Cancer is a dreaded disease of mankind and is increasingly recognised to be a global problem. This is predominantly a disease of older age group, its relative magnitude in any country depends on the age composition and the size of population. It has been estimated that every year throughout the world more than 6 million new cases of cancer occur and about 4 million people die from cancer. The number of cases occurring in the world have been divided almost equally between the developed (49.3%) and the developing nations (50.7%). In the developed countries lung cancer is the predominant form of cancer (22.3%) among men while breast cancer is the most common cancer among women (22.9%) (see Table 1). In the developing countries cancer of the uterine cervix is the major cancer, accounting approximately 24% of cancers among women.

In India, it has been estimated that every year more than 7 million new cancer cases occur. Cancer of the oral cavity, esophagus, lung and stomach are the predominant types among males while in females cancer of the uterine cervix and the breast are the dominant ones (Fig 1).

Cells, the building blocks of our body grow in a fixed pattern but if a cell or a group of cells start multiplying in an uncontrolled fashion, it is said to be cancerous growth. Such abnormal growth results in the formation of a cell/tissue mass known as tumor which has apparently no relation to physiological demands of the organ involved.

Tumors may be benign or malignant (cancerous). In benign tumors, the neoplastic cells closely resemble those of parent tissue and grow slowly by expansion usually resulting in a well encapsulated lesion and have limited growth potential. In contrast, malignant tumors are composed of poorly differentiated cells characterised by the capacity for progressive growth and invasion to the surrounding tissues. The cancer cells eventually may enter lymphatic and blood vessels and spread to other parts of the body where they produce secondary growth or metastases.
Table 1: Prevalence of different types of papillomaviruses in male and female genital tracts. (Gissmann, 1984)

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Clinical Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV - 1</td>
<td>Plantar warts</td>
</tr>
<tr>
<td>HPV - 2</td>
<td>Verrca vulgaris</td>
</tr>
<tr>
<td>HPV - 3</td>
<td>Flat warts</td>
</tr>
<tr>
<td>HPV - 4</td>
<td>Plantar warts</td>
</tr>
<tr>
<td>HPV - 5</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 6</td>
<td>Genital warts and laryngeal papillomas</td>
</tr>
<tr>
<td>HPV - 7</td>
<td>Common warts meat handlers</td>
</tr>
<tr>
<td>HPV - 8</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 9</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 10</td>
<td>Flat warts</td>
</tr>
<tr>
<td>HPV - 11</td>
<td>Laryngeal papillomas and genital warts</td>
</tr>
<tr>
<td>HPV - 12</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 13</td>
<td>Oral focal epithelial hyperplasia</td>
</tr>
<tr>
<td>HPV - 14</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 15</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 16</td>
<td>Cervical dysplasia, Bowenoid papulosis, and cervical carcinoma</td>
</tr>
<tr>
<td>HPV - 17</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 18</td>
<td>Cervical dysplasia and carcinoma</td>
</tr>
<tr>
<td>HPV - 19</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 20</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 21</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 22</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 23</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 24</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 25</td>
<td>Macular lesions in EV</td>
</tr>
<tr>
<td>HPV - 26</td>
<td>Flat warts</td>
</tr>
<tr>
<td>HPV - 27</td>
<td>Verrca vulgaris</td>
</tr>
<tr>
<td>HPV - 28</td>
<td>Flat warts</td>
</tr>
<tr>
<td>HPV - 29</td>
<td>Verrca vulgaris</td>
</tr>
<tr>
<td>HPV - 30</td>
<td>Genital warts and laryngeal carcinoma</td>
</tr>
<tr>
<td>HPV - 31</td>
<td>Cervical dysplasia</td>
</tr>
<tr>
<td>HPV - 32</td>
<td>Genital intraepithelial neoplasia and cervical carcinomas</td>
</tr>
</tbody>
</table>
FIG. 1 CANCER INCIDENCE BY SITE AND SEX

(Estimates based on crude incidences rates for Bangalore, Bombay, Madras and Delhi, 1989)
CANCER OF THE UTERINE CERVIX

Like all other cancers carcinoma of the uterine cervix is a multistep disease developing over a long period of 10 to 15 years. Invasive cancer preceeds a well-defined preneoplastic stage commonly known as 'dysplasia' which either progress to cancer or regress to normal depending upon various factors. During the process of cervical carcinogenesis, most, if not all of the initial events occur in the transformation zone or squamocolumnar junction. This is an area of epithelium in which columnar epithelium is readily replaced by squamous epithelium, resulting in the process known as squamous metaplasia.

According to the CIN histological classification dysplastic lesions are graded primarily on the basis of proportion of epithellium occupied by basaloid, undifferentiated cells. CIN I, II and III are the three different preneoplastic stages which have been well recognised. According to WHO classification the precancerous lesions are subdivided as mild, moderate and severe dysplasia.

Natural history of cervical lesions

The natural history of cancer of the uterine cervix is still not clearly understood. While some workers have postulated progression from dysplasia to invasive cancer either directly or through carcinoma in situ during a variable length of time, others have envisaged a more "dynamic" concept of dyplasias, with some progressing to higher grades of dysplasia or even cancer; others persist unchanged and still others regress to normalcy (especially mild dysplasias). A certain proportion of women with dysplasia do present a continuity from precancerous lesions to invasive carcinoma. Unfortunately, with current knowledge, it is not possible to accurately predict the behaviour of any given lesion.

In India, Luthra et al.,(1987) reported cumulative progression rate from dysplasia to malignancy at the end of 84 months of follow-up as 11.7%. The progression of moderate dysplasia to cancer (28.9%) was reported considerably higher than the progression of mild dysplasia cases
The rate of progression of dysplasia reported elsewhere to vary from (Luthra et al; 1987) 4.4% to 65.5% (Noda et al., 1976). Regression rates between 30% and more than 60% have been reported by various investigators. (see Luthra et al; 1987). It has been suggested that exposure of squamous epithelium to HPV in combination with an altered state of the immune defence system leads to subclinical or clinical HPV infection. Sunclinical, possibly latent clinical infections may progress to intraepithelial or invasive neoplasia if certain preconditions are met namely, infection with high-risk HPV types such as HPV 16 or 18, integration of the viral genome, and a failure of host-cell control of the persisting viral genes. (zur Hausen, 1986, 1989 a,b). Several risk factors which are important in this scenario have been identified but their mode of action is not yet clearly understood.

**HPV infection and cervical lesions:**

HPV DNA has been regularly demonstrated in vulvar, penile and cervical neoplastic tissues (Green et al 1982, Das et al., 1992a). Both experimental and epidemiological studies have shown a possible oncogenic role for HPV in anogenital cancer. In permissive lesions such as condyloma acuminatum positive for HPV 6 or 11, the amount of viral DNA increases towards the epithelial surface with the highest amount of viral replication confined to the superficial layers (Stoler et al., 1989). Different viral messages are expressed at the various levels of the infected epithelium. The early (E) open reading frames (ORF) are transcribed before onset of vegetative viral DNA replication and are continuously expressed with increasing amounts coinciding with cellular maturation. E4 and E5 ORF transcripts are most abundant and their transcription starts just above the basal cells. The late messages appear simulataneously or after initiation of viral DNA replication and can be detected only in the upper epithelial layers. The viral DNA is packed in viral capsid proteins and infectious viral particles are released on the surface of the epidermis. The viral E4 protein—as shown for HPV 16-interacts and the intracellular cytokeratin network may be essential for efficient release of the viral particles (Doorbar et al., 1991).
The biology of subclinical / latent infections with respect to localization and activity of viral nucleic acids, proteins or virus particles is ill-defined. It is not known if the infection can be primarily latent or if it can only become latent after regression of a lesion. Additionally, it is not known if latently infected tissue is truly infectious. Recent data from morphologically normal laryngeal tissue positive for HPV 11 showed fewer and shorter RNA transcripts compared to active infection. However, the functions of these transcripts for the establishment or maintenance of latency is not clear at present.

**Diagnosis of papillomavirus infection**

Until recently HPV infection of the cervix was considered uncommon because microscopically visible HPV infection was less common than clinically apparent HPV infection of the vulva, vagina, perineum and anus. These macroscopically invisible lesions are referred to as flat condylomas (Meisels & Fortin 1976), subclinical papillomavirus infection or non-condylomatous wart virus infection. Although HPV can not yet be grown in cell culture and no type specific antibodies are available, various techniques are currently available to demonstrate papillomavirus infection.

**Cytology and Histology:**

In the Papanicolaou (Pap) smears of cervical scrapes, human papillomavirus (HPV) infections of the uterine cervix are recognised by the presence of koilocytes and dyskeratocytes (Meisels and Fortin 1976). These cells show mild degrees of nuclear pleomorphism with often smudged, indistinct chromatin and binucleation. The cytoplasmic changes consist of a distinctive prenuclear cavitation, with augmented cytoplasmic density in the periphery in the case of koilocytes, and keratinization in the case of dyskeratocytes. These prepathogenic/pathogenic cell changes suffice to accurately predict the presence of HPV, which nearly in one-half of the cases can be demonstrated by electron microscopy or immunoperoxidase staining (Meisels et al., 1984). Population screening for cancer of the cervix and its precursor lesions has allowed the identification of a large number of patients with HPV infection.
Histology has been used as the gold standard for diagnosing HPV infection in many studies, and diagnostics features are koilocytes, dyskeratosis, basal-cell hyperplasia and mild nuclear atypia (Meisels et al 1982). When this constellation of changes is present, the diagnosis of HPV infection is generally agreed upon by all pathologists. However, in cases where in many lesions these changes are less pronounced or are associated with significant nuclear atypia, a general consensus may be difficult to achieve. The histological continuum between HPV infection and CIN is well appreciated and, as Reid (1984) and others have pointed out, the histology of subclinical HPV infection may differ from that of dysplasia only quantitatively rather than qualitatively. A typical mitoses, abnormalities of the basal cell layer, grading of nuclear atypia, polarity and determination of ploidy are just a few of the criteria helpful in establishing whether a lesion is a benign HPV infection or a higher grade CIN lesion associated with or perhaps due to HPV.

**Immunohistochemistry**

Studies of typical 'florid' condylomas have demonstrated positive antigen staining to papillomavirus genus specific structural antigen (Woodruff et al., 1980). Since it is now known that many lesions that were called CIN I 10 years ago have features suggestive of condylomas, it is not surprising that Kurman et al (1982) found that a higher percentage of CIN lesions showed positive immunoperoxidase staining to the structural antigen than did lesions classically associated with CIN II-III. As more biopsies are examined by immunological staining or by other techniques to identify viral DNA, the histological spectrum of HPV infection will become clear. It is clear from the identification of HPV DNA in dysplastic lesions which lack the typical morphology traditionally associated with condylomas that the role of light microscopy in the diagnosis of HPV infection is limited.

**Colposcopy:**

Colposcopy has traditionally been used to identify typical epithelial changes to allow confirmation of Pap smear findings by directed biopsy.
Colposcopy was introduced by Hinselmann (1925). He believed that leukoplakia and acetowhite areas were a feature specific for cancer and that colposcopy could provide a method for diagnosis of cervical cancer (Hinselmann, 1925). Further, Schneider et al., (1988) have suggested that all acetowhite epithelia with minor changes showing histological features of mature metaplasia (benign acanthotic epithelium) could represent early or discrete HPV-associated lesions.

The colposcope provides magnification and high intensity illumination for direct examination of the cervix through a vaginal speculum. The vulva and vagina can also be viewed.

The transformation zone:

Preclinical squamous cancer of the cervix develops inside the transformation zone, characterized as the portion of the ectocervix that has been covered by glandular epithelium, then replaced by squamous metaplasia. Virtually all cervical precancers and cancers originate in the transformation zone, at the squamo-columnar junction, during the metaplastic process. In these cases, such epithelia exhibit colposcopic characteristics that constitute the atypical transformation (ATZ) zone. The common, normal appearances, exhibiting a great variety of features, constitute the typical transformation zone (TTZ). The cellular activity of squamous metaplasia is of significance. The transformation zone is the preferred target of HPV.

Acetowhite Epithelium:

The characteristic whiteness of the atypical transformation zone (ATZ), developing after careful cleansing of the cervix with diluted acetic acid (5%) may be slow to appear in subclinical papillomavirus infection (SPI). Acetowhite epithelium is the commonest presentation of SPI and forms the essential basis of its recognition.

Electron microscopy:

Electron microscopy has successfully been used to identify
characteristic HPV particles in biopsy specimens, and occasionally in smears with changes suggestive of HPV infection (Reid et al 1984).

The first supporting evidence that koilocytosis was associated with the presence of HPV particles was provided by Laverty et al.,(1978) by electron microscopy of cervical biopsy material. Using a modification of a technique developed by Collman et al., (1977), Hills and Laverty (1979) documented the presence of koilocytes in a cervical smear. Several additional reports pertaining to electron microscopic observations in cervical smears and biopsies (Meisels et al., 1984) have documented that in about 50% of the patients afflicted with intraepithelial cervical lesions showing koilocytosis, viral particles may be observed in mature squamous cells. In tissue sections the HPV-positive cells are confined to the superficial layers of the epithelium. Meisels et al.,(1984) also emphasized the presence of virus in atypical squamous cells with homogeneous and hyperchromatic nuclei but without the perinuclear zone (dyskeratocytes). In intact nulcei of koilocytes, the viral particles usually form crystalline lattices located in between coarsely clumped nuclear chromatin. On close inspection the particles measure about 50nm in diameter and show a denser core and somewhat lighter periphery. The crystalline particles have been shown to be icosahedral, i.e., have 20 faces. By ultrastructural studies alone, the precise type of virus cannot be determined, except that it belongs to the family of papillomaviruses.

**Molecular methods for HPV detection:**

Papillomaviruses need a differentiating cell layer for their circular double-stranded DNA molecule to replicate and their capsid proteins to be synthesized and assembled to form mature virus particles. Due to these special requirements, papillomaviruses cannot be cultivated in the laboratory and no antibody could be produced. Therefore methods for detecting a papillomavirus infection differ from the usual serological methods that are commonly used for detection of other viral infections, and instead have relied mainly on viral nucleic acids detection methods.
In-situ hybridization:

This method is often preferred since both the changed morphology of cells as well as presence of viral genome can be visualised under microscope. The morphology of the tissue can be distinguished after hybridization and the signal can be located within the nuclei of cells. The sensitivity of this method is almost similar to Southern blotting if a single copy gene localization method is used (Das et al, 1993). If a regular in-situ with radio-labelled probe is used a cell has to contain at least 20 to 50 genome copies in order to give a visible signal upon hybridization (Schneider et al, 1991), whereas the sensitivity decreases 50 to 350 genome copies or more per cell if non-radioactive methods are used (Beckmann & Myerson, 1989).

Filter in situ hybridization:

In this method (Wagner et al., 1984), scraped cervical epithelial cells, are filtered onto a membrane, denaturated in situ and hybridized by radiolabelled HPV DNA probes. Although hundreds of samples can be tested with relative ease, the method has several disadvantages. These are:

(1) Very low sensitivity (only 50 HPV genome copies per cell or more can be detected).

(2) High background combined with non-specific hybridization, in many cases due to contaminating bacteria or blood and mucus in the sample.

Southern blot hybridization:

For routine diagnosis of HPVs, this method though time-consuming and labour-intensive (Southern, 1975) is generally used. It is regarded as the test from which most information can be obtained. Tumor DNA is digested with appropriate restriction enzyme(s) (Pst I for HPV16, for example) and the fragments are separated on an agarose gel. The sensitivity ranges between 0.1 and 0.01 HPV genome copies per cell. Information on episomal versus integrated DNA, deletions, HPV type involved in single or even double infections, relatedness of one HPV type to another, detection of unknown HPV types and presence of bacterial infection can be obtained with
a minimal number of experiments through a suitable choice of hybridization conditions and probes. Using Southern blot hybridization HPV DNA sequences could be detected in more than 90% of cervical carcinomas. (Pfister et al., 1984, Gissman et al., 1976, Das et al., 1992a).

**Dot/blot hybridization:**

This method differs from the preceding ones in that the DNA to be fixed onto the membrane is not separated, but fixed in the form of a "dot" or "slot" depending on which Manifold apparatus is used. It is not possible to estimate the degree of non-specific hybridization, and therefore adequate controls must be run in parallel with probes that do not contain contaminating vector sequences.

**Polymerase Chain Reaction (PCR):**

PCR is basically an enzymatic process that is carried out in discrete number of cycles and temperature profile of amplification. Each such cycle doubles the amount of DNA present in the sample. This method was discovered by Kary Mullis, (U.S. patent 4,6,83,195, July, 1987 (Saiki et al., 1985, Muller et al., 1986, Millis & Faloona, 1987). It is a sensitive way of synthesizing millions of copies of a single nucleic acid sequence of interest in a few hours (Saiki et al., 1985,1988). This method has recently been employed as a highly sensitive technique to detect HPV DNA sequences (Ting & Manos, 1990; Das et al., 1992a).

The great sequence diversity of the HPVs has spurred the development of two distinct types of PCR-based systems: consensus primers and type specific primers. Consensus primers also called general or generic primers, facilitate amplification of a broad spectrum of HPV types using a single set of primers (Snijders et al., 1990) or a mixture of primers (e.g., degenerate primer systems; Manos et al., 1989). In contrast, type-specific primers are designed to amplify DNA from only one specific type of HPV. Type specific primers chosen from various regions of the HPV genome. Generally, the conserved L1-ORF region or E6/E7 region or the upstream regulatory regions (URR) are often the choice of majority investigators.
This technique is superior over traditional hybridization methods by increasing the amount of target material, leading to easier, often less ambiguous data interpretation. However, the entire process (from sample collection to PCR set-up) is susceptible to contamination by minute amounts of recombinant plasmids, PCR product carry-over, and other patient specimens which give rise to false positive results (Kwok & Higuchi, 1989). Therefore with all precautions, strict negative controls must be strategically interspersed during every step of the assay (Bauer et al., 1991). PCR based methods have revealed that over 80% of invasive cervical cancer and advanced preneoplastic lesions contain specific types of human papillomavirus (HPV) (Riou et al., 1990, Van den Brule et al., 1991, Higgins et al., 1991, Lorincz et al., 1992, Das et al., 1992a). In asymptomatic normal women the frequency of HPV infection as detected by PCR is found to vary from 20 to 70% (de Villiers et al., 1987, Meanwell et al., 1987).

RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF CERVICAL CANCER

Physical factors:

The average annual incidence of invasive cervical cancer varies widely by geographic area (Durst et al., 1983). The highest rates have been reported from Latin America, where the risk is approximately six times to that of US whites, whose rate is one of the lowest in the world. It is well recognized that cervical cancer occurs disproportionately in women of the lower socio-economic classes. Furthermore, there are distinct trends with race; for instance, in the United States, rates tend to be approximately twice as high for blacks and Hispanics than for whites or orientals (Brown et al., 1984).

A number of epidemiological investigations (Harris et al., 1980, Slattey et al., 1989) have found that women having sexual exposures at early ages are at higher risk in developing cervix cancer than either virgins or women whose sexual experiences begin later in life. In view of suggestions
that the cervix may be more vulnerable at early ages perhaps because of immature epithelium the number of sexual partners at different age intervals has been of interest. Peters et al., (1986) found that the effect of lifetime number of partners was totally attributable to effects associated with number of sexual relationships before the age of 20. They also found some evidence that subjects with short intervals between menarche and initiation of sexual intercourse were at elevated risk (with relationships stronger than those observed with age at first intercourse alone). The risk of cervical cancer has been shown to be influenced by the number of sexual partners, often indexed by promiscuity, multiple marriages, separations or divorces. Several of the studies (Brinton., 1987, Harris 1980., Herrero., 1990) found that women with cervical cancer more frequently report to have multiple sexual partners than controls.

More recently, the role of the male in the etiology of cervical cancer has been examined by comparing sexual and other behavioural characteristics of husbands of cervical cancer patients with husbands of women free of cervical disease (Brinton et al., 1989b; Buckley et al., 1981; Kjaer et al., 1991; Paridan & Lilienfeld, 1971;). In all of these studies, the husbands of cases reported significantly more sexual partners than husbands of controls.

The available epidemiological data provide reasonably convincing evidence that cigarette smoking increases the risk of cervical cancer although the absence of any dose-response relationship in some studies is a little surprising. On balance, it appears that the risk is elevated in ex-smokers as well as in current smokers. In this context, it is interesting that Sasson et al., (1985) have recently shown that nicotine and cotinine can be detected in the cervical fluid of smokers.

Low cervical cancer risks have been recorded among Catholic nuns (Fraumeni et al., 1969), the Amish (Cross et al., 1968), Mormons (Gardner & Lyon, 1977) and Jews (Graham & Schotz, 1979). The extent, however, to which these lower cancer risks reflect a reduced prevalence of sexual promiscuity is still unclear.
There is little evidence that risk of cervical cancer is linked to age at menarche or age at menopause (Brinton et al., 1987; Rotkin, 1967) or to hygienic factors (Brinton et al., 1987; Herrero et al., 1990), although Zhang et al., (1989) noted significant elevations in risk associated with poor hygiene (absence of genital washing and use of sanitary napkins) and extended days of menstruation. There is growing evidence that multiparity may significantly relate to cervical cancer risk (Brinton et al., 1987, 1989a; Parazzini et al., 1989), a four-fold excess risk of invasive cervical cancer having been found associated with 12 or more births compared to 0-1 birth in Latin America, an association that persisted after adjustment for a variety of socioeconomic and sexual factors (Brinton et al., 1989a). Possible explanations for the association include cervical trauma during parturition and hormonal or nutritional influences of pregnancy.

Possible interactions of hormonal oral contraceptives and HPV are of interest, especially in view of studies which have demonstrated that the transcriptional regulatory regions of HPV DNA contain hormone-recognition elements and that transformation of cells in vitro with viral DNA is enhanced by hormones (Crook et al., 1988). The effect of oral contraceptives on cervical cancer and precursor lesions might operate through enhanced viral carcinogenicity, since studies have shown increased HPV expression in oral contraceptive users (Hildesheim et al., 1990; Lorincz et al., 1990). In a number of studies, users of barrier methods of contraception (diaphragm or condom) have been shown to be at low risk of cervical cancer (Slattey et al., 1989), presumably because of reduced opportunity for exposure to infectious agents.

It has long been known that cancer of the cervix is much more common in women of low socio-economic class than it is in upper class women (Luthra et al 1975). This is true whether "social class" is measured in terms of the woman's own occupation, her husband's occupation, the type of dwelling occupied, family income, or educational status achieved. It is generally considered that differences in risk between occupational groups are explicable in terms of other variables that predict cervical cancer risk, such as sexual behaviour (of the women and her consort), cigarette smoking, and contraceptive practices. In the United Kingdom, Robinson (1982) has
suggested that occupational exposure of the male to carcinogens might play a part in the etiology of cancer of the cervix.

**Biological factors:**

**Presence of HPV**

In all countries where studies have been carried out, HPV 16 has been found to be the predominant type in invasive cervical cancers, (20%-60%). In India, while HPV 16 is the most predominant type in squamous cell carcinoma of the cervix (Das et al., 1992a), the frequency of HPV 18 is extremely low not only in squamous cell carcinomas but also in adenocarcinomas (Das et al., 1993). In asymptomatic normal women the frequency of HPV infection as detected by PCR is found to vary from 20 to 70% (de Villiers et al., 1987, Meanwell et al., 1987).

**Role of infectious agents other than HPV:**

The association of cervical neoplasia with sexual risk factors has motivated the search for a venereal infectious agent. Although the possible chemical carcinogenicity of semen and sperm has been suggested (Coppleson, 1970; Malhotra, 1971), most attention has focused on infectious agents, particularly herpes simplex virus type 2 (HSV 2), HPV and bacterial infection.

**Herpes Simplex Virus 2:**

Of particular interest has been the relationship of risk to infection with HSV2, since laboratory studies have demonstrated that this virus can transform cells in culture and that HSV2, proteins and integrated DNA can be found in some cervical cancers (Mc Dougall et al., 1986). Although the relationship of HSV-2 to cervical cancer risk was of intense interest in the 1960s, interest in the oncogeneic potential of HSV2 declined when HSV 2 DNA and protein were not detected consistently in cervical tumor specimens (Seth et al., 1988) and a large follow-up study of Czechoslovakian women failed to demonstrate a significantly increased risk of cervical neoplasia
related to HSV-2 serology at enrollment (Vonka et al., 1984).

**Other infections:**

Other infections besides HPV and HSV2 may play independent or supporting etiological roles during the process of cervical carcinogenesis (Alexander, 1973). Chlamydia has been suspected, based on case-control comparisons of serology (Schachter et al., 1982) and of chlamydia-associated changes seen on stored cervical smears (Allerding et al., 1985). Additional infections that have been studied included syphilis, gonorrhea, cytomegalovirus, Epstein-Barr virus and bacterial vaginitis. No consistent association with cervical cancer has been observed for any of these agents. However, one investigation noted a rise in risk of cervical cancer with multiple, concurrent infections (Schmauz et al., 1989). Most recently, infection with human immunodeficiency virus (HIV) has been observed to be correlated with the detection of HPV-related cytological changes (Bradbeer et al., 1987; Feingold et al., 1990). It is possible that the study of HIV-induced immunosuppression may clarify the interrelationships of cell-mediated immunity, HPV infection and cervical neoplasia.

**Dietary factors:**

The role of nutritional status to risk of cervical neoplasia has been of interest for quite some time. Initial case-control studies in New York State (Marshall et al., 1983) and in Italy (La Vecchia et al., 1988) suggested a protective effect of vegetables and fruits rich in carotenoids, with no apparent effect of animal foods containing vitamin A, although both studies had rather limited dietary data. More recently, two case-control studies (Slattery et al., 1990; Verraeault et al., 1989) with more detailed dietary data confirmed a relationship of dietary factors to cervical cancer risk; reduced risks being associated with high dietary intake of vitamins A, C and E, as well as beta-carotene.
THE HUMAN PAPILLOMAVIRUSES:

The Papillomaviruses are a group of small DNA viruses which induce squamous epithelial tumors (warts and Papillomas). The first papillomavirus described was the cottontail rabbit papillomavirus (Shope 1933). Subsequently, many HPV genotypes have been isolated and characterized from other vertebrate species, including humans. So far more than sixty five human papillomavirus (HPV) DNA types described have been associated with variety of epithelial cancers at different organ sites in human (de Villiers, 1989)(Table-1). Among them about 20 types have been associated with anogenital cancers (de Villiers 1989). Standard virological approaches to the study of these viruses have been limited, due to lack of tissue culture system for their in vitro propagation. This lack may, in part, be because the productive functions of the papillomaviruses are expressed only in fully differentiated squamous epithelial cells. To date, tissue culture systems for keratinocytes have not permitted the full expression of the papillomavirus life cycle. Investigators generally believe that the viral genome is present in the epithelial cells of the basal layer and that the expression of specific viral genes in the basal layer and in the lower layers of the epidermis is responsible for the proliferation of epithelial cells that is characteristic of a wart or a papilloma. The control of papillomavirus late gene expression, appears tightly linked to the state of differentiation of the squamous epithelial cells.

Organization of the papillomavirus genome:

Papillomavirus genome consists of superhelical double stranded DNA molecules, having a length of approximately 8000 bases (bp) (Danos et al., 1982, Chen et al., 1982, Schwartz et al., 1983, Giri et al., 1985). Papillomaviruses have a different genome organization from other members of the papovaviridae family like simian virus 40 and poliomavirus (Danos et al., 1982, Chen et al., 1982). A characteristic feature of papillomavirus genome is that all major ORFs are located on the same DNA strand. (Fig 2).
FIG. 2 GENOME ORGANISATION OF HUMAN PAPILLOMAVIRUS (HPV16)
Like most DNA viruses HPV genome could be subdivided into two functional groups of genes: the early genes (E1-E7), which are expressed before the onset of viral DNA replication, and the late genes (L1-L2), which are expressed after viral DNA replication has commenced. The coding regions cover approximately 90% of most papillomavirus genomes. A region which appears to be non-coding since it lacks ORFs of significant size does, however, exist. It is located between the end of the L region and the beginning of the E region.

**Papillomaviruses and the neoplastic process**

The studies with the HPVs associated with human cancers would indicate that there is a continued requirement for the presence of the HPV and presumably, its expression. Although in the case of the EV-associated cutaneous carcinomas, apparently the DNA can remain extrachromosomal, in the case of the genital tract carcinomas, the viral DNA is usually integrated (Durst et al., 1985, Cullen et al., 1991, Das et al., 1992a). A substantial number of HPV genotypes that infect the anogenital tract have been isolated and characterized (Gissmann, 1984). Cloning and characterization of HPV DNA from a variety of human genital tumors have determined the prevalence of the different viral types in male and female genital tract.

HPV types 6, and 11 considered to be 'low risk' types are found in 90% of condylomata acuminata and lower grade dysplasias as HPV-16 and 18 are found more frequently in severe dysplasia (CIN III), and in invasive carcinomas. The apparent affinity of HPV-16 and 18 for malignant tissue raises the probability that these two virus types, unlike HPV-6 and HPV-11, are involved preferentially in cervical carcinogenesis and hence formed as "high risk" types. Support for this hypothesis has been drawn from several studies of HPV DNA in warts, dysplasia, invasive cancer and cervical cancer cell lines (Zur Hausen 1989a,b,c, Pfister et al, 1984). In invasive cancers and cervical cancer cell lines such as HeLa, SiHa, the viral DNA is integrated into the genome of the cancer cells, whereas in benign warts and in dysplasia the viral DNA is found in episomal form.
These high-risk types of HPV (16 & 18) are thought to be causally involved in the pathogenesis of cancer of the uterine cervix (Zur Hausen, 1989a,b). This is assumed on the basis (i) that viral DNA is found in close of 90% of such tumours (most frequently DNA of HPV 16). (ii) the majority of the malignant virus-positive tumors contain integrated viral DNA (Durst et al., 1985, Das et al., 1992b). The viral circular episome is disrupted within a specific region (E2 open reading frame) for integration. No consistent pattern has been observed for intrachromosomal localization. A small percentage of malignant tumors harbors non-integrated copies of viral DNA (Matsukura et al., 1989, Das et al., 1992b). (iii) that vast majority, if not all, of HPV positive cancer biopsies and all HPV containing cervical cancer cell lines reveal specific transcripts originating uniformly from two specific open reading frames, E6 and E7 of the persisting HPV genome (Schwartz et al., 1985).

The E6 and E7 genes of high-risk types of HPV (but not of low risk types) immortalize human foreskin, and cervical keratinocytes (Munger et al., 1989), or epithelial breast cells after in vitro transfection in tissue culture (Band et al., 1990). In organotypic cultures of these immortalized cells share growth characteristics with intraepithelial neoplasias (McCance et al., 1988). Although HPV E6-E7 immortalized human cells are initially nontumorigenic in nude mice, long time in vitro cultivations may lead to malignant clones (Hurlin et al., 1991). This result demonstrates that these HPV infections alone can induce malignant growth, provided a sufficient number of cell generations permit the manifestation of additional spontaneous or virus gene induced modifications. Chromosomal instability of immortalized cells has been regularly observed in such cultures and may be the source of the additional progressive changes.

ONCOGENES AND HUMAN CANCER:

To date, over dozen oncogenes have been identified in human cancer (Barbacid et al., 1985). Among them, the most frequently found are members of the ras gene family: H-ras, K-ras and N-ras. H-ras and K-ras are the cellular homologues of the oncogenes present in the Harvey and Kirsten strains of murine sarcoma viruses (Der et al., 1982, Parada et al., 1982,
Santos et al., 1982). The N-ras locus has so far not been transduced by retroviruses (Shimizu et al. 1983). These oncogenes become activated at some stages of tumor progression as a random consequence of the genetic disarray of tumor cells. Although such events may occasionally happen (Albino et al., 1984), available experimental evidence strongly suggests that ras gene activation plays a causative role in the development of many a cancer.

The ubiquitous expression of ras oncogenes and their extreme degree of conservation during evolution indicates that ras genes are also indispensable for normal cellular functions. If so, it is not inconceivable that mutations that turn ras genes into oncogenes may trigger neoplastic behaviour in many cell types.

The Functions of Proto-Oncogenes and Oncogenes:

There are almost 40 proto-oncogenes and oncogenes, yet we can so far name only four biochemical mechanisms by which this rich diversity of proteins may act: protein phosphorylation, with either tyrosine or serine and threonine as the substrate amino acids (Varmus et al. 1984); metabolic regulation by proteins; and control of gene expression and replication of DNA (Nau et al., 1985). The details of these mechanisms have been reviewed (Powell et al., 1971, Klein et al., 1983).

Ras oncogenes and Cervical cancer:

The inherent simplicity with which ras oncogenes acquire transforming properties suggests that activation of these oncogenes cannot be sufficient to elicit the full spectrum of phenotypic changes that occur during tumor development. Experimental evidence gathered at the Frederick Cancer Research Facility and by Allan Balmain at the Beatson Institute suggests that ras oncogenes are involved in the initiation of carcinogenesis in at least certain animal model tumor system. Balmain and coworkers have identified Ha-ras oncogenes in pre-neoplastic skin papillomas initiated by DMBA and promoted by phorbol esters suggesting that oncogene activation occurred early in tumor development and that additional secondary changes
are necessary to achieve the full malignant phenotype characteristic of skin carcinomas (Balmain et al., 1984). This hypothesis is supported by recent studies utilizing retroviral ras oncogenes. In the past Riou et al. (1988) have reported that the c-myc and the c-Ha-ras proto-oncogene co-operate in the progression of cervical cancer.

**Myc oncogenes and Cervical cancer:**

Another gene family, myc has been strongly implicated in a variety of human malignancies. So far, three loci designated as c-myc, N-myc and L-myc have been characterized at the molecular level (Varmus et al., 1984, Barbacid et al., 1985). They share significant sequence homology, particularly within two small domains located in their respective first coding exons (Schwab et al., 1983, Nau et al., 1985) and appear to code for nuclear proteins with similar in vitro biological properties (Weinberg et al., 1985). In human tumors, the c-myc oncogene was first identified in Burkitt's lymphoma. In these B-cell tumors, c-myc is translocated from its normal residence in chromosome 8 to the vicinity of immunoglobulin loci (Klein et al., 1983). The exact mechanism of activation of the c-myc oncogene still remains to be elucidated. It has been proposed that perturbances in its regulatory domain may result in constitutive c-myc expression throughout the process of B-cell differentiation leading to neoplastic development. C-myc has also been found to be amplified in several human tumor cell lines and in the highly tumorigenic variants of small cell lung carcinomas. N-myc and L-myc are also often amplified in this type of malignancy (Nau et al., 1985). Previous studies (Riou et al., 1984, 1985) on c-myc proto-oncogene in a limited number of cervical tumors had shown that this oncogene was activated in the most evolved cancers, suggesting that c-myc activation was associated with tumor progression.

**Papillomaviruses, anti-oncogenes and cervical cancers**

Recently it has been showed that specific cellular proteins can bind efficiently to the HPV E6-E7 oncoproteins of high risk types of HPV (16 and 18) (Dyson et al., 1989). The E7 oncoprotein binds to the products of the retinoblastoma susceptibility gene (Rb), whereas E6 binds to the p53 protein.
(Eliyahu et al., 1989). The E6 binding to p53 promotes the degradation of p53 (Scheffner et al., 1990). The Rb and p53 are considered to be the tumor suppressor genes for many a cancer. Interference with their function may lead to deregulation of the cell cycle and to chromosomal instability and aneuploidy that are regularly observed in individuals with high risk HPV infections (McCance et al., 1988, Pirisi et al., 1987). E6 or E7 proteins from persons with low risk HPV infections (HPV 6 or 11) not only appear to bind either less actively or not at all to these host cell proteins (Dyson et al., 1989); but they also fail to induce chromosomal changes. Thus, the interaction of high risk HPV E6-E7 gene products with cellular Rb and p53 protein may represent an endogenous progression factor and may be important for the progression of premalignant lesions to malignant stages of carcinogenesis as the consequence of the induction of mutation and chromosomal instability. p53 mutations have been noted in HPV negative cervical carcinoma cell lines but not in HPV 16 or 18 positive lines (Scheffner et al., 1991).

Immunosuppression and cervical cancer

There is growing evidence linking immunosuppression to increased risks of cervical cancer. Among the populations showing elevated rates of cervical cancer are renal transplant patients; possibly explaining the excessive rates is the observation that these patients are unusually prone to developing genital infections with both papillomavirus and herpesvirus (Matas et al., 1975 a; Scheneider et al., 1983; Sillman et al., 1984). However, these patients are in general susceptible to infection by a variety of organisms, leading to questions regarding the specificity of the association.

Assessment of the relationship of immunosuppression to cervical cancer risk particularly in view of a recent publication shows excessive rates of both cervical intraepithelial neoplasia and infection with both herpes virus type 2 (HSV 2) and papillomavirus (Adam et al. 1985), an additional group of whom there is evidence of immunosuppression.
Azadirachta indica commonly known as Neem plant is reputed to have many curative properties in traditional medicine. In the past, the anti-viral activity of various parts of neem has been demonstrated by various workers. Rao et al., (1985) used aqueous flower extracts of Azadirachta indica, for inducing resistance to potato virus (PVX) in the hyper sensitive Chenopodium amaranticolor. Srivastava et al., (1986) found that crude margosa(neem) oil inhibited the transmission of cucumber mosaic virus (CMV). Kareem et al (1988) studied the effect of mixtures of neem seed kemel or neem cake powder and carbofuran granuled for controlling green leaf-hopper (GLH) and rice tungro virus (RTV). Singh et al.,(1988) showed the anti-viral activity and studied physical properties of the extracts of Azadirachta indica. The leaf and bark extracts inhibited infection of Chenopodium amaranticolor by cowpea mosaic comovirus. Anti-viral activity of neem leaf extract has been also reported against pox viruses in cell culture (Rao et al., 1969).