REVIEW
OF
LITERATURE
2. REVIEW OF LITERATURE

2.1 Cancer of uterine cervix:

Cancer of uterine cervix is the most prevalent cancer in Indian women and is the second most cancer after breast in worldwide. Large percentage of these (about 80%) occur in the developing countries (Parkin, 1984). It is the most frequent cancer in China, Africa, India and other South East Asian countries. India, alone accounts for approximately 16% of the global incidence. It has also been evaluated that this load of cancer of cervix will increase by 1.6 fold in absence of any control programme (Murthy et al., 1990).

2.1.1 Incidence and mortality rates of cervical cancer:

The incidence of cervical cancer changes throughout the world. In the Israel, the incidence and the mortality rate for cervical cancer tend to be lowest in the world (Day, 1976) and have dropped about 50% over the last 30 years or 3-4% per year. The highest incidence rates have been reported from South America, where the incidence is about 6 times that of the US. Surveys in UK (Cook et al., 1984; Doll, 1985) and Newzealand (Green, 1979) have shown recent increase in the incidence and mortality rates among young women. Henson (1977) compared age adjusted and age specific rates for US and UK. The age adjusted rates reported to be identical, whereas the age specific rates
increased at an earlier age in US when compared with UK. In another WHO report, Hill (1975) has reported the highest mortality rate in Chile while Egypt, Israel and Spain have low rates (0.3, 0.8, 0.8 per 100,000 respectively). In India, it has been estimated that annually about one lakh women develop cervical cancer which constitutes about 16% of the world's annual incidence (Luthra, 1983; Luthra et al., 1987; WHO Report, 1986). It accounts for about 20 to 43% of all female cancers and 20 to 85% of all female genital cancers (Jussawala et al., 1971; Wahi et al., 1972).

The age adjusted incidence data available on cervix cancer from population based registries (Bombay, Madras and Bangalore) were 17.0, 38.5 and 23.2 per 100,000 women. These values are higher than the incidence rates of Thailand (11.6), UK (9.1) and Sri Lanka (9.0) (Luthra, 1983).

2.1.2 Natural history of cervical cancer:

The natural history of cervical cancer has been established (IARC 1986), however comparative informations on this subject are lacking from the developing countries. World Health Organisation (WHO) in a meeting held in 1986, recommended the generation of such information from the developing countries. Dysplasia, in the context of the uterine cervix refers to an abnormal epithelial growth without further
indicating the biological characteristic of the lesion. Fundamentally, the dysplastic reaction is characterised by combination of hyperplasia and a block in the normal differentiation of the component cell (viz. parabasal, basal, intermediate & superficial) as they reach the uppermost cell layer (Reagen et al., 1953). Dysplastic lesions are subdivided into three groups according to the extent to which undifferentiated cells occupy the full thickness of the epithelium. Therefore all other (than CIS) disturbances of the differentiation of the squamous epithelium lining of the surface and glands are to be classified as dysplasia.

Cervical cancer consists of squamous cell carcinoma in 85-90% of cases and usually arises around the junction of cervical canal and the ectocervix. In this area, the columnar epithelium is replaced through a metaplastic process with squamous epithelium during the late fetal life, adolescence and pregnancy. Most cervical pathology occurs close to the squamocolumnar junction, known as transforming zone. Cancer of uterine cervix does not arise from a normal epithelium but it is preceded by a spectrum of abnormal epithelium changes most commonly termed as 'cervical dysplasia' (Gellman, 1976; Briggs, 1979). The changes of normal epithelium to invasive carcinoma over a variably prolonged period is a four stage process as follow-
The "dynamic" natural history modeled by Knox (1973) involved 2 components (i) the possibility that some cases of carcinoma in situ as well as dysplasia regress and (ii) the possibility that some patients who develop invasive cancer do so after so short a natural history that any reasonable screening programme would miss the preceding stages of dysplasia or CIS. The findings from the British Columbia cohort study (Boyes et al., 1982) indicate that regression is part of the natural history of CIS, especially in younger women. In 1976 report, the Task Force evaluated cross-sectional data from the British Columbia screening programme to develop a new interpretation that suggested a somewhat longer natural history of CIS than had been suspected previously. This interpretation was dependent on an assumption of the progressive nature of the natural history.

In Indian women, the natural history of cervical cancer was reported first time on a sizable cohort on a long term basis. According to this report, CIS and invasive cancer which were detected did not arise de novo. All of them preceded by various grade of dysplasias (Luthra et al., 1987). Therefore cervical dysplasia is an important precancerous lesions. The
comparative risk of development of CIS from different grades of dysplasia was 1:20 for mild dysplasia, 1:4 for moderate and 1:2 for severe dysplasia. The journey time from dysplasia to CIS was 36 to 48 months which is consistent with the duration as reported from the developed countries. Moreover, this period was not affected by either age or known risk factors. The median age at the detection of dysplasia, CIS and invasive cancer was found to be as 34.4, 38.6 & 47.8 years respectively (Luthra et al., 1987). The overall progression of dysplasia to malignancy was found to be 11.7% at the end of 54 months. Progression to cancer was the highest in severe dysplasia and the lowest in mild dysplasia.

Any cancer of the cervix that extends beyond the anatomic boundaries of the surface epithelium and the adjoining endocervical glands must be considered as INVASIVE CANCER. Although this definition is clear enough, but it is sometimes difficult to determine the anatomic boundaries with accuracy. Invasive carcinoma has been defined in international forums as a clearly invasive carcinoma of the uterine cervix in which the depth of the invasion of the stroma exceeds 5 mm. Burghardt (1979) clearly showed that invasive carcinoma may originate from well differentiated precancerous lesions of squamous epithelium that according to the
international definition would be classified as mild or moderate dysplasia. Microinvasive carcinoma is generally asymptomatic and the process of its discovery is similar to that for intraepithelial lesions.

2.1.3 Classification of cervical neoplasia:

2.1.3.1 WHO classification:

It is widely recognised cytological (Riotton and Christopherson, 1983) and histological (Poulsen and Taylor, 1975) classification of precancerous lesions of uterine cervix. According to this, the precancerous lesions are divided into two groups, dysplasias and carcinoma-in-situ (CIS). Again dysplasias are subdivided into three viz mild, moderate and severe dysplasia.

2.1.3.2 CIN Classification:

Despite the serious drawbacks of the international definition of CIS and dysplasia, the concept of two disease system has become anchored. The concept that underlines separation of dysplasia from CIS is that the latter has been established as to be an intraepithelial neoplasm, which if untreated progress to invasive cancer in 60 (Boyes et al., 1982) to 70% of cases. In contrast, dysplasia has not been established as the precursor of CIS. It has been claimed that dysplasia if undisturbed either (Burghardt, 1979 and
Table-1. Comparison between CIN and WHO classification.

<table>
<thead>
<tr>
<th>CIN-classification</th>
<th>WHO-classification</th>
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<tbody>
<tr>
<td>CIN I</td>
<td>Mild dysplasia</td>
</tr>
<tr>
<td>CIN II</td>
<td>Moderate dysplasia</td>
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<tr>
<td>CIN III</td>
<td>Severe dysplasia and Carcinoma in situ</td>
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Koss et al., 1963) persist or regress and seldom progress to CIS (Kirkland, 1963). Therefore the evidence on which the separation of dysplasia from CIS is based largely derives from histologic observations. In more recent years, a variety of sophisticated clinical and laboratory experiments have appeared concerning the natural history of cervical cancer precursors and the relationship between dysplasia and CIS. The results of the data obtained seems to be disagreement with the traditional concepts of dysplasia and CIS. Convincing evidence has been presented regarding lesions currently named dysplasia and CIS as part of a single disease system (Richart, 1967; Richart et al., 1969). It has been suggested that the continuous nature of the process be designated by a generic term, Cervical Intraepithelial Neoplasia (CIN), because the use of a compound term (dysplasia-CIS) implies a two disease system. CIN is defined as a spectrum of intraepithelial changes that begins as a generally well differentiated intraepithelial neoplasm which has tradionally classified as very mild dysplasia and ends with invasive cancer. CIN is classified into three groups namely CIN I, CIN II, and CIN III which are compatible with the WHO-classification (Table-1). The only difference is dysplasia and CIS has been replaced by CIN.
2.1.3.3 Bethesda classification:

Atkinson (1989) found the lack of consistency in subclassifying squamous epithelial dysplasia, cervical intraepithelial neoplasia (CIN) and other epithelial abnormalities of the cervix. He introduced a new system for reporting the cervical cytologic diagnosis which includes the term 'Squamous Intraepithelial Lesions' (SIL). The lesions are divided in two groups in The Bethesda System: Low Grade SIL and High Grade SIL. These encompass the spectrum of terms currently used for squamous cell precursor to invasive cancer, including the grades of CIN, the degree of dysplasia and CIS. While Low grade SIL and High grade SIL are preferred, use of these new terms does not preclude the addition of the degree of dysplasia or grade of CIN

a. Low Grade SIL:

(i) Cellular changes associated with HPV infection.

(ii) Mild dysplasia and cellular changes associated with HPV infection.

b. High Grade SIL:

This group includes moderate (CIN II), severe dysplasia and CIS (CIN III).

In this present study, CIN-classification has been used for the classification of various cervical morphologies.
2.1.4 Important risk factors:

2.1.4.1 Sexual behaviour:

Rotkin (1973) exhaustively reviewed the various epidemiologic studies on cervical cancer and concluded that the carcinoma of the cervix has a higher incidence in women with many pregnancies, in women with a young age at first marriage and those with a number of sexual partners. These factors have been examined for their possible etiological significance and some of them are highly correlated. Therefore the development of cervical cancer is strongly linked with human sexual behaviour. Studies have revealed that women who reported more than one sexual partner were at greater risk, and the risk appeared to be increased directly with the number of lifetime partners (Zunzunegui et al., 1986). In addition, women who have onset of early sexual relations are at high risk than whose sexual experiences begin later in life (Terris et al., 1967; Duncan et al., 1990). It was also found from the various parts of India (Jussawala et al., 1971; Luthra et al., 1987) that women with cervical cancer, appears to have begun sex at early age. It was also observed that women with consummation of marriage (ACM) < 18 years of age had 2.8 fold higher progression to malignancy than those with ACM>18 years.
The role of male behaviour in the etiology of cervical cancer has also been discussed. Recently cervical and penile cancers were reported to cluster geographically (Cartwright, 1980). In Indian context, for three population based cancer registries viz. Bangalore, Bombay, Madras, for the period 1982-85, a higher degree of correlation ranging from 0.6 to 0.8 (p<0.02) was observed between the age specific incidence of cancer cervix and penis (Annual Report, NCRP, 1982-85). Further support for a male associated factor was derived from studies showing elevated rate of cervical cancer in wives of men with penile cancer (Martinez, 1969; Graham et al., 1979; Smith et al., 1980) and were a several fold increase in the incidence of cervical cancer found whose husbands had been married previously to women with cervical cancer (Kesseler, 1977). The male partner study of our Institute revealed that male promiscuity alone increased the risk of cervical cancer in women by 4.7 fold and the promiscuity of either partner increased the risk by 8.7 folds (Menon et al., 1989).

2.1.4.2 Viral agents:

Viral etiology of cervical cancer has been discussed by several reviewers. A possible role of Human papilloma viruses (HPVs) has been discussed in
genital cancers (zur Hausen, 1977) and the data strongly pointed out as the causative agent which could be transmitted sexually (Reeves et al., 1989). Genital HPV infections are flat or inverted condylomata, and a number of studies have shown that 50-70% of condylomatous lesions of the cervix are associated with the spectrum of CIN (Koss, 1987). More than 60 HPV type are recognised so far and of these approximately 20 are detected in anogenital region but only 15 are found with any appreciable frequency. Type 6, 11, 16, 18, 31, 33, & 35 are most consistently associated with genital infections and also with CIN, especially 6 and 11 in lower grades of dysplasia and 16 and 18 in higher grades of dysplasia and invasive carcinomas (Crum et al., 1985).

In a case-control study, HPV 16/18 sequences were detected in 67.3% of dysplasia subjects which progressed to CIS as compared to dysplasia which failed to progress (Murty et al., 1990). Multivariant analysis showed significant association of HPV as risk factor (Das et al., 1989).

Regarding the Herpes simplex virus (HSV), there are several lines of evidence to support a possible association of HSV-2 with genital cancer. Experimental studies have shown the oncogenic potential of HSV-DNA or subgenomic viral DNA fragments. In vitro studies
have shown the oncogenic potential of partially inactivated HSV in rodent cells (Duff & Rapp, 1971). Subsequently transfection by specific restriction enzyme fragments of herpes virus DNA into cells, resulted in transformation in vitro and identification of genome that may be involved in transformation (Rapp & Howett, 1984). The two regions of HSV-2 genome has been identified as having transforming potential viz. Bgl II N HSV-2 DNA fragment (u 0.058 - 0.63) and Bgl II c fragment (u 0.42 - 0.58) contain both immortalizing and transforming potential (Jariwala et al., 1983; Iwasaka et al., 1983).

Venereal mode of Human Immuno Deficiency Virus (HIV) is also documented from the HIV infection of the uterine cervix (Roger et al., 1988). Additionally it is supported from its detection in cervical secretion of HIV infected women. An increased risk of HIV infection of the cervix may be present in the women with pre-existing inflammation or genital ulceration (Greenblatt et al., 1988). Although there is high frequency of CIN in women with HIV seropositive (Bradbeer, 1987; Hennry et al., 1989; Adachi et al., 1993), there is no evidence about the role of HIV in cervical cancer.
2.1.4.3 Bacterial and Protozoal agents

There are other venereal agents like bacterias, protozas, chlamydia which are also the risk factors for cervical cancer. Most commonly bacterial agents known are N. gonorrhea and T. pallidium. However the etiologic association of these bacterias are yet not been proved. Significantly higher seropositivity of gonorrhoea has been found in women with cancer of cervix (Duncan et al., 1990). The metabolic products of bacterial infection may have the mutagenic potential (Brinton, 1990), which increases the risk of cancer of cervix. The role of chlamydia in cervical carcinogenesis is still unclear (Schacter et al., 1982). Recent studies on the prevalence of chlamydia infection of the female genital tract has attracted the attention. Isolation of chlamydia was reported more frequently from women with CIN. The atypical changes in cervix were seen in women with chlamydial infection. In the protozoal infection, Trichomonas vaginalis is the most common sexually transmitted protozoal infection leading to cervical/vaginal discharge and nonspecific infection of female lower genitalia.

The frequency of T. vaginalis was found 13.98 % in Indian women where the more than 50% of chronic cervicitis is due to repeated infections (Bang et al., 1989). However, recent reports do not provide any
evidence (direct or indirect) about its role in cervical carcinogenesis.

2.1.4.4 Non venereal agents:

There are several other non venereal agents which are synergistically associated with viral etiology of cervical cancer viz. smoking, oral contraceptives, pregnancy and nutritional factors.

2.1.4.5 Smoking:

There are several studies which have shown the direct relationship of smoking to the risk of cervical cancer (Clarke et al., 1985; Lyon et al., 1983; Brinton, 1990). Thomas (1973) and also found smoking to remain as risk factor for CIS and other cervical neoplasm after adjustment for several established risk factors. Recent studies have detected cotinine, nicotine, and mutagenic activity in the cervical mucus of smokers (Sasson et al., 1985) and thus support a direct carcinogenic effect. Alternatively, smoking may depress the immune system and allow the carcinogens to contribute in an abnormal cellular development leading to onset of cervical dysplasia (Brinton, 1990; Winkelstein, 1990). Clarke et al. (1982) have shown the risk of cervical cancer in long term smoker women.
2.1.4.6 Oral Contraceptives

The relationship between use of oral contraceptives and cervical neoplasia has been investigated extensively. Early studies generally failed to find an association between pills use and cervical abnormalities (Worth & Boyes, 1972; Boyce et al., 1977; Melamed et al., 1973). But some studies have shown an increased risk of non-invasive cervical abnormalities for long term pill users (Peritz et al., 1977; Stern et al., 1977). Further studies have indicated that oral contraceptives use is an independent risk factor and long term users are at high risk (Veesey et al., 1983).

2.1.4.7 Dietary Factors:

Dietary intake has been etiologically implicated in a number of common cancer viz. colon, rectum, breast, ovary, and cervix. It has been estimated that 80-90% of human cancer is due to environmental factors and nutrition and diet accounts for 30% of attributable risk (Doll & Peto, 1981). Epidemiological studies have shown that deficiencies in consumption of preform of vit A or its precursor, β-carotene may increase the risk of cervical cancer and CIN (Romney et al., 1981; Marshall et al., 1983; La Vecchia et al., 1984). An increase association has been found for β-carotene, especially in women with preinvasive disease (Palan et
Vit E has also been found to be inversely related to the risk of cervical cancer (Knekt, 1988; ICPO Ann Report, 1990-91). Vit C has been examined in relation to precancerous lesions of cervix and has been found to have a protective effect (Wassertheil-smoller et al., 1981; Romney et al., 1985; Ann Report, ICPO 1990-91). Studies on trace elements (Se, Cu, Zn) showed the significant association in cervical carcinogenesis. Elevated levels of Cu and lowered Zn were found in CIN as well as in invasive cervical cancer (Grail et al., 1986; ICPO Ann Report, 1990-91). Serum Se has also been found to be lowered in women with CIN (Sundstrom et al., 1984), but Brock et al. (1991) could not find any difference in serum Se in all grades of CIN when compared to normal women.

2.2 Glutathione:

Glutathione (GSH), an ubiquitous tripeptide in which N-terminal glutamate is linked to cysteine via a non-α-peptidyl bond (Fig-1) is present in nearly all the living cells. It is usually the most abundant sulphydryl compound present in animal tissue (Meister, 1975). The biosynthesis, utilisation and function of GSH have been subjects of several reviews (Flohe et al., 1974; Arias & Jakoby, 1976; Meister, 1975). GSH is known to function directly or indirectly in many
Fig-1. Glutathione molecule (γ-glutamylcysteinylglycine).
important biological phenomenon including the synthesis of proteins and DNA, transport, enzyme activity and protection of cells from many toxic molecules. Thus the multifunctional properties of GSH are reflected by growing interest in this small molecule on such diverse subjects as radiation, oxygen toxicity, environmental toxins and Cancer (Meister, 1983).

2.2.1 Metabolism of glutathione:

It is important to realize that the synthesis of this tripeptide takes place in the absence of the normal protein synthesizing system (involving mRNA, t-RNA, ribosome and enzyme system). Therefore GSH can consequently be formed in anucleate, mature erythrocytes. GSH is synthesized intracellularly by the consecutive action of γ-glutamylcysteinylsynthetase (γ-GCS) and GSH-synthetase. The first step in the synthesis of GSH is the formation of a peptidyl bond between γ-carboxyl group of glutamate and the amino group of cysteine in a reaction catalysed by γ-glutamylcysteineyl synthetase. Formation of this peptidyl bond requires the activation of γ-carboxyl group, which is achieved by ATP (Fig-2). The resulting acylphosphate intermediate is then attacked by amino group of cysteine. This reaction is feed back inhibited by GSH. In the second step, ATP activates the carboxyl group of cysteine to enable it to combine
Fig-2. \( \tau \)-Glutamyl Cycle- The \( \tau \)-glutamyltranspeptidase enzyme is located in cell membrane and all other enzymes are cytosolic.

1. Dipeptidase
2. \( \tau \)-glutamylcyclotransferase
3. 5-Oxoprolinase
4. \( \tau \)-glutamylcysteinyl synthetase
5. GSH-synthetase.
\[
\text{AMINO ACID (A.A)} \\
\text{Y-GLUTAMYL TRANSPEPTIDASE} \\
\text{ADP + PI} \\
\text{ATP} \\
\text{GSH} \\
\text{Gly} \\
\text{Cys-Gly} \\
\text{Cys} \\
\text{AMINO ACID} \\
\text{H3N-C-H} \\
\text{CH2} \\
\text{CH2} \\
\text{O} \\
\text{5-OXOPROLINE} \\
\text{ATP} \\
\text{ADP + PI} \\
\text{Y-GLUTAMYL CYSTEIN} \\
\text{GLUTAMATE}
\]
with aminogroup of glycine. This reaction is catalysed by GSH-synthetase (Fig-2). The breakdown of GSH is catalysed by \( \gamma \)-glutamyl transpeptiase \( (\gamma\text{-GTP}) \) which catalyses the transfer of the \( \gamma \)-glutamyl moiety to acceptors viz aminoacids. GSH occurs mainly intracellularly and a major fraction of the transpeptidase is on the external surface of the cell membranes. GSH transported across the cell membranes interacts with \( \gamma\text{-GTP} \). \( \gamma \)-Glutamyl aminoacids formed by this enzyme are transported into the cell. This \( \gamma \)-glutamyl aminoacid is cyclized to 5-oxoproline and transported aminoacids is released into the cell. The ATP dependent conversion of 5-oxoproline to glutamate is catalysed by 5-oxoprolinase. The cysteinylglycine formed is split by dipeptidase to give free cysteine and glycine which are again recycled to form GSH. These reactions give rise to a cycle known as \( \gamma \)-Glutamyl Cycle (Fig-2). Therefore GSH also participates in the transport of aminoacids through this cycle.

2.2.2 Transport of glutathione:

The intracellular levels of GSH in mammalian cells is in millimolar range (0.5-10 mM), whereas micromolar concentration are typically found in blood plasma. Several lines of evidence (Meister, 1975)
indicate that $\gamma$-GTP is accessible to external substrate, and that enzyme is largely bound to the outer surface of the cell membrane. But many findings indicate that intracellular GSH is the major substrate of GTP. The finding of an enzyme and its substrate on opposite side of the membrane led to postulate that intracellular GSH is transported to the membrane bound GTP (Griffith et al., 1981). In the absence of significant GTP deficiency, substantial amount of GSH appears extracellularly. When the inhibitors of GSH synthesis e.g. buthionine sulfoximine are given to mice and rats, plasma GSH levels decrease substantially (Griffith & Meister, 1979a; Griffith & Meister, 1979b). The rapid and marked increase in plasma after inhibiting GTP, and the considerable decrease seen soon after inhibition of GSH synthesis indicate the active turnover of plasma GSH. The finding suggest that there is normally an appreciable flow of GSH from liver into plasma. Liver GSH is transported in substantial amounts to the hepatic vein plasma and to the bile.

Intracellular GSH is normally over 99% reduced GSH and this form is the major transported form. Analyses of mouse blood plasma (Griffith, 1986) and rat blood plasma (Anderson & Meister, 1980) and rat bile show that about 90% is in reduced form. Under condition of marked toxicity or oxidative stress, intracellular
GSSG increases substantially, and there may be a mechanism for its export. It has been concluded that the export of GSH from erythrocytes involves the transport of GSSG (Kondo et al., 1981); however, normal transport of GSH is difficult to exclude. GSSG transport was reported in erythrocytes whose GSSG level (normally, <0.1%) was artificially raised by a GSH-oxidizing agent.

2.2.3 Antioxidant Function:

2.2.3.1 Glutathione reductase:

GSH cycles between the reduced form (GSH) and an oxidized form (GSSG) in which two GSH molecules are linked by a disulfidryl bond. Glutathione reductase (GR) catalyzes the reduction of GSSG to GSH (Fig-3).

\[
\text{GR} + \text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{FAD}} 2\text{GSH} + \text{NADP}^+ 
\]

This reaction is virtually irreversible and thereby high GSH:GSSG ratio. GR is a flavoprotein and utilizes the reducing potential (NADPH) generated by glucose metabolism (Pentose phosphate pathway), to maintain GSH in reduced state. This enzyme has been extensively investigated, and numerous successful purifications have been made from a large variety of sources such as microorganism (Woodin et al., 1968; Mavis et al., 1968), plants (Mapson et al., 1963) and erythrocytes (Scott et al., 1963). The substrate
specificity of this enzyme is rather limited. It has a high specificity for NADPH over NADH. The native enzyme usually a dimer of MW of 110 KD to 120 KD.

The primary and ambiguous role of GR is to regenerate reduced GSH that has oxidized-

(i) nonspecifically by oxygen radical or peroxides
(ii) enzymatically through GSH-peroxidase reaction and
(iii) spontaneously or enzymatically by means of thiol-sulfides exchange reactions.

In human erythrocytes, the capacity of this enzyme seems large enough to meet this demand ever under extreme condition as long as sufficient supply of NADPH available (Beutler, 1974). However, in case of impaired NADPH production, as in genetic glucose-6-phosphate dehydrogenase (G6PD) deficiency, GR activity tends to be elevated. This observation suggests that under special condition the overall production rate of GSSG may be regulated through the activity of GR. This enzyme is rapidly and irreversibly inactivated by the antitumor agent BCNU by apparently alkylating an essential sulfydryl on the enzyme (Frischer et al., 1977).

2.2.3.2 Glutathione peroxidase:

Selenium (Se) containing glutathione peroxidase (GPX) catalyses the conversion of reduced GSH to
oxidized GSH by reducing peroxides in a general reaction (Fig-3).

\[
\text{GPX} \quad \text{ROOH} + 2 \text{GSH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O}
\]

where R may be \(^\dagger\)H or an organic residue. These acceptors substrate comprise a variety of biochemically important compounds such as unsaturated lipids, steroids, nucleic acid and prostaglandins (Nugstersen & Hazelhof, 1973). This enzyme to be common in mammals has also been detected in bacteria. In higher animals, the enzyme is found in most of the tissues (Mills, 1960; Pirie, 1965) including liver, kidney, erythrocyte, stomach and spleen.

The homogeneous human erythrocyte enzyme has a native MW of 95 KD and consists of 4 identical subunits of approximately 23 KD. This enzyme is a selenoprotein containing one residue of selenocysteine per subunit (Flohe et al., 1973). This enzyme acts complementarily to catalase in eliminating H\(_2\)O\(_2\), especially in tissue devoid of catalase, such as lens of eyes. Although H\(_2\)O\(_2\) might be a physiological product of minor toxicity, it may contribute efficiently to the maintenance of free radical chain via the Harber weiss cycle or similar reaction (Azzi et al., 1974). Enzyme acting on H\(_2\)O\(_2\) may thus be of critical importance in preventing oxidative damage to biological structure. In this
respect this enzyme is obviously helpful to terminate free radical chains. A further function of GPX may be seen in its possible role in the biosynthesis of prostaglandins (Nugstersen & Hazelhof, 1973). The hydroxyl group at position 15 of prostaglandins is derived from 15-hydroxy group, which is reduced with GSH in the presence of GPX.

Selenium, which is the integral part of the GPX enzyme, is an essential trace element in mammals. This element has been most extensively studied in recent years (Shamberger, 1983; Stadtman, 1974; Diplock, 1976). The nutritional importance of Se has been reviewed recently by Combs & Combs (1986). Naturally occurring Se in foodstuff is mostly found as organic form that are bound to aminoacids such as selenocysteine and selenite bound to protein. Se content of plant foods varies according to the concentration of Se in the soil. Some areas are known to be Se deficient (Combs & Combs, 1984). Se intake varies among populations from less than 60 to 200 ug/person/day. Se deficiency results in characteristic disease; example include liver necrosis in rats and pigs, necrosis of heart, liver, kidney, pancreas in mice. Deficiency of Se is manifested in two ways, one involves GPX and other involve a protein(s) containing acid labile Se.
Two mechanisms were proposed for the incorporation of Se into GPX; one is the de-novo biosynthesis of the enzyme molecule with selenocysteine and other is incorporation of Se by post translational modification (Sunde et al., 1980). Se may enhance the activity of certain mixed-function oxygenases (Capel, 1980; Chow & Garirola, 1984). Although there is no evidence for involvement of Se in hormone production or action, there is some basis for considering 'Se' as an affector of immune function (Combs & Combs, 1986d). This includes the findings of impaired humoral immune responses in Se deficient animals.

2.2.3.3 Glutathione S-transferase:

GSH also interacts with foreign compounds to form GSH conjugates. This is catalysed by Glutathione S-transferase (GST). GSH conjugates are typically converted to mercapturic acids by a series of reactions initiated by \( \gamma \)-GTP (Chasseud, 1979) in which \( \tau \)-glutamyl moiety of the conjugate is transferred to an acceptor (Fig-3); the resulting cysteinylglycine conjugate is converted by the action of dipeptidase to the corresponding cysteiny1 conjugate which is N-acylated to form a mercapturic acid. This may be excreted in the urine or bile.
GSTs occur in substantial quantities in liver and other mammalian tissues e.g. erythrocytes (Marcus et al., 1978), and intestine (Pinkus et al., 1977; Clifon & Kaplowitz, 1977). Like GSH, GSTs are located principally in the cytosol. Crude differential centrifugation experiments showed that at least 80% of GST activity was present in soluble supernatant fraction of rat liver. GSTs from rat liver are dimeric enzymes that may contain 4 types of subunit viz Ya (Mw22,000), Yb (Mw23,500), Yb(Mw23,500) and Yc (Mw25,000). They may combine to form six isozymes. Therefore GSTs are a family of multifunctional proteins which play a crucial role in the protection of cells against the harmful effect of cytotoxic and genotoxic chemicals (Chasseaud, 1979).

The mammalian cytosolic GSTs enzymes can be classified by their substrate specificity, isoelectric points, amino acid sequence homology and immunological relationship into three species independent classes as acidic, ξ; basic, η; and near neutral, μ (Mannervik et al., 1985). The GST-ξ class isozymes are of considerable interest because of their potential role as early markers for cancer and their association with the development of antineoplastic drug resistance (Waxman, 1990). The GST-ξ is the predominant form in the most extra hepatic tissue, including brain (Polidoro et al.,
1984), placenta (Mannervik et al., 1981), erythrocytes (Marcus et al., 1978) and breast (Di Ilio et al., 1985). The erythrocyte GST has a native molecular weight of 47.5 KD and is composed of two identical subunits. GST also catalyses the Se independent GPX activity which have relatively low activity towards organic hydroperoxides, but none at all towards H2O2 (Ketterer et al., 1986).

2.2.4 Pentose phosphate pathway:

Pentose phosphate pathway is an important branch point in carbohydrate metabolism in which D-glucose 6-phosphate (G6P) is converted into D-ribose sugar (Fig-4). It is a multicyclic process in which three molecules of G6P give rise to three molecules of CO2 and three 5-carbon residues. The latters are arranged to regenerate two molecule G6P and one molecule of glyceraldehyde 3-phosphate (Gly 3 P). Since 2 molecule of gly 3-P can regenerate G6P, glucose may be completely oxidized by this pathway.

\[
\begin{align*}
3 \text{G6P} + 6 \text{NADP} & \rightarrow 3 \text{CO2} + 2 \text{G6P} + \text{Gly 3 P} + 6 \text{NADPH} + 6 \text{H} \\
\end{align*}
\]

This pathway is the source of D-ribose for nucleotide synthesis and the major source of cytoplasmic NADPH. This pathway is composed of two parts (Fig-4).
Fig-4. Pentose phosphate pathway and its related enzymes.

1. Glucokinase (GK)
2. Glucose 6-phosphate dehydrogenase (G6PD)
3. Gluconolactone hydrolase (GLH)
3. 6-phosphogluconate dehydrogenase (6PGD)
D-GLUCOSE
(I) GK
D-GLUCOSE 6-PHOSPHATE

(2) G6PD

D-GLUCOSE 6-PHOSPHATE ^----------NADP+
ir

NADPH+H+

6-PHOSPHOGLUCONOLACTONE

(3) GLH

6-PHOSPHOGLUCONATE

H2O, Mg^2+

(4) 6PGD

6-PHOSPHOGLUCONATE NADP^+

NADPH+H+

3KETO-6-PHOSPHOGLUCONATE

CO2

RIBULOSE 5-PHOSPHATE

PART-I
OXIDATIVE PHASE

PART-II
NON OXIDATIVE PHASE

XYLULOSE 5-PHOSPHATE RIBOSE 5-PHOSPHATE

D-FRUCTOSE 6-PHOSPHATE

D-GLUCOSE 6-PHOSPHATE
Part 1: Oxidative Phase: Oxidation of the reducing +
+C' of G6P to CO2 by two equivalents of NADPH + H.

Part 2: Non-oxidative Phase: Reorganisation of the PP-
pathway products back to G6P.

2.2.4.1 Dehydrogenases:

The dehydrogenases of the pentose phosphate
pathway provide NADPH which is used by GR to reduce
GSH. The common metabolic pathway involvement of these
dehydrogenases with GR suggests that the structural
and functional properties of the individual proteins
should provide some interesting correlation (Rosemeyer
et al., 1931). Glucose 6-phosphate dehydrogenase
(G6PD), one of the dehydrogenases of pentose phosphate
pathway is uniformly found as a soluble or cytosolic
enzymes. This enzyme dissociates to subunits of
relative molecular mass of near 50-60,000 (Levy,
1979). Estimates for the enzyme from human
erythrocytes are within the range at 52,000, 53,000, or
59,000 D (Rattazi, 1968; Shreve & Levy, 1977). This
enzyme is specific for the α-anomer of G6P. The
optimum pH for this reaction is 8 and it requires
magnesium ions (Levy, 1979). The specific activity is
often in 300-500 U/mg range for microbial enzyme when
assayed at 25 C. The human erythrocyte enzyme has been
frequently isolated with a specific activity near 180 U/mg. Human G6PD is the product of a single gene located on X-chromosome (Krikman et al., 1965). About 185 variants of this enzyme have been tabulated (Beutler et al., 1973). 6-Phosphogluconate dehydrogenase (6PGD) is the second dehydrogenase of pentose phosphate pathway. But this enzyme has not received as much attention as the first enzyme, G6PD. In the human tissue, the first isolation of this enzyme was from erythrocyte (Pearse et al., 1975). This is a soluble enzyme located in cytosol in animal tissue. Under denaturing conditions, the enzyme dissociates into subunits between 50-60,000 D in relative molecular mass in microorganisms (Scott et al., 1973; Rippa et al., 1969). The native enzymes are dimers of 100-120,000 d. This enzyme has the specific activity of 15-20 U/mg in mammals and Kms are in the range of 10-70 uM for NADP (Betts et al., 1975; Toews et al., 1976).

2.3 Glutathione and carcinogenesis:

Despite of intensive research efforts throughout the world, cancer still remains a mystery and is a universal problem. Of the global 6.5 million new cases of cancers occur and almost half of these occur in the developing countries. Carcinogenesis is the multistage process in which the basic structure of DNA is the
target for the initiation followed by promotion with various factors such as viruses, chemicals and/or radiations and ultimately to progression to neoplastic cells (Miller et al., 1971). Though the tumor virology is a major focus of attention these days, but epidemiological studies have suggested that 80-90% of all human cancers are caused by various factors present in the environment. Environmental factors, acting in presence of possible genetic factors are recognised as important determinants of human cancers. Chemicals, man-made and naturally occurring comprise the predominants environmental carcinogens (Higgson, 1969; Higgson & Muri, 1973). For carcinogenesis and other toxic effects, most of the compounds, being inert in their native state require metabolic activation by the enzymes in the host tissue. The accepted hypothesis is that chemical carcinogens are converted to electrophilic metabolites which have a very high affinity towards nucleophilic centers containing electron rich atoms (Miller, 1973; Heidelberger, 1975). The interaction of these electrophilic sites of the ultimate carcinogens with nucleophilic center of one or more of the informational molecules (DNA, RNA, or protein) of the target tissue leads to the activation of the oncogenes and eventually causes initiation, the first step of carcinogenesis (Miller et al., 1971;
Miller, 1973). Therefore it makes it essential to protect man by excluding and to elucidate the molecular mechanism involved in the induction of cancer by chemicals and their detoxication.

2.3.1 Glutathione and glutathione reductase:

Potentially significant connection between GSH and carcinogenesis have attracted the attention of several reviewers in recent years (Meister & Griffith, 1979; Cammeron et al., 1978; Richards & Astrup, 1982). Antitumor potential of GSH has been assessed by several authors (Cook et al., 1984; Novi, 1981). It has been suggested that GSH may be important as anticarcinogenic as a free radicals quencher. The free radicals are thought to be closely connected with a variety of pathological events, cancer and aging (Pryor, 1976-84). Therefore GSH provide some protection against the potentially mutagenic and carcinogenic effect of irradiation by quenching these free radicals. The GSH might also repair radiation-induced damage to nucleic acid and other site of free radical attack. GSH detoxify peroxides and superoxides which are thought to be toxic to the cell membrane and other genetic material by Se-dependent GPX. GSH also detoxify various categories of carcinogens catalysed by GST enzyme (Chasseaud, 1979).
Recently increasing attention has been paid to the presence of GSH and its related enzymes activities in tumor cells and tissues. In most of the cases differences in the activities of several GSH metabolising enzymes and in the levels of GSH have been found between normal and malignant cells (Peskin et al., 1977; Bauer & Wendel, 1980; Bozzi et al., 1979; Siegers et al., 1984). Administration of certain carcinogens have been found to increase the levels of GSH and γ-GTP (Cammeron et al., 1978). Novi (1981) has recently found that GSH may also prevent tumor growth or cause tumor regression when given to rats bearing aflatoxin-B1 induced hepatocellular carcinoma. GSH levels have been shown to increase also in liver following carcinogen administration (Meister & Griffith, 1979). Elevated GSH was found in the erythrocytes of patients with adenocarcinoma of gastrointestinal and with leukemia (Engine, 1975; Ozsoyulu, 1970). Significantly higher GSH content was found in the transitional cell carcinoma of the urinary bladder. Conversely, various other authors reported decreased levels of GSH in several malignancies (Mohandas et al., 1984; Drozdzcz et al., 1988; Corrocher et al., 1986). Beutler & Gilbert (1985) showed decreased plasma GSH levels in several malignancies. Low GSH content was reported in the gastric
adenocarcinoma and in urinary bladder cancer (Engine, 1990; Mohandas et al., 1984). Drodzdz et al. (1988) has also found lower GSH content in the neoplastic tissue of larynx. Similar results were obtained in the hepatoma (Corrocher et al., 1984). Low GSH levels have been found in many tumor cell lines (Ali-osman et al., 1990; Carmicheal, 1988).

Anticarcinogenic effect of GR enzyme is thought to be through its role in providing reducing potential to maintain the GSH/GSSG and NADPH/NADP ratios. Lauderberg et al. (1982) have shown that under condition of toxicity and oxidative stress, the intracellular levels of GSSG increases substantially. Lower activity of GR enzyme has been found in the genesis of urinary bladder cancer (Mohandas et al., 1984). In contrast, higher activity of GR was found in the neoplastic tissue of breast (Di Ilio et al., 1985) and in the neoplastic mammary tissue of mice (Hilf et al., 1970). However the erythrocyte GR is a flavin enzyme that has an important role in the stablization of erythrocyte membrane. The GR activity, in presence and in absence of FAD has been used to assess the riboflavin status. Several studies indicated that the riboflavin deficiency inhibits the tumor growth in experimental animals and possibly in man. Variety of epithelial changes including atropy, hyperkeratosis,
ulceration have been thought to be produced in animal and man as a result of riboflavin deficiency. Azo-dye induced carcinogenesis in liver has been thought to be a special case in that riboflavin deficiency increases the potency of these drugs in tumor causation. In tumors that have been induced by azo dye, FAD concentration has been found to be markedly reduced and that may be due to increased riboflavin excretion and enhanced FAD degradation (Yang et al., 1966). In a clinical study of patients with cancer of larynx, the riboflavin concentration in blood found to be decreased.

2.3.2 Selenium (Se) and selenium dependent glutathione peroxidase (GPX):

2.3.2.1 Selenium:

Nelson et al (1943) reported first time that the relatively high dietary levels of Se could produce the liver cell adenomas or low grade carcinoma in rats. In contrast, Clayton & Bauman (1949) indicated that high levels of Se protected against the azo-dye-induced hepatic tumors in rats. Therefore a decade before it was unclear whether Se could be carcinogenic or anticarcinogenic. In latter 1950's, the independent work of Schwartz et al (1957) and Patterson et al (1957) showed the nutritional role of Se related to vit
E. This recognition and the consideration of the use of Se compounds as a nutritional supplement to animal feeds again put focus on the possibility of a relationship of Se and carcinogenesis (Scott, 1973).

Several studies with rats finally confirmed the anticarcinogenic role of Se and therefore it might function in somehow to reduce the risk of cancer. Se probably affects both tumor initiation and promotion (Ip, 1981) and various in vitro studies have investigated the effect of Se on the growth of the tumor cell lines. It has been reported that Se has a biphasic effect on the growth response and DNA synthesis in mouse mammary epithelial cell line YN4; at low concentration (50 nM), it enhanced the cell growth (Medina et al., 1981; Medina et al., 1988). In contrast, higher dose (5 uM) inhibited the cell proliferation.

Several mechanism have been proposed for the anticarcinogenic action of Se. Various studies have examined that under some circumstances the constitutive activities of cytochrome P450 and at least hepatic mixed-function oxygenases may be enhanced by Se (Capel et al., 1980; Chow & Gairola, 1984). Medina (1986) suggested that the anticarcinogenic effect of Se results from the cellular responses based upon the endocrine and/or immune system. While there is no
evidence for involvement of Se in the hormone production or action, there is some basis for considering Se as an affector of immune function (Comb & Comb, 1986d). This includes the findings of impaired humoral immune responses in Se-deficient animals. Since such responses are dependent upon the function of T-cell and macrophage in addition to the B-cell, it is possible that they indicate the secondary effect on the cell of first and/or seconds types.

Griffin (1979) suggested that anticarcinogenic activity of Se through its role as an essential component of glutathione peroxidase (GPX) enzyme which is the important protection from the oxidative stress. Several studies have shown that blocking of the promotional phase of carcinogenesis in the skin of mice is thought to be mediated by the Se's antioxidant properties (Shamberger, 1970) which may be due to the changes in the activity of GPX enzyme. Anticarcinogenic effect of Se also involved the perturbation of normal metabolism due to the presence of large amounts of Se compounds (La Boeuf & Hoekstra, 1985).

Several studies have shown high levels of Se to inhibit cell proliferation in way in related to effect on protein synthesis (Vernie et al., 1980; La Boeuf & Hoekstra, 1985). The studies of Vernie et al (1974)
suggested that such effects may be mediated by an intermediate similar to GSSG i.e. metabolic product of reduction of selenite by GSH, GSSeSG. The anticarcinogenicity of high levels of Se may also involves the formation of other more stable selenotrisulfides, such as those of protein, which impair the the tumor cell metabolism (Schwartz, 1975). In summary, it is possible that anticarcinogenic action of Se may be mediated through one or more several mechanisms which have been shown experimentally to be plausible.

Both animal and ecological studies have supported the hypothesis that low intake of Se is associated with an increased risk of cancer (Medina & Morrison, 1988). Ecologic studies of mortality rates by area have shown an inverse correlation between the Se levels in forage crops, diet or blood, and cancer mortality (Schrauzer et al., 1977; Clark, 1985; Yu et al., 1985). Schrauzer et al. (1977) reported that age-adjusted mortality rates within US for cancer of breast, colon, rectum were inversely related to the blood Se status. Clark et al (1984) studied the relationship of Se status and the incidence of nonmelanomous skin cancer in a free living population of Eastern North Carolina. Salonen et al (1984) showed that mean concentration of Se was significantly lower in cancer cases than in controls in
a case-control study of Finland. The study of Willett et al (1983) revealed a relative risk of 2.0 for cancer subjects in lowest versus highest quantiles of serum Se levels. Other researchers have also shown the blood Se in patients with neoplastic disease (Robinson et al., 1979). Decreased blood Se levels were found in the cancer patients (McConnell et al., 1975) and patients with chronic lymphocytic leukemia (Calautti et al., 1980) in US. But these findings are not consistent. Peleg et al, (1985) could not find any difference in the serum Se levels between cancer and the age-matched controls. Similarly, Robinson et al. (1979) could not find any difference in blood Se and GPX activity between cancer patients, healthy subjects and noncancer patients living in New Zealand.

2.3.2.2 Glutathione peroxidase (GPX):

Early studies have shown the blocking of promotional phase of carcinogenesis in the skin of mice thought to be mediated by Se's antioxidant properties (Shamberger et al., 1966; Riely, 1966). The antioxidant action may be due to the changes in the activity of Se dependent enzyme GPX. This enzyme is responsible for the destruction of H2O2 and organic peroxides which are responsible for the induction of cellular damages (Mills, 1960). Peskin (1978) have found lower activity of GPX in experimental tumor,
hepatoma 27 and the activity was found relatively very low in the cytosol and the mitochondrial matrix of hepatoma 27. Decreased activity of GPX has also been found in larynx carcinoma (Drodzdz et al., 1988). In contrast, Di Ilio et al (1985) found higher activity of GPX in the neoplastic human breast tissue. Capel et al (1980) also found elevated activity of GPX and Se levels in the erythrocytes of breast cancer patients. Bauer & Wendel (1980) measured the activities of peroxide metabolising system in human colon cancer and found that carcinoma specimen had higher activity of GPX than healthy tissue. However, Robinson et al (1979) could not find any difference in the activity of GPX between the cancer and the normal individuals in the Se deficients area, New Zealand.

2.3.2.3 Glutathione S-transferase (GST):

Recently increasing attention has been paid to the GST enzymes in carcinogenesis for several reasons. They may confer an advantage on preneoplastic cells and neoplastic cells over their normal neighbors. They may also confer resistant to chemotherapeutic drugs and may have a diagnostic value as a tumor markers. GST isozymes are involved in the conjugation of GSH to a wide variety of xenobiotics, electrophilic drugs, toxins and carcinogens, and such are believed to be
responsible for the detoxication (Clapper et al., 1987; Jakoby, 1978 & 1980). The relationship of GST isozymes (μ, μ, α) and cancer, however is complex. Many human tumors contain increased concentration of various GST isozymes (Batist et al., 1986; Buller et al., 1987; Mannervik et al., 1987; Singh et al., 1988). This increased expression of GSTs in tumor cells may be a marker of acquired drug resistance or other late changes accompanying carcinogenesis. Alternatively, increased activity may play the direct role in the early events of cell transformation. However, anionic form i.e. GST-μ has been implicated in the acquisition of a drug-resistant phenotype as a part of the progression to tumor after exposure to carcinogens (Solt & Farber, 1976).

In many experimental tumor system, θ-class is regarded as a specific marker for preneoplasia (Sato, 1989; Kodate et al., 1986). Recent reports have shown the strong expression of GST-θ in gastric cancer (Tsutsumi et al., 1987), malignant melanomas (Mannervik et al., 1987), lung cancer and a range of human cancers (Shea et al., 1988). The increased expression of GST-θ has also been noted in multidrug resistant human breast cancer cells (Batist et al., 1986; Cowan et al., 1986) and in a cis-dichlorodiamine platinum (II)-resistant human squamous carcinoma cell lines (Moscow & Cowan,
Kodate et al. (1986) and Sato (1989) have shown immunohistochemically the enhanced expression of GST-κ in cancer of colon, stomach, and pancreas. Increased expression of GST-κ has also been found in leukemia (Holmes et al., 1990). Elevated levels of GST-κ mRNA have been found in a variety of hematological malignancies (Mcquaid et al., 1989; Moscow et al., 1989). Tsuchida et al (1989) revealed a 6-fold elevation of GST-κ content in colon cancer tissue. He also found the elevated GST-κ expression in liver cirrhosis, colonic adenomatous polyp. However Seidegard et al (1986) showed that person deficient in GST-κ had been reported to be a high risk for smoking induced lung cancer.

Many tumor cell lines also have been shown to have elevated expression of GST-κ e.g. both small cell and nonsmall cell lung carcinoma cell lines and the EJ6 bladder carcinoma (Wolf et al, 1987). Batist et al. (1986) have found 45-fold increase in the expression of GST-κ in adriamycin-resistant MCF-7 mammary carcinoma cell line. Ali-Osman et al (1990) investigated the GST activity in three human malignant astrocytoma cell lines (UW R1, UW R2, UW R3) of varying 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) resistance. He found that GST activity was directly correlated with the increasing BCNU resistant cell lines. Recently Wang et
al (1989) reported that acquisition of BCNU resistance in a human malignant melanoma cell lines were associated with elevated GST-κ. Wang and Tew (1985) found increased activity of GST in a Walker 256 carcinoma cell lines resistant to bifunctional nitrogen mustard. But Carmicheal et al (1988) could not find any significant difference in GST activity between small cell lung cancer cell line and nonsmall cell line. Di Ilio et al (1987) also showed the elevated activity of GST in renal cortex tumors by isoelectric focusing and in breast tumors. Beside breast, elevation of GST activity has also been found in tumors of brain (Polidoro et al., 1984), bladder (Whyte et al., 1984) and sigma but not in colon and rectum (Siegers et al., 1984). On the other hand, a trend towards higher GST activity in tumor tissue of colon compared with normal segments has been reported by Bauer and Wendel (1980). Conversely, in human liver GST activity was found to be lower in tumor tissue than in nontumor tissue.

GST activity in human tissue can be expressed by several isozymes (Mannervik et al., 1983). But Di Ilio (1985) could not establish whether the elevation of GST in breast was accompanied by variation in the isozyme composition. However it has been reported that certain forms of GST are inducible in rat liver during chemical carcinogenesis (Kitahara et al., 1984).
2.4 Pentose phosphate pathway and carcinogenesis:

For many years it has been observed that malignant tumors usually exhibit a high rate of glycolytic activity in presence of oxygen (Warburg, 1956). In certain human cancers alterations in the glycolytic enzymes have been successfully used as markers with diagnostic or prognostic values (Verhagen et al., 1985a; Verhagen et al., 1985b; Balinsky et al., 1984). Therefore the study of selected enzymes activities in neoplasia as markers of tumor status in terms of predictable pathological evolution, metabolic activities and indexes of growth hormone dependency has been an active field of research.

The glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) of the pentose phosphate pathway (PP-pathway) have received considerable attention (Goldberg et al., 1965; Weber, 1977). These enzymes produce both NADPH and the precursor of nucleic acid synthesis (Pentose sugars) (Evans et al., 1980). Three major aspects of functional properties of G6PD and 6PGD are involved:

(i) They control the PP pathway, the activities which are needed for cell growth and thus be increased in malignant tissue (Boxer & Devlin, 1961).

(ii) Their activities are stimulated by a number of hormones (steroid hormones) (Chayen et al., 1974)
Their activities are the major source of NADPH which is needed for steroid and drug metabolism via the cytochrome P450 pathway (Chayen et al., 1974).

Therefore these dehydrogenases deficiencies interfere with normal cell function and replication (Beconsfield et al., 1964 & 1965) and lead to a protective effect against the development of cancer. An explanation for these findings might be found in the block of metabolic pathway of glucose degradation via PP pathway. This would cause slower formation of ribose and hence of nucleic acid, with a subsequent obstacle to neoplastic proliferation.

The study of G6PD activity in tumor is therefore particularly relevant. Nevertheless, conflicting results have been reported on the levels of this enzymes in tumors (Smith et al., 1966; Hilf et al., 1970; Hilf et al., 1976). A first impulse to study the relation between G6PD deficiency and the incidence of cancer came from the observation of the geographical variability of the both factors, sometimes inversely related (Beaconsfield et al., 1965). In the year 1968-72, several authors supported the hypothesis of a lower incidence of cancer in G6PD deficient subjects. Beconsfield et al (1965) reported standardized mortality rates for four tumors (stomach, kidney,
colon, pancreas) in Occidental and Oriental Jews; in all the cases, and more clearly in males, the incidence of cancer was higher in Occidental than in Oriental Jews. Since Occidental Jews were found G6PD deficient. In a further report, Sulis (1972) reported a case record of 320 male affected by cancer of all sites, resident in the area near Cagliari, 13% of whom were G6PD deficient. Naik & Anderson (1970) reported their study on erythrocyte G6PD deficiency in 241 Negro patients (66 males and 175 females) affected by cancer and compared them with 266 negro males blood donors and 142 females attending the planned parenthood and maternity clinics. The different prevalence among female sex in two groups compared did not allow any interference without a stratification by sex. Another study by Long et al (1967) did not reveal a significant negative relation between G6PD deficiency and cancer. A more recent study by Cocco et al (1987) in Sardinian males did not reveal a final conclusion on the hypothesis of a negative association between G6PD deficiency and the incidence of cancer.

In recent years, different authors discovered a relationship between G6PD activity, cell proliferation and cancer. Schwartz et al (1975) repeatedly have shown that inhibition by dehydroepiandrosterone (DHEA) protects against the growth of chemically induced
tumors and is also able to block the cell division and proliferation. On the other hand, it was shown that the inhibition of the tumor induction may be due to the failure of G6PD deficient cells to metabolize chemicals to the ultimate form that is able to induce cancer (Feo et al., 1984). Despande et al. (1981) showed a correlation of an enzyme ratio with relapse after mastectomy. A low ratio of 3-glycerolphosphate dehydrogenase (3-GPD) to 6PGD signified a considerable increased risk of recurrence. Weber & Lea (1966) reported that poorly differentiated carcinomas (grade III) showed significantly higher activity of G6PD than well differentiated (grade I) tumors. Hilf et al. (1970) studied the activities of various enzymes in normal breast tissues, fibrocystic disease and infiltrating ductal carcinoma. He found that infiltrating ductal carcinoma of the breast demonstrated 2- to 8-fold elevations in the activities of pyruvate kinase, G6PD, hexokinase per mg of DNA compared with normal breast tissue. Smith et al. (1966) reported that malignant breast tissues were characterised by elevation in the activities of LDH, 6PGD and isocitrate dehydrogenase per mg of DNA compared with the normal breast tissue. Cohen (1964), using histochemical procedure, reported the increased activity of G6PD in carcinoma of the breast.

50
2.5 Glutathione and its related enzymes in cervical carcinogenesis:

Cervical cancer has been shown to be associated with a number of risk factors and the aetiology of this cancer is complex (Ferenczy et al., 1987). Human papilloma viruses (HPVs) are currently thought to be implicated as an important etiologic risk factors in the cervical carcinogenesis (Brescia et al. 1986; Youngs et al. 1989; Mc Cane, 1886). It has also been speculated that phagocytosis (Babior, 1982) as a consequence of viral infection may generate free radicals which might be one of the contributory factors in the pathogenesis of cervical lesions. The free radicals have been found to have the oxidizing properties, and thus would have the disturbing effects on the redox balance, including the ratio of thiol-disulfide group like GSH:GSSG. Large difference in the free radicals content has been found between samples of normal and cancer tissue of human uterine cervix (Tomasi, 1984). The possibility for this difference may be associated with detectable perturbation of redox balance. Therefore glutathione (GSH) and its related enzymes have attracted the attention in relation to cervical carcinogenesis during recent years. Very few studies have appeared which have reported the low GSH content in the exfoliated
cells as well as in the tissue of cervical cancer women (Basu et al., 1990; Guinchard et al., 1990). Slater in 1985 measured the protein thiols including the GSH and GSSG content on the sections of normal cervix and or women with dysplasia, CIS and invasive cancer. He showed the substantially reduced ratio of GSH:GSSG in the epithelium to stroma in the pathological conditions compared with normal and in apparently adjacent area. Sieron et al (1988) reported the decreased GPX activity and the low GSH content in the cervical cancer biopsies. Selenium has been found to be protective to develop cervical cancer (Sundstrom et al., 1984), though he could not find any difference in the Se concentration between the various age groups or various clinical stages of cervical cancer. In another study from Australia by Brock et al (1991) could not find the protective role of Se in in-situ cancer of cervix.

Most of the studies conducted on GSH and cervical cancer are related to the GSH/GST system. GSH/GST system is a major route of detoxication of carcinogens by conjugation with GSH. Therefore altered expression of GST has been implicated in the progression to the tumor after exposure to carcinogens. Increased expression of GST, especially the \( \pi \)-form has been found in all grades of CIN as well as in invasive cancer compared with no expression in normal epithelium.
(Shiratori et al., 1987; Randall et al., 1990). Similarly Cardner (1990) showed that the increased expression of GST-\(\kappa\) in all grades of CIN as compared with normal women was paralleled by a reduction in the expression of microsomal GST. In another study, Maguire (1990) showed the positive staining with monoclonal as well as polyclonal antibodies of GST-\(\kappa\) in squamous intraepithelial and invasive lesions of cervix as compared to the negative staining for normal ectocervical squamous epithelium. Riou (1991) estimated the GST-\(\kappa\) transcripts in invasive cervical cancer and found the increased levels of GST-\(\kappa\) transcript which was not related to the presence or absence of HPV (Riou et al., 1991).

2.6 Pentose phosphate pathway enzymes in cervical carcinogenesis:

The changes in the activities of certain glycolytic enzymes occur at early stage of malignancy (Bannasch et al., 1982) and hence are detectable before the morphological changes appear. Few studies have analysed the enzymatic alterations, especially in G6PD and 6PGD during the process of cervical carcinogenesis (Dutu et al., 1980.; Kolstad et al., 1967). Palaoro et al (1988) investigated the variation in the activity of cytoplasmic enzyme of PP pathway, G6PD and reported the
increased levels in dysplasias and carcinoma, however low enzymatic levels in condyloma. Dutu (1980) carried out a large battery of cytoenzymatic tests to determine their potential value in the diagnosis of carcinomas. He also reported the increased activities of G6PD and 6PGD in the malignant cells of the uterine cervix. Smith et al (1971) used electrophoretic variants of X-linked enzyme, G6PD to study the possibility of single cell or multicellular origin of human uterine cervical neoplasia. He reported a heterogeneous etiology of invasive cancer of uterine cervix.

Most of the reports on 6PGD enzyme showed the consistent elevation of above the normal levels in the vaginal fluid of patients with invasive cervical cancer (Bonham & Gibbs, 1962; Cameron & Hussain, 1965). Cameron & Hussain reported the false negative results in CIS. Kolstad et al (1967) found the raised 6PGD levels in 63% of dysplasia cases, 71.2% of intraepithelial carcinomas, 94.2% of early invasive cancer, and in 97.8% of frank invasive carcinoma. Similarly Pfleiderer & Barun (1969), in another study showed histochemically the increased levels of G6PD and 6PGD in the epithelium endometrial cancer.