estimation of lipid peroxidation product in bile from patients with cancer of gall bladder as a preliminary study. They observed higher concentration of Lipid peroxide product 4-hydroxynonenal in the bile of patients with Ca-of gall bladder when compared with normal. (122)

Oberley TD et. al. (1994) studied antioxidant enzyme activities (AE) in normal hamster kidney peroximal tubules and in estrogen induced hamster kidney cancer. In vivo kidney tumor had lower activities of Mn superoxide dismutase Cu-Zn superoxide dismutase, catalase and GPx compared to activity found in normal kidney. (95)
Durak I et. al. (1994) measured activities of ADA, XO, superoxide dismutase, CAT enzymes in cancerous and cancer free adjacent bladder tissues from 36 patients with bladder cancer and in control bladder tissues of 9 noncancerous persons. Increased ADA and decreased superoxide dismutase, CAT activities were found in cancerous bladder tissues compared with those of controls. Differences were also found between enzyme activities in the bladder of different disease stages and grades free radical metabolising enzyme activities were depressed in cancerous bladder tissue which indicated exposure of cancerous tissues to more radicalic stress. (42)

Baisubramanyam et. al. (1994) estimated Lipid peroxide, Glutathione content and activity of antioxidant enzyme in patients having cancer of cervix and values were compared with normal. Results show remarkable reduction in glutathione content and in the activities of superoxide dismutase, CAT in neoplastic tissue in stage II, III and IV. Activity of GPx and reductase were significantly lower in stage III and IV than that of normal controls. The tissue level and Lipid peroxide and activity of glutathione transferase found significantly higher than that of normal from stage II onwards. These observations suggest impaired antioxidant status in Ca-Cx. (7)

Gaucher A. S. et. al. (1995) attempted in this study to evaluate the effects on the antioxidant and Lipid peroxide status of the growth of human malignant tumors xenografted in to athymic mice. One brain tumor and two bladder tumors were xenografted into athymic mice. After growth establishment blood collected and Superoxide dismutase, Glutathione peroxidase, Lipid peroxides, selenium levels were measured. In mice xenografted with bladder tumor GPx, Se levels were found decreased and Lipid peroxide increased. Such effects were absent in mice xenografted with brain tumors. It indicates that an oxygen mediated stress exists in the animal bearing implanted tumor compared with the control group and that tumoral tissue itself is able to induce oxidative stress into its host. (52)
Bhuvaramuthy V et al. (1996) studied the effect of radiotherapy and chemotherapy on the antioxidant system of human uterine cervical carcinoma. Circulating lipid peroxide, antioxidant components, and activities of defense enzymes were estimated before and after radiotherapy and chemotherapy and compared with controls.\(^{(13)}\)

Antioxidants such as selenium, glutathione, and vitamin E are reduced in Ca-Cx. The reduced levels were normalized after treatment. Erythrocyte lipid peroxide and membrane lipid peroxide were found to be increased in all stages and ca-cx. Erythrocyte superoxide dismutase, GPx, and catalase were found to be decreased in Ca-Cx. These altered parameters were reversed to normal.

Arivazhagans et al. 1997 analysed lipid peroxide and antioxidant status in erythrocytes from 24 adult male gastric cancer patients and an equal number of age and sex matched normal subjects. LPO was markedly increased and enzymic, nonenzymic antioxidants were decreased in erythrocytes of gastric cancer patients. The study highlights the occurrence of LPO and possible breakdown of antioxidant status in patients with gastric carcinoma.\(^{(5)}\)
Trace Elements

Trace elements play an active role in various metabolic processes of body. In recent years there has been an enormous awareness and understanding of the role of trace elements in health and disease. Trace elements catalyze and control bio-chemical reactions within the body. More than half of the body enzymes have one or more of these elements incorporated at their active sites, many more of the enzymes depend upon loose association with trace elements for their activity.

Several trace elements are of great importance in a no. of biochemical processes mostly through their action on activator or inhibitor of enzymatic reactions by completing with other elements and proteins for binding sites, by influencing the permiability of cell membrane or through other mechanisms.

Trace elements are required in small concentration as essential components of biological enzyme system or of structural portion of biologically active constituents. They constitute, in total, less than 0.01 % by the weight of the total body composition and those thus for defined an essential in arrival deficiency expt. include iron, iodine, fluorine, copper, manganese, zinc, cobalt, chromium, selenium, molybdenum tin, Vanadium and possibly silicon and nickel.

The trace elements acts as a cofactor in enzymes like iron is an important constituent of succinate dehydrogenase as well as a part of heme, myoglobin and cytochromes. Zinc is involved in carbonic acid (carbonic anhydrase) and in alcohol (alcohol dehydrogenase) formation, and in proteolysis (carboxy peptidase, leucine aminopeptidase).
Copper is present in many enzymes involved in oxidation (tyrosine, ceruloplasmin, amino oxidase, cytochrome oxidase) and manganese is a part of enzymes involved in urea formation, pyruvate metabolism, and galacto transferase of connective tissue biosynthesis. Cobalt is also a cofactor of enzymes involved in urea biosynthesis and amino acid metabolism, in addition to its role in Vit B12. Molybdeum plays an important role in purine metabolism, selenium is related to glutathione peroxidase, and enzyme involved in the protection of hemoglobin, against injurious effects of H₂O₂ and vanadium blocks cholesterol biosynthesis.¹¹²

In animal deficiency studies, it has been determined that Se can prevent muscular dystrophy and linear necrosis in rats and white muscle diseases in Cattle. Chromium is needed for growth of rats and its deficiency leads to reduced life span, Corneal lesions and interference with insulin action producing a diabetic state with removal of glucose from the blood at a ratio that is 1/2 that of animal on a chromium containing diet. In other animals deprivation studies the growth of rats was only 2/3 that of control animals if the diet contained less than 1 to 2 ppm of tin or less than 0.1 ppm of Vanadium, fluoride, is essential for the development of skeleton and features of young chicks and nickel is essential for the growth of wing and tail feathers.

Variation in diet and nutrition practices among different populations have been studied by Gabriel and Venkatraman, have been associated with increased or decreased cancer risk depending upon the availability of foods and the level of individual nutrients and caloric intake or their close introduction.
Certain micronutrients may act as antioxidants and inhibit activation of proto-oncogens, detoxify carcinogenic agents, prevent formation of carcinogenic substances. Micronutrients may also enhance the functional activities of the immune system and its interacting mechanism composed of T cells, B cells, macrophages and natural killer (NK) cells of the host by enhancing various kinds of cytokine production.

Biochemical laboratory tests help in the management of patients with cancer. In some instances, a direct relationship has been found between changes in trace element contact and the severity, duration and incidence of diseases. These trace elements estimated in biochemistry laboratory can be used on biological markers of the diseased state.

According to Mar Czynski "energetic" biological trace elements [Gallium III, Germanium II, Silicon (Silica), Arsenic (V), Selenium (IV)] occurring in DNA of eukaryotic cells may improve the semiconductor properties of DNA and may influence the mechanism that control genetic expression at the electronic level. Their roles are postulated as follows -

(i) To maintain the level and direction of free sliding electron in DNA.
(ii) To modulate the electron conductivity and whole conductivity of DNA. This specific electronic nature of DNA take the form of magnetic pigeon holes in which an electric pulse is or is not stored as an area of local magnetization. These types of conductivity occurring in different parts of DNA of different cells could participate in the switch ON and switch OFF of genetic transformation in gene expression. This model may help to elucidate the mechanism of action of these naturally occurring antitumor agents and may help in understanding the role of trace elements in charge transport of DNA and in carcinogenesis. (84)
Copper :

Copper was first identified in biological materials by Bucholtz (1816) and Meissner (1817) when found it in plants. (31)

At this time the universal distribution of Copper in plant was established and it was suggested that the element participates in life process as a catalyst. It is an essential nutrient, the absence of which can lead to severe derangement in growth, physiology and metabolism and which is detected in an increasing number of natural products of biochemical importance.

Average body Copper concentration in adult vertebrates is of the order of 1.5 - 2.5 \( \mu \text{gm./gm.} \) of fat free tissue. The liver, heart, kidney, hair and brain contain the higher concentration of Copper spleen, lung, muscle and bone contain intermediate concentration. (8) While pituatory thyroid and thymus have the lowest concentration. Over 90% copper in mammalian plasma is associated with \( \alpha_2 \) - globulin, cerulopasmin, while the bwk of erythrocyte. Copper is presumed to be associated with erythrocuprein.

Copper is primary found in serum (95%) as part of oxidative enzyme Ceruloplasmin, an glycoprotein with molecule weight about 150000, the remainder present in an ionic form loosely bound to albumin. The daily adult requirement of copper is about 2 mg./day and the majority of ingested copper is rapidly converted in the liver to ceruloplasmin. Cellular copper is primarily found in mito. only 10 - 60 micro gm. of copper are excreted in the urine each day. (112)

The copper level in a normal individual is very constant. In various pathologocal conditions increased serum copper concentrations are caused by increased in both fractions especially an increase in ceruloplasmin.
The role of copper, hypocupremia and elevated liver copper concentration in Wilson's disease is well documented and the effects of copper toxicity have been clearly described. Deficiency status have been reported in malabsorption (Kwashiorkor, Sprue, Celiac disease), Marasmic infants, infants with mentes kinky hair syndrome and Nephrotic syndrome.

Elevations of serum copper have been reported in multiple sclerosis, infection, myocardial infraction, acute and chronic liver disease and Schizophrenia. There is a direct correlation of serum copper with C-reactive protein. In patients with lymphomatous diseases the role of copper has been the subject of considerable investigation. Elevated serum copper has also been reported following administration of estrogens or thyroid and pitutary hormones and after surgery.

Significantly elevated copper levels were found in congestive heart failure, pneumonia, myoma, of the uterus, rheumatic heart diseases, bronchitis, cholelithiasis, asthma, pelvic inflammatory disease, cerebral arteriosclerosis, arterial heart disease and diabetes mellitus. Hrgovicic and associates reported that measurement of copper represents valuable tool for monitoring the clinical response in Hodgkins disease.

Zinc :-

The presence of zinc in living organism and its role as an essential nutrient for plants and animals has been recognised by Raulin in 1869. Its occurrence in biological matter was first described by Lecharitter and Bellamy (1877). In same year Recault and Breton (1877) described the presence of zinc in human liver.
The recent discovery of zinc in many highly purified enzymes has revealed the diversity of its function in protein and carbohydrate metabolism, owing to its presence in several dehydrogenases of many species a peptidase and phosphatase, jointly covering the whole evolutionary spectrum by vallee (1955). The discovery of zinc deficiency as the basis of percin parakeratosis was the long expected implication of this nutrients in a natural pathological process of a higher animal species by Tucker's and Salmon.\(^{(31)}\)

In 1955 Vallee reviewed the following zinc metaloenzymes - Glycine - Glycine dipeptidase, alanyl and leucyl glycine dipeptidase, tripeptidase, glycyl-L-Leucine dipeptidase, carnosinase, aminopeptidase, arginase, dehydropeptidase, histidine deaminase, alkaline phosphatase, lecithinase, enolase, yeast and clostridium aldolase, oxaloacetic dicarboxylase.

Zinc is present in human in quantities varying from 10 to 200 micro. gm./gm. of tissue weight. (Koga 1935, Leiner 1941, Vallee and Altschule 1949). An extensive study has presented results of the mineral zinc composition of whole human bodies on a fat free basis (Widdowson et. al. 1951).

Zinc is essential for the function of several enzymes as mentioned above. Zinc has special promotional role in skin and connective tissue metabolism. Both deficiency of zinc and excessive zinc inhibit, chemical tumor induction in animals, Zinc is also associated with several immunological function. The role zinc in both T and B cell interaction as well as cytotoxic T cell and NK cell function was investigated and reported by Fernandes and Venkatraman.\(^{(44)}\)
Decreased zinc connection have been observed in acute and chronic alcoholism and Lannec's cirrhosis. Low concentration were found in bronchoitis, pneumonia and pregnancy. Decreased plasma zinc concentration have been reported in pernicious anemia, leukemia, thalassemia major, certain malignancies of tumors, acute myocardial infarction, psoriasis and other dermatoses and venous ulceration of leg. \(^{121}\)

In some pathological condition such as myoma of the uterus, schizophrenic reaction, rheumatic heart disease and focal encephalopathy. The mean value was formed to be slightly higher than normal. Elevated serum zinc has also been reported in certain cases of hyperthyroidism, hypertension, polycythemia and eosinophilia. \(^{121}\)

Reports of zinc values in biological field of cancer patients have been variable. Serum yields higher zinc levels than does plasma, presumably due to platelet break down.

In addition there is decline in zinc levels with increasing age and women demonstrate lower levels than men.

Normal red blood cells contain about 10 times the concentration of zinc in serum and about 30\% of serum zinc is bound to albumin. Zinc is presumably transported bound to a specific \(\alpha_2\)-glycoprotein. Low serum zinc levels have been observed in serum of patients with cancers of bronchous and colon. Hypozincaemia is a non specific observation and decreased serum zinc levels have been found in cirrhosis, hepatitis, lung infection, including tuberculosis, myocardial infarction, renal insufficiency, disease producing increased muscle metabolism, acute tissue injury and pregnant woman. Steroid therapy, including administration of P.O. contraceptives also produce hypozincaemia.
Patients with cancer excrete as much as three times more zinc in their urine than do normal persons. Zinc levels are lower in prostatic cancer tissue than in normal prostate tissue. But they are increased in benign prostatic hypertrophy tissue. Liver, kidney or lung harbouring metastasis has a higher zinc contact than does the corresponding normal tissue or the tumor itself.\(^{(112)}\)

It has been reported that cancerous lung and breast tissue have a higher zinc concentration than does the normal tissue. Poswillo and Cohen found that ingestion of zinc inhibited the development of tumor.

**Selenium**

Selenium approximately as rare as gold, was discovered in 1817 by the Swedish chemist Berzelius. The recent discovery that selenium is an essential nutrient completes the complex portrait gone of the most versatile mineral element.\(^{(31)}\)

It has been shown to be essential for several animal species. (McCoy and Weswing 1969, Thompson Scott 1970) and although essential has not been proven for man, several lines of reasonably from existent data suggest it. 1) Selenium has shown to be essential constituent of erythrocyte glutathione peroxidase in several species. (Rotrucke et. al. 1973) and also in human red cells. 2) Some of the urinery metabolites of selenium seen to be produced by man and by the rat, a species in which selenium is known to be essential. 3) Accumulated evidence suggest that there is homeostatic regulation of blood selenium level. 4) Finally there is evidence that selenium binds specifically to certain human plasma protein.

Several reports of the use of seleneomethionine in the diagnosis and staging of lymphomas have appeared (Herrea et. al. 1965, Ferrucci et. al. 1970). The study indicated a 70 - 80% probability that disease was present.
Most recently the relationship between methionine, vitamin E and selenium as an essential nutrient has been appreciated and glutathione peroxidase, an enzyme essential for protection, is selenium dependant.\(^{(31)}\)

Weisberger and associates (1956) have attempted to treat human leukemia and certain types of rodent tumor by making use of the competition for cellular uptake between selenocystine and cystine.

Selenite and other selenium compounds affect metabolism in a variety of ways, depending upon the concentration of selenite and the particular metabolic system concerned. The evidence considering the essential nature of selenium leads one to wonder if the nutritional effects of selenium are mediated merely through metalloactivation of possible inhibition of some enzymes system or if selenium is incorporated into an important organic metabolite in the body, Schwarz (1955) and associates (1958).

In view of the varying effects of selenium on the different vitamin E deficiency diseases, it is also possible that selenium possesses more than one beneficial metabolic effect in the animal body. The many metabolic effects already uncovered serve to demonstrate that selenium must be considered among those element which are known to have profound effects upon animal health.\(^{(31)}\)

Selenium deficiency causes cardiac necrosis in mice but not in rats. In dogs on the other hand injections of selenium in trace amounts produce cardiac damage and blood pressure changes.

Selenium levels were lower in the whole blood of leprosy patients compare to controls. The metabolic role of this enzyme is thought to be the destruction of intracellular \(\text{H}_2\text{O}_2\) and other hydroperoxidase (Lewand 1988).
Thus the finding lower selenium in patient compared to controls may be related to the specific defect in leprosy patients due to loss of ability to oxidatively kill bacteria intracellularly. A therapeutic trial in selenium suplimentation in leprosy patient would right in these patients.

Copper, Zinc and Selenium in Cancer:

Dejorge and et. al. (1965) studied 23 patients with mammary carcinoma and observed mean copper, 229 µgm./dl. which was significantly increased.

Williasalo M. A. and Halkone M. (1966) studied serum copper levels in 35 patients with lung carcinoma. They found elevated concentration of copper in patients as compared to the healthy controls.

Shamberger R. J. and Rudolph G. (1966) studied protection against carcinogenesis by antioxidant. In their experimental study they use sodium selenite as antioxidant against carcinogenesis. Sodium selenite applied as concomitantly with croton oil (tumor promoter) markedly reduced tumor formation.

Sinha S. N. and Gabrieli E. R. (1970) studied copper levels in various pathological conditions. They reported decreased plasma zinc in leukemia and certain other malignancies.

Shamberger R. J. (1970) established inhibitory effect of selenium on carcinogenesis. In their experiments of nondietary tumors promotions, sodium selenite significantly reduced the number of tumors in mice.

Hrgovic and et. al. reported high conc. of copper in 80 patients with Hodgkins disease. Level of copper conc. increased as the stage of disease advantage.
Hrgovcic and et. al. (1973) reported significant high copper conc. in their study of 236 patients with a variety of malignant lymphomas. The extent of elevation appeared to be much greater in patients with generalized disease compared to those with localised disease.

Shamberger (1973) and et. al. measured blood selenium in patients with cancer and in patients with other disease. Blood selenium values for 48 normals were 22.9 ± 3.52 µ gm./dl (S.D.) patients with gastrointestinal cancer or metastasis to gastrointestinal organs had significantly lower blood selenium values. This was true for 6 patients with Hodgkin's disease and those with hepatitis and cirrhosis. (116)

Broghamer W. L. and et. al. (1976) determined serum selenium levels on 110 patients with carcinoma. The mean serum selenium level of the 110 patients with carcinoma was 1.268 ± 0.034 µ gm./gm. of dry serum. A mean serum selenium level of control group was 1.481 ± 0.073 µ gm./gm. (15)

K. Habib Dembinski T. C. and Stich S. D. (1980) studied serum copper contents from 41 patients with benign prostatic hypertrophy and 44 patients with carcinoma of prostate. They found plasma copper levels were significantly higher in benign and malignant categories. (63)

McConneli and et. al. (1980) collected serum from 35 woman with breast cancer and evaluated serum selenium values from these patients. These levels were compared with control group which is free from breast cancer. He found low selenium levels in patients with breast cancer than those of controls. (87)
Habib F. K. Dembinski T. C. Stitch S. R. (1980) studied the Zn and Cu content of blood leucocyte and plasma from patients with benign and malignant prostate. Zn and Cu levels in plasma have been measured in 44 patients with Cancer of prostate, 41 patients with benign prostatic hypertrophy, 24 of whom were receiving some form of hormonal therapy. We found plasma Zn / leucocyte Zn levels were not affected by age or disease, whereas plasma Cu levels were significantly higher (p < 0.01) in the benign and malignant categories (124 ugm / dl). When compared to a younger normal population. Hormonal therapy induced a rapid rise in the plasma Cu concentration and a concomitant marginal fall in Zn levels of Cancer patients. (83)

Garofalo J. A., Erlandson E, Strong E. W., Lesser M., Gerold F., Spiro R., Schwartz M., Good R. A. (1980) evaluated Sr. Zn; Sr. Cu levels and Cu / Zn ratio in 50 patients with epidermoid cancer of head and neck to treatment with respect to site and stage of disease. There is a trend towards decreasing SZL and increasing SCL and Cu / Zn ratio with advanced stage of disease. (51)

Jssell B. F. (1981) determind serum zinc conc. in 26 extensive squamous cell lung cancer patients. The mean serum zinc conc. in patients was 43.2 μ gm. / dl. ± 3.6 S.E.M. (normal 80 to 100 μ gm. / dl.

Brandes J. M. and et. al. (1983) determined serum zinc levels in 73 woman hospitalized for suspected gyneocological tumor, 25 of which were having malignant tumor and 48 had benign growth. They reported significantly decreased serum zinc levels in malignant group than in benign group. (16)
Ashwi Bidri and et. al. (1983) estimated serum copper concentration in carcinoma of cervix uteri as an indicator of successful radiotherapy. They studied patients with squamous cell carcinoma of the cervix. They found the human serum copper level has been significantly elevated in those patients than control. They also reported that, there is a trend of elevation in serum copper level as the disease progresses. (4)

Pras P. and et. al. (1983) studied zinc levels in various diseases and found decreased zinc conc. (101)

Miller E. and et. al. (1983) investigated serum selenium conc. in patients with selected diseases, they found decreased conc. of selenium in serum with alcoholic cirrhosis, malignancies and chronic renal failure. (69)

Sundstrom and et. al. (1984) were determined serum selenium concentration in 37 patients with cervical cancer and 64 patients with endometrial cancer. The patients had lower (p < 0.001) serum selenium concentration than the control women. (128)

Sundstrom H. and et. al. (1984) estimated serum selenium concentration in patients with uterine, ovarian or vulvar cancer. He reported lower selenium concentration (1.15 ± 0.04 S. E. micromol / lit, p < 0.05) in patients with gynaecological cancer. (127)

Chrakrawarty P. K. and Choudhary J. R. studied (1984) serum copper levels in malignant neoplasia with special reference to the cervix uteri. They reported the serum copper level was elevated in all the different types of tumor examined. (19)

Jendryczko et. al. (1986) studied copper and zinc conc. in malignant and nonmalignant tissues of female reproductive organs. They reported in malignant sample the mean copper conc. was 110% higher for Ca-Cx, 76% higher for endometrium and 38% higher for ovary than nonmalignant, while zinc conc. in malignant group was lowered than in nonmalignant group. (75)
Fu Y. L. (1989) estimated serum zinc levels in 155 patients with
triphloblastic tumor and in 114 patients with benign or malignant
gynaecological tumor. He reported lower zinc concentration in patients with
invasive mole and choriocarcinoma.\(^{(50)}\)

Chung Hua. (1989) studied 155 patients with tropheoblastic tumor, 114
patients with benign or malignant gynaecological tumor for serum copper
estimation. He found significantly higher copper concentration than that of
controls.\(^{(23)}\)

Hokkaido (1989). Reported elevated copper in patients with
malignancy of stomach than normal. Plasma and whole blood copper conc. in
stage IV showed a significant higher levels than that of stage I.\(^{(66)}\)

Gerg A. N. and et. al. (1990) estimated copper level in cancerous
breast tissue and reported elevated copper in these patients.\(^{(53)}\)

Kobayashi M. (1990) performed his study to find out selenium
concentration from patients with stomach cancer. Plasma selenium
concentration of stage III showed a significant lower level than that of
stage I.\(^{(77)}\)