424

INFLUENCE OF SOME CHEMICAL AND HORMONAL MODIFIERS ON THE DEVELOPMENT OF MURINE CERVICAL CARCINOMA

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TO

MY SPIRITUAL FATHER

YOGIRAJ VETHATHIRI MAHARISHI AVL

PREFACE

The research work embodied in this thesis has been carried out in the School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067. The work is original and has not been submitted so far, in part or full , for any other degree and diploma of any University.

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CONTENTS

PRELUDE

GENERAL INTRODUCTION

Cancer:

Life would like to grow and multiply but when cells cooperate in the construction of complex organism their growth must be regulated in the interest of the whole. A higher organism is possible only by repressive growth politics. These politics must be very rigorous, for a mammalian cell too - not counting exceptions - could double itself every 24 hours. Even the smallest amount of freedom would be fatal; if the production of the epithelial cells which cover the digestive tract were to increase only a few percent, the system would be completely blocked within a short time. Therefore, production and sloughing off must

be adjusted to each other in the finest degree. In a tumor this adjustment is out of order; a tumor cell no longer reacts to the regulatory impulses of the tissue or the whole organism. It appears to be deaf to the growth regulating signals. This is one of the important characters of the cancerous growth. But defining 'cancer or tumor' is a difficult task. Nevertheless, the main characteristics of the too frequent neoplasms of man are well known as a result of clinical observations extending back for several millennia and of the study of their cellular pathology for more than a century.

Foulds (1975) discusses extensively about the problem of defining 'Cancer'. He points out that the most distinctive character of neoplastic proliferation is that it is uncoordinated, independent of the structural and functional pattern of the organism and indefinitely progressive. In this, it differs fundamentally from reparative growth, hyperplastic growth and the growth of malformations in all of which the cellular multiplication is limited in amount and duration. It is clear that neoplasia involves some permanent cellular change manifesting in itself in excessive multiplication, that the change is transmitted to the descendants of the first affected cells to an infinite number of generations and

that it persists in these without the continuance of the stimuli which initially evoked it. So a tumor is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of normal tissues and persists in the same excessive manner after cessation of stimuli which initiated the change.

Causes of Cancer and Mechanisms of Cancerogenesis:

The principle known causes of cancer in man are radiation, chemicals and viruses. The exact mechanisms of carcinogenesis by these agents are not completely known, but there is substantial data which gives some clarification of the processes involved.

Radiation is an universal carcinogen as it is present everywhere in the biosphere. Ionizing radiations may elicit somatic mutations, activate oncogenic viruses or protoviruses or misrepair the nucleic acids and thereby induce neoplastic changes (see Hiatt et al., 1977). Ultraviolet radiation induces the formation of pyrimidine dimers in DNA (Maher and McCormick, 1979).

Seventy percent of the human cancers are suspected to be caused by environmental chemicals (Epstein, 1977). The majority of the chemical carcinogens require metabolic activation by enzymes in the target organs or elsewhere in

order to induce cancer. The terms precarcinogen, proximate carcinogen and ultimate carcinogen have been introduced to describe the relatively inactive parent compound, metabolites with greater activity and the final product that reacts with the crucial cellular target to cause the malignant change. The ultimate carcinogens are highly reactive electrophiles that react with nucleophilic centers in DNA, RNA and proteins (Fig. 1).

Oncogenic viruses may contain either DNA or RNA. After entry into the cell, the DNA of the DNA viruses becomes stably integrated into the genome of the host cell and this is followed by the change to a cancer cell. The RNA of the RNA tumor viruses acts as a template for the RNA dependent DNA polymerase that is present in the virion. The DNA product is then incorporated into the cellular DNA as with oncogenic DNA viruses and replicates with the host DNA. Recent researches indicate that the possibility of the involvement of oncogenic viruses in several human cancers (see Hiatt et al., 1977).

Geographical Distribution of Cancer and the Importance of Cervical Cancer in India:

Cancer is one of the most dreaded diseases all over the world. Epidemiological studies show that

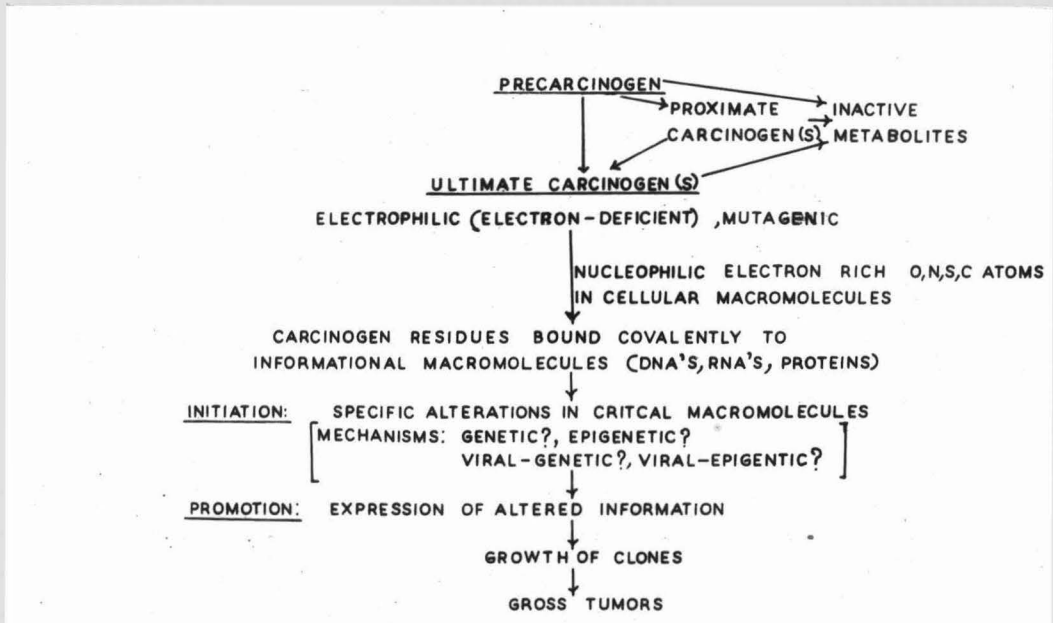
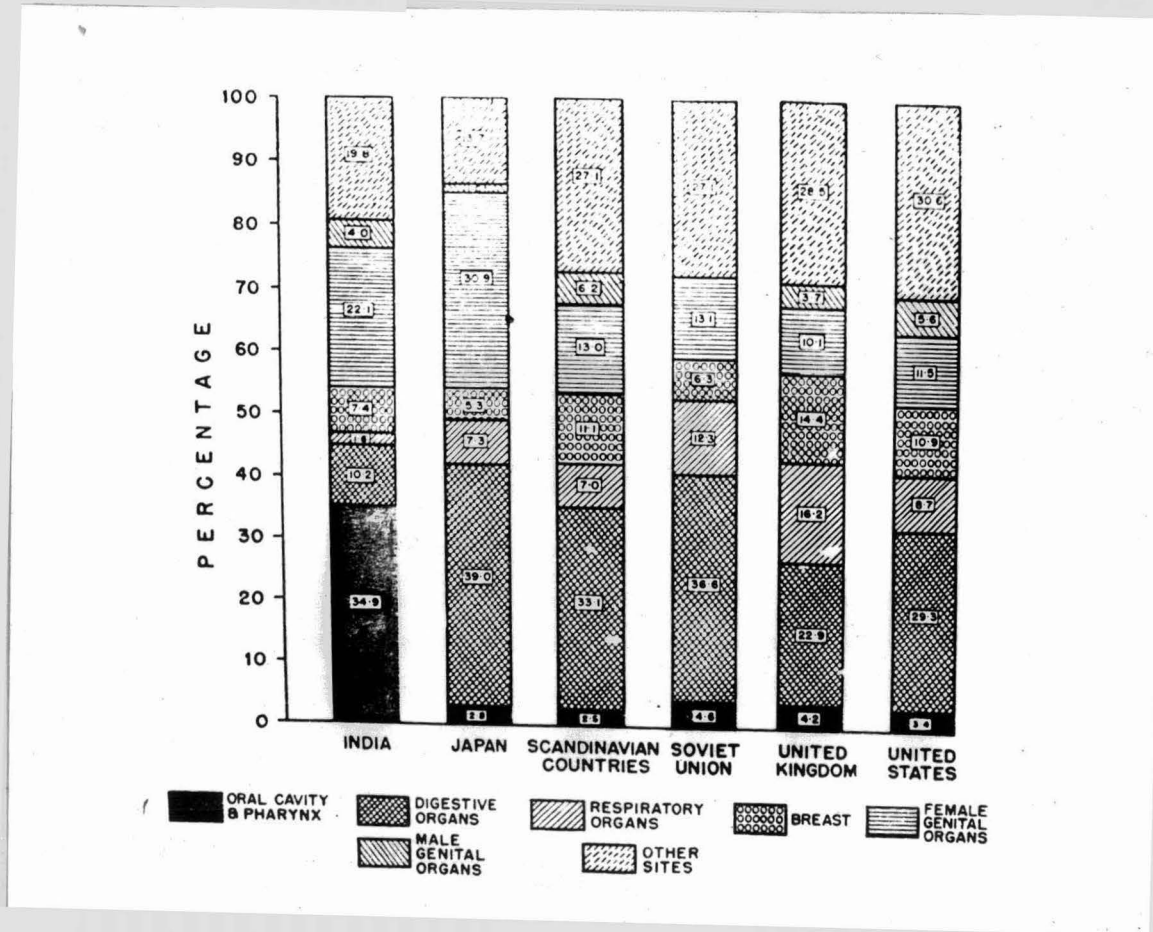


FIG.1 GENERAL ASPECTS OF THE METABOLISM AND MECHANISMS OF ACTION OF CHEMICAL CARCINO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UNITED KINGDOM
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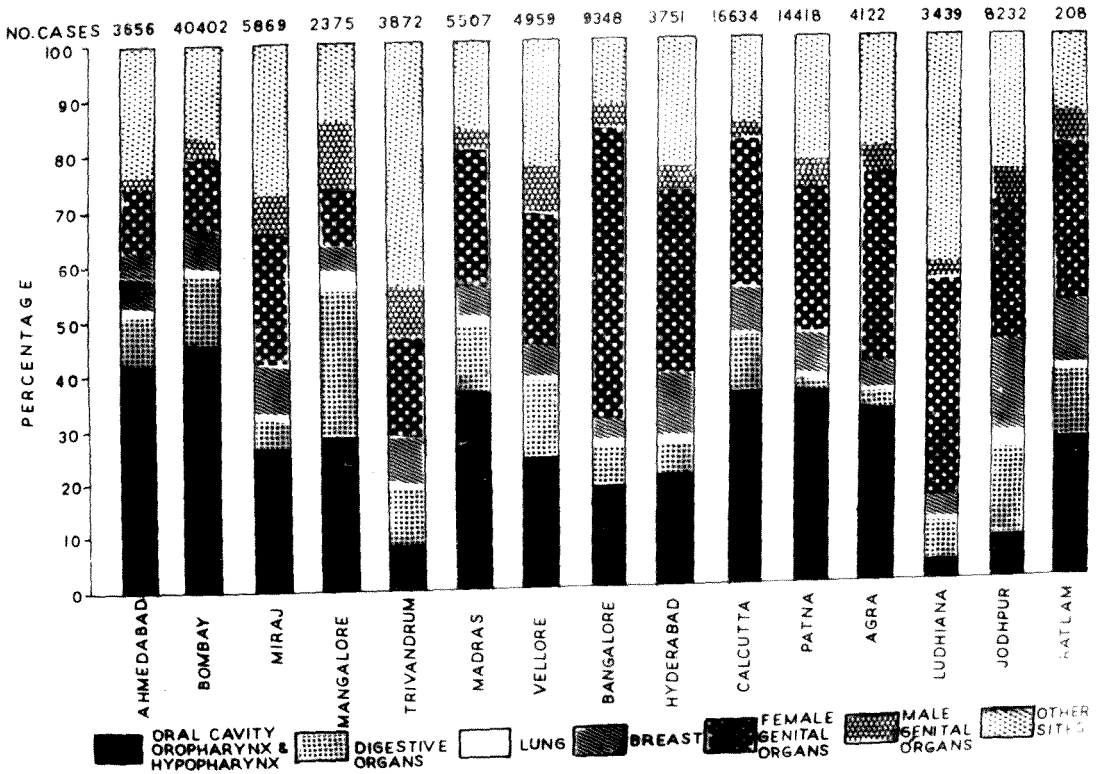

Fig. 3: Incidence of different types of cancer in India. (Paymaster, 1964).

always a particular type of cancer predominates in a region. The distribution of cancers in some important countries, and in India are illustrated in the Figures 2 and 3. It is to be noted that almost 60-70% of the cancer deaths of the females is in the genitourinary region, the uterine cervical cancer taking an important share (Paymaster, 1964). According to Luthra (1970) the estimated crude average annual prevalence rate of the cancer of the uterine cervix in Agra district, India is 210 per 100,000 women. This places Agra the first among populations of higher risk for uterine cervical cancer in the world (Luthra, 1970). Early marriage and multiple pregnancies are significant factors in the high prevalence of cervical cancer in Agra families.

Cervical cancer forms 28.6% of all cancers and 51% of all female malignancies at the Cancer Institute, Madras. It had a prevalence rate of 1.8 per 1000 population in a sample survey carried out in the Madras hinterland (Shanta et al., 1977). Oral cancer comes only second with a prevalence rate of 1.4 per 1000 population in that region. According to Bhargava (1981) a total number of 11,607 cancer patients were admitted in Kidwai Memorial Institute of Oncology, Bangalore during the period 1973 to 1980. Of these, 2840 were cervical cancers, thus accounting for one-fourth (24.5%) of all cancers.

Bombay cancer registry shows uterine cervical cancer forming 24.7% of all female cancers (Jussawalla, 1976). In Kurnool, (Andra Pradesh) the cervical cancer forms 36.11% of all female cancers (Reddy et al., 1967).

Thus, it is very clear that cervical cancer constitutes a major oncologic probelm in India. The influence of environmental factors on the incidence and frequency of cervical cancer was stressed by many workers in the field (Stocks, 1955; Wynder, 1955; Shanta, 1977; Luthra, 1970). It is common in women from low socioeconomic groups. There is a significant correlation of early marriage and the birth of first child with the incidence of cervical cancer. Further conflicting reports have been reported on the role of factors like number of pregnancies and the circumcisions of the husbands etc. Low incidence of cancer of the cervix in nuns and among Jewish women is noteworthy (Boyd and Doll, 1964).

Owing to the wide prevalence and importance in India present study is undertaken on the cervical cancer at the experimental level. There are some reports on the induction of experimental cervical cancer by chemical carcinogens (Murphy, 1953; Forseberg, 1974). Based on studies with exfoliative cytology the development of clinical and experimental cervical cancer was also discussed in the

literature (Koss, 1978; Kehar and Wahi, 1967). However, there is no report on a standardized animal model system with detailed histopathological observations at different stages of the cervical cancer development. One of the tasks of the present study is to make an attempt to solve this problem.

The multiplicity of the etiological factors implicated in the literature shows the importance of extrinsic and intrinsic modifying factors in cervical cancer. So the influence of aging and some chemical and hormonal modifiers on the development of cervical cancer is also investigated in the present study.

Chapter I deals with the sequential analysis of murine cervical cancer development.

Chapter II deals with the influence of aging on the cervical carcinogenesis.

Chapter III deals with the influence of some chemical modifiers like butylated hydroxyanisole, retinoic acid and 2-mercaptopropionyglycine, on the development of cervical cancer.

Chapter IV deals with the influence of some exogenous hormones like estrogen, progesterone, prolactin and testosterone on the cervical cancer development.

CHAPTER - I

SEQUENTIAL ANALYSIS OF CERVICAL CANCER DEVELOPMENT

INTRODUCTION AND REVIEW OF LITERATURE

A Forward Look for Cancer Development:

Most human cancers are discovered only when they have grown to a recognizable size or invaded or metastasized. Hence different methods of evoking neoplasia experimentally in animals are of great value for studying many aspects of neoplastic development from the early stages. Experimental carcinogenesis has been achieved mainly by chemical, physical and viral agents. They may act directly or locally at the point of application or remotely on a distant target tissue. The mechanism of action of these agents were briefly discussed in the earlier chapter.

First in 1914, Yamagiwa and Itchikawa, reported the development of papillomas by the repeated applications of coal tar to the inner surface of rabbits' ears. From that time onwards studies on experimentally induced cancer have been progressing, with the hope that these observations will extrapolate to the situations in man. Unlike the human situations most rodent tumors do not invade or metastasize but their cells are anaplastic and have the ability to kill the host by cachexia as those of the metastasizing tumors of man.

As was clearly pointed out by Foulds (1975) the majority of cancers develop as a multistep process. He considered carcinogenesis as a 'process of sequential neoplastic development' extending over a long period of time which in man might amount to several decades and be manifested by a wide variety of lesions that might emerge contemporaneously or consecutively at various times and places. As with any multistep process at any level of organization from the molecular to the cellular to the organismic an understanding of the process is dependent upon the identification of the essential nature of the major steps and the factors of their emergence and fate. Carcinogenesis is no exception to this general principle. The study of the development of cancer "from beginning to end" is one of the important aspects of cancer research.

"Historically" as pointed out by Foulds (1975), "neoplastic diseases have been studied from end to beginning, which has been encouraged a backward way of thinking". Given the apparent complexity of the processes through which malignant neoplasia develop and the enormous variations among individual cancers in even a single organ or tissue, it seems reasonable that an analytical approach "looking forward" might provide insights not really obtainable when looking backward.

As emphasized by Foulds (1969, 1975) and Farber (1973) in most examples of cancer development new cell populations represent stages in the cellular evolution from normal through initiated, preneoplastic and pre-malignant cells to highly malignant neoplasia. In otherwords such new cell populations create the material continuity of suspected sequential lesions essential for our ultimate understanding of how cancer develops.

According to Farber (1980) the process of carcinogenesis consists of a number of biological phenomena that represent fundamental response patterns in biology and as such deserves intensive and penetrating study. Although not completely but partially the success to date in the control of cancer of the skin and of the uterine

cervix by removing noncancerous presumptive precursor lesions such as dysplasia and carcinoma in situ and thus interrupting cancer development attests to the practical benefits that are likely to occur from a "forward looking" approach. This success has occurred despite the rather superficial knowledge and understanding of the carcinogenic process.

Sequential Cellular Events in a Typical Carcinogenic Process:

A composite and idealized diagrammatic representation of neoplastic development is shown in the Figure 4. It summarizes the possible stages in the process. Burnet's stochastic approach (Burnet, 1971) as opposed to a deterministic approach for the study of neoplastic disease should be emphasized here. Stochastic processes are those which appear random when observed in individuals but manifest statistical regularities when sufficiently large populations of those individuals are studied. In the Figure 4, the first initiatory phase (Phase A) is either clinically silent or manifested only by apparently trivial and dubiously neoplastic lesions designated as group 'A' lesions. The first clues to the recognition of initiating circumstances usually come from the observation of an interruption of the normal random scattering of afflicted patients through the whole population by a clustering of

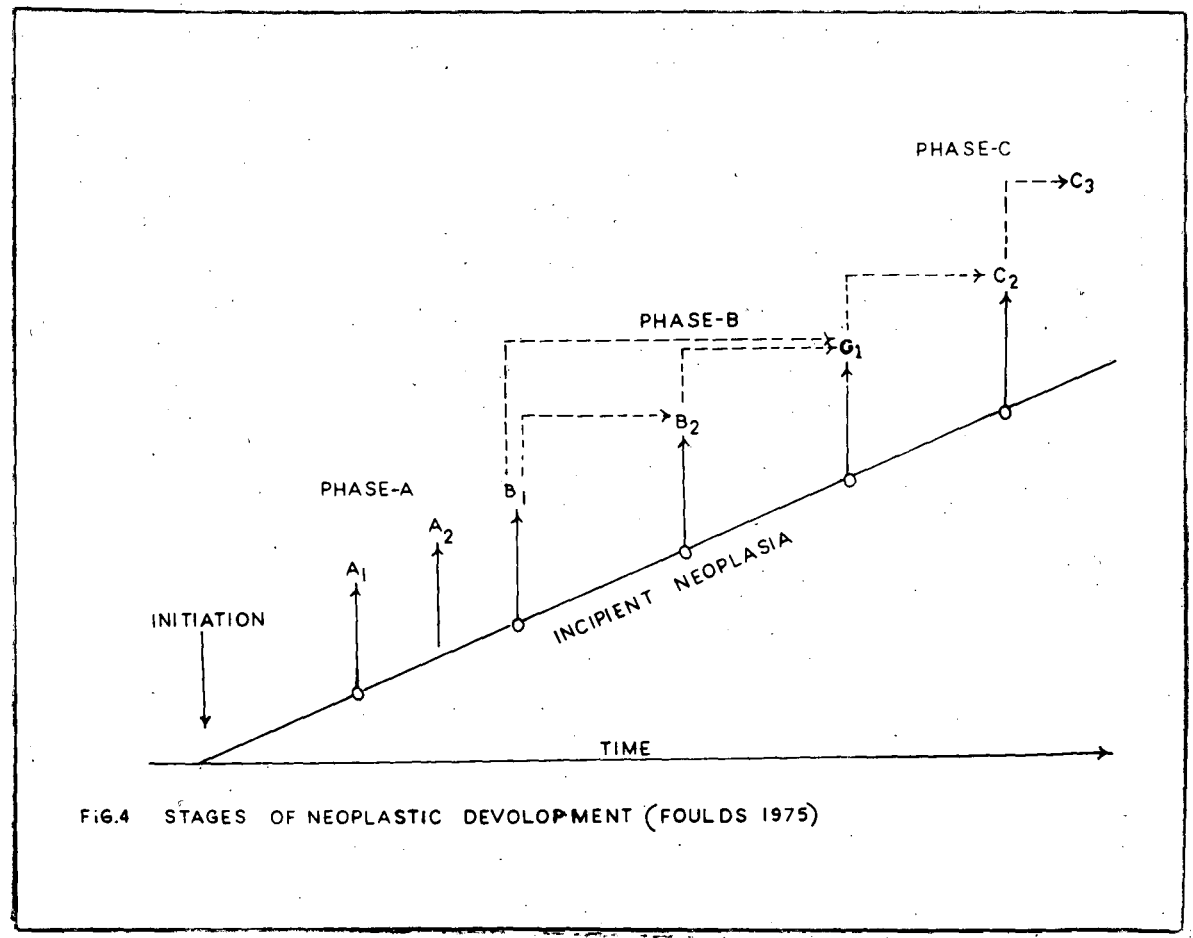

FIG.4 STAGES OF NEOPLASTIC DEVOLOPMENT (FOULDS 1975)

diseased patients in certain identifiable groups of people within it. The recognition of chimney sweeps cancer in the eighteenth centuary England and of the excessive incidence of lung cancer in twentieth century heavy cigarette smokers provide an old and modern example. The initiatory circumstances establish a diffuse state incipient neoplasia coextensive with the region of exposure to those circumstances and characterized by having a finite capacity for neoplastic development. The incipient neoplasia once established is an enduring state that persists throughout the remaining life. And it is an evolving state whose neoplastic capacity can be augmented by intensification or prolongation of the initiating circumstances and probably like embryonic competence may change autonomously with mere lapse of time. In Figure 4, the upward starting line indicates the augmenting neoplastic capacity of the region of incipient neoplasia. The vertical block lines indicate the emergence of discrete neoplastic lesions from the incipient neoplasia at various times. The emergent lesions increasing in gravity with the passage of time as indicated diagrammatically by their increasing height above the base line.

The group 'A' lesions are in the developmental state and are not consistently manifested by any specific histological

changes. The 'A' lesions are the damaging side effects of the initiating circumstances. The group 'A'_2 lesions comprise a medley of proliferative lesions some or most of which are innocuous hyperplasias whereas others may be equally harmless abortive neoplastic lesions that have reached dead ends of neoplastic development. Possibly a few are capable of further neoplastic development.

The second intermediate phase (Phase B) is manifested by a variety of neoplastic lesions including those commonly described as "precancerous" that cannot be plausibly assigned to either 'benign' or 'malignant' categories of the orthodox classifications of neoplasms but are in some sense intermediate between them. The group 'B' lesions are subjected to four main fates: (i) they may undergo progression to form either another group 'B' lesions with a higher degree of neoplastic capacity or to produce a group 'C' lesions; (ii) they may grow progressively, although slowly, for a long time without any qualitative change; (iii) they may persist indolently for a long time with minimal growth and no qualitative change; or (iv) they may regress completely. The relative frequencies of these four prospective fates vary widely in various situations. The diagram shows the two group 'B' lesions i.e. 'B_1' and 'B_2'. The 'B_2' lesion is presumed to have a higher degree of neoplastic capacity than 'B'_1'. Both the lesions may derive independently of one

another directly from the region of incipient neoplasia as indicated by the vertical solid lines. And 'B_2' may develop indirectly from the incipient neoplasia by progression in 'B_1'. The dotted lines indicate paths of such direct development by progression in intermediate lesions.

The advanced phase 'C' is characterized by the presence of the malignant carcinomas and sarcomas of the orthodox classifications. The C_1 and C_2 lesions are shown to indicate the different degrees of malignancy and the progression to increasing malignancy within phase 'C' itself. The 'C_3' lesions have no counterpart in man but indicate the further aggravation of malignancy that may occur in transplanted tumors in laboratory animals. The diagram shows the direct origin of 'C_1' or 'C_2' but not 'C_3' from the region of incipient neoplasia. It also shows the origin of 'C_1' by indirect development through an intermediate stage 'B_1' or 'B_2'. The possible indirect development through 'B_1' and 'B_2' to 'C_2' are omitted to avoid over complicating the diagram. And some of the group 'C' lesions emerge directly from the region of incipient neoplasia with all their malignant properties established from the beginning. The relative frequencies of direct and indirect paths of development are impredictable and can be assessed statistically only on the basis of abundant empirical observations.

Present work relates to cervical cancer development with known suspected lesions like, mild dysplasia, moderate dysplasia, marked dysplasia and early and advanced carcinoma. These lesions were categorized mostly by clinical as well as experimental studies of the exfoliated cells during different stages of the cervical cancer development.

Cellular Events in the Pathogenesis of Human Cervical Cancer:

The human cervix (Fig. 5) is a continuation of the lower end of the corpus uteri extending from the level of the internal os to the wall of the vagina. The endocervix extends from the internal to the external os and is lined by columnar mucus secreting epithelium. The portio of the cervix or ectocervix projects into the vagina and is covered by stratified squamous epithelium. The term squamous columnar junction is used usually to describe the junction of the two types of epithelium which ideally coincides with the location of the external os.

With the advent of Pap smear test (Papanicolaou and Traut, 1943) new concepts of the pathogenesis of cancer were introduced, particularly for uterine cervical cancer. Abnormalities noted in exfoliated cells of the Pap smear correlated in the biopsy specimen with histological changes consistent with neoplasia but confined to the mucosa. The intraepithelial lesions, dysplasia and preinvasive cancer,

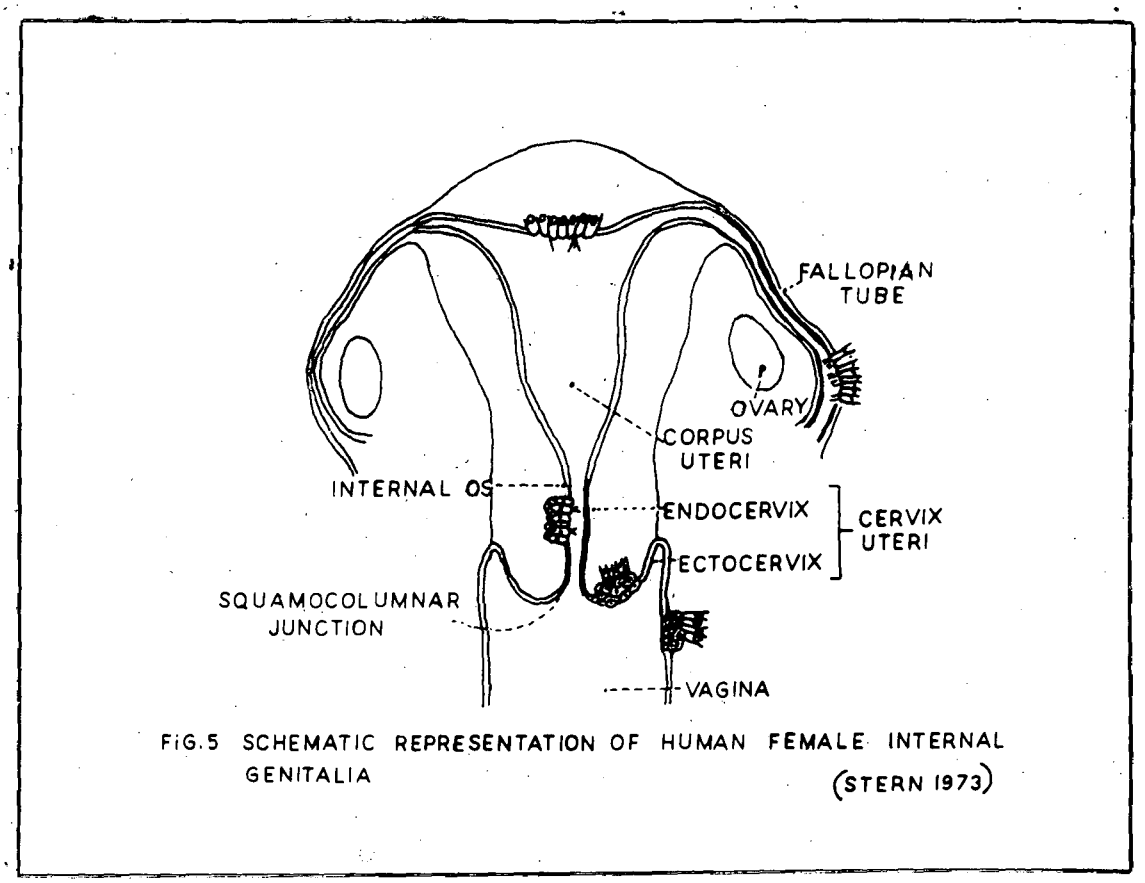

FIG.5 SCHEMATIC REPRESENTATION OF HUMAN FEMALE INTERNAL GENITALIA (STERN 1973)

were thus identified and the concept was formulated that dysplasia is a precursor of cancer and that there is an 'in situ' or preinvasive stage of the disease. It was postulated by Stern (1973) that a gradient of changes at the site of origin in the endocervical mucosa-reserve cell hyperplasia, squamous metaplasia, dysplasia, preinvasive, and invasive cancer occurs in the pathogenesis of squamous cancer of the cervix.

Reserve cell hyperplasia results from proliferation of the reserve cells normally present in patches and serving as depots for regeneration of columnar lining cells. In the next change squamous metaplasia the cells become polygonal and resemble stratified squamous epithelium. These cells may begin to produce glycogen while cells at surface may retain columnar shape and continue to secrete mucus. Neither reserve cell hyperplasia nor squamous metaplasia noted in 40 to 90% of adult cervices can be regarded as precursors of squamous cancer but rather as nonspecific preliminary change of the epithelium.

In dysplasia, the cells are definitely abnormal and show criteria similar to cancer cells. It is considered as a transitional state in the pathogenesis of cancer of the cervix with cellular attributes which permit progression to cancer as well as regression towards normality. In a prospective study of women with dysplasia

Stern (1973) found 12% progressed from dysplasia to cancer. Annually about 34% per year regressed from dysplasia towards normality and of these 30% per year showed a recurrence of dysplasia. Further in following a population of women previously negative for cancer of the cervix Stern (1963) found that almost all new cases of cancer were discovered in patients with dysplasia while very few were found in much larger fraction of the population which did not show dysplasia. In preinvasive (in situ) cancer the malignant process is confined to the epithelial layers. In the invasive stage, the disease spreads locally and to distant organs.

Experimental Studies on Cervical Cancer:

Spontaneous malignant neoplasms of the uterine cervix appear to be quite rare in both wild and domesticated mammals. No primary malignant cervical tumors were encountered in 3,400 wild mammals autopsied at the Philadelphia Zoological Gardens (Ratcliffe, 1933). Primary carcinoma of the cervix is similarly rare in laboratory animals. According to Slye (1934) the incidence of spontaneous uterine tumors in mice is less than 0.06%. However, in mice of the PM strain Gardner and Pan (1948) found 13 spontaneous malignant tumors of the uterine cervix, vaginal fornices and upper vagina in 56 mice. Unfortunately,

this strain was lost because of high incidence of steribity. Because of the rare incidence of the spontaneous uterine cervical cancer in animals, the induction in laboratory animals for experimental study has been necessitated.

Induction of cervical cancer:

Attempts to induce carcinoma of the uterine cervix experimentally began shortly after the discovery of the carcinogenic properties of crude tar by Yamagiwa and Itchikawa (1947). The earliest developments consisted of intravaginal application of crude tar by instillation or implantation into the vaginal vault, cervix and lower uterine segment. Although, squamous metaplasia was readily induced, invasive carcinoma was observed in only a few cases.

Later Gardner and coworkers (1959) studied the influence of exogenous estrogens on the mouse uterus. They found that weekly injections of estrogens continued for over a year led to carcinoma of the cervix in 50 to 60% mice. All strains responded in this way if they survived long enough, the higher tumor incidence occuring at 450 to 600 days (Allen, 1941).

Cervical and vaginal tumors could also be induced in adult mice by pellets of stilbestrol-cholestrol attached to nylon threads dipped in colloidin and placed in the vagina.

They were also seen in some mice after intravaginal instillation of stilbestrol three times weekly. Estradiol benzoate administered weekly was also effective. Other substances under consideration for use in intravaginal contraceptives (urea, adipic acid and carboxymethyl cellulase) also induced invasive lesions of the vagina and cervix after 50 days or more (Gardner, 1959). Experiments by Dunn (1963) confirmed the effectiveness of estrogens administered on the day of birth. Injections of diethylstilbestrol in three mouse strains led to the formation of astonishing concretions in the vagina in 12 out of 30 after 13 months as well as carcinoma of the cervix and vagina (Dunn, 1963). The antifertility drug, Enovid administered in liquid diet to new born BALB/C mice produced endometrial lesions similar to estrogen-induced ones and some animals continued on Enovid for around two years had lesions of the cervix and diagnosed as early cancer (Dunn, 1969). Subcutaneous implants of testosterone pellets twice a week in female C57B1/DBA mice starting at 6 to 13 weeks of age induced uterine tumors in 26 out of 42 in 15 to 18 months; mostly in the cervix or impaired uterine part (VanNie et al., 1961). When benzo(a)pyrene (B(a)P) in cholesterol was broken into fragments of about 5 mg and placed into the vagina of 10 mice, two or three times a week, tumors appeared in all after 10 to 14 months.

They were first seen in the vaginal wall and all were infiltrating squamous cell carcinomas (Fishman et al., 1942).

Other methods of applying carcinogens were devised such as painting acetone solutions on the cervix with cotton tipped wire loops (Von Haam and Scarpelli, 1955) and later aided by the use of an infant-sized otic speculum (Iijima et al., 1964) or by impregnating string that was then threaded into place and secured with knots or stiches at laparotomy (Koprowska et al., 1958).

Rats appear to be less susceptible than mice to cervical tumor induction by B(a)P painting of the cervix (Steinwerblowsky, 1960). 3-methylcholanthrene was shown to induce cervical carcinoma when uteri of young female mice were removed, the carcinogen crystals were inserted, and the uterus transplanted subcutaneously on the upper abdomen of the hosts (usually brothers and sisters) of the same strain. Of 104 mice of various genetic types 55 developed 61 tumors (32 carcinomas and 29 sarcomas) the majority appearing by the eighth week. Most tumors occured in the cervix than in the uterine horns and the tumors included epidermoid carcinoma, adenocarcinoma, adenoacanthoma and sarcoma (Pan and Gardner, 1948).

An investigation (Forsberg, 1972) using threads with three knots impregnated with methylcholantherene (MCA) in bees wax in the ratio of 1:3 and suspended in the vagina of C_3H mice led to malignant neoplasms of the cervix developing from seven weeks onward in the majority of animals. In all the animals with carcinoma, the cervix was involved and there were extensions of tumor into the uterus and the upper vagina. The carcinogen dimethylbenz(a)anthracene (DMBA) was also used in the knotted silk threads to induce the cervical cancer by some investigators (Joneja and Carlson, 1973; Meisels, 1966).

In a review of the subject by Scarpelli and Von Haam (1957) a comparison was made of the string and painting methods with benzo(a)pyrene and methylcholanthrene in C_3H female mice. The highest incidence of cervical tumors was obtained at 11 to 33 weeks by using methylcholanthrene string method (85%). Then in order came B(a)P string, MCA painting and B(a)P painting. With the string method most tumors were induced in the cervix, but painting gave many vaginal lesions. Many tumors were invasive and extended to pelvic nodes with metastasis seen in lung and liver. The lesions showed striking resemblances to human cervical cancer.

Experiments with virally induced cervical cancers are very rare. Virus-like particles were seen in examination

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of the ultrastructure of cervical carcinomas induced in mice (C_3H x A) by treatment with B(a)P. They were not seen in any of the normal tissues of the mice that were examined and it was speculated that the viral particles seen in the tumors might be the result rather than a cause of the carcinomatous process (Thiery et al., 1959). Further investigations have been done to induce cervical cancers in animals with the viruses(Munoz ,1973). The animals were infected by applying the virus on cotton swabs to the cervix. Out of 19 BALB/C mice treated with HSV-2 alone only two cancers and one precancerous lesion was seen. One of these cancers was transplanted with success (Munoz, 1973).

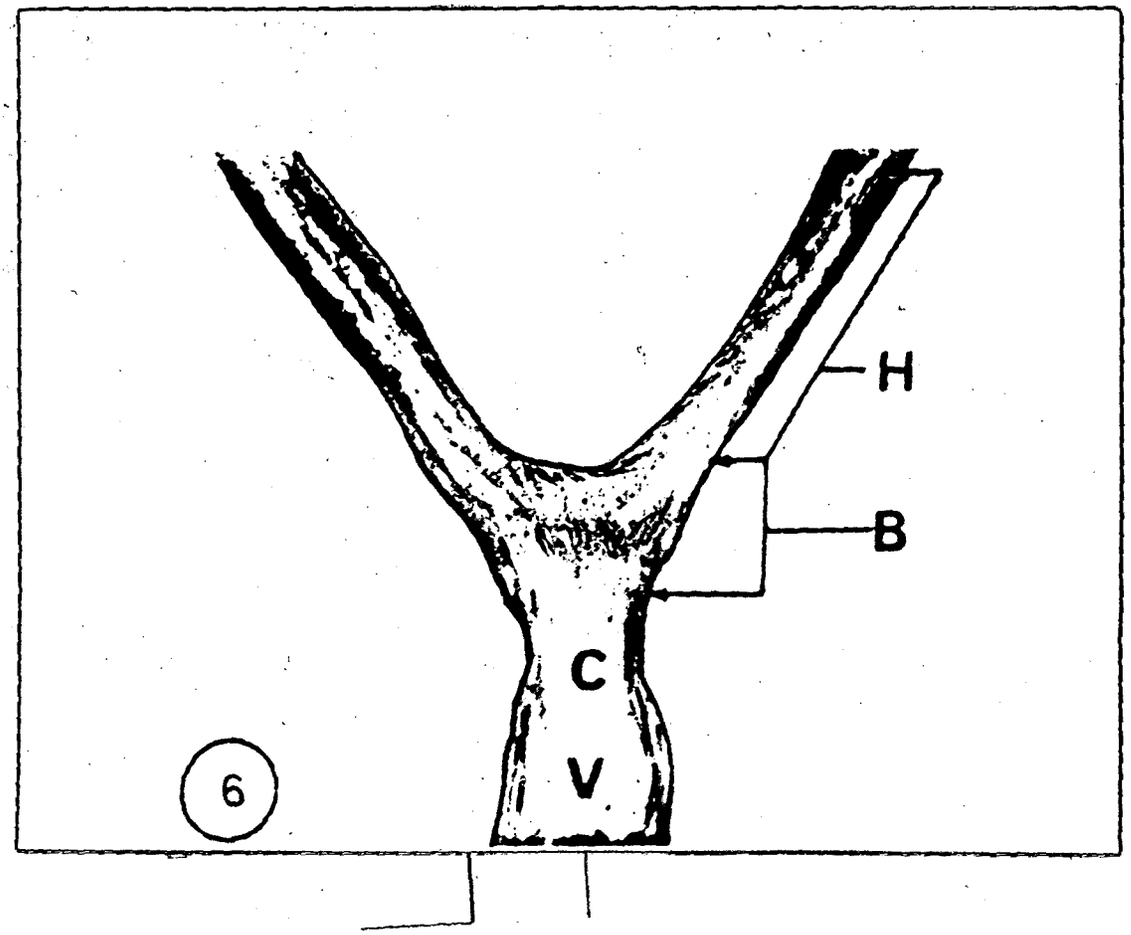
Pathogenesis:

Experimental studies on the pathogenesis of cervical cancer were made mostly on mice. So it will be appropriate to explain the histology of an uterine cervix in a normal mouse.

The mouse uterus (Fig. 6) is composed of two horns 3-10 mm in length which fuse in a 'Y' fashion to form an undivided caudal segment, the corpus uteri. The mid dorsal and mid ventral outer surfaces of the cervix were found with the adjacent inner surfaces of the vagina to form dorsal and ventral frenula, the former extending

Fig. 6 : Caudal portion of the reproductive tract of the adult female mouse (diagrammatic). The two uterine horns or tubes (H) join to form the body of the uterus (B) and uterine cervix (C) which is obscured by the cranial portion of the vagina (V). X 12. (Leppi, 1964).

Fig. 7: Representative diagram of a mid coronal section of the genital tract of an adult mouse showing the lumen of each uterine horn (H) continuous with those of the uterine corpus (B). These lumens are divided by a mid line septum (S) whose caudal edge marks the cranial limit of the uterine cervix (C) which contains a single lumen and projects into the upper vagina (V) forming deep vaginal fornices (F). Circularly arranged smooth muscle is represented by cross hatching, stroma of the uterine horns and body is indicated by (R), cervical and vaginal stroma by (A) and simple columnar epithelium by interrupted lines, and stratified squamous epithelium by continuous lines. X 15. (Leppi, 1964).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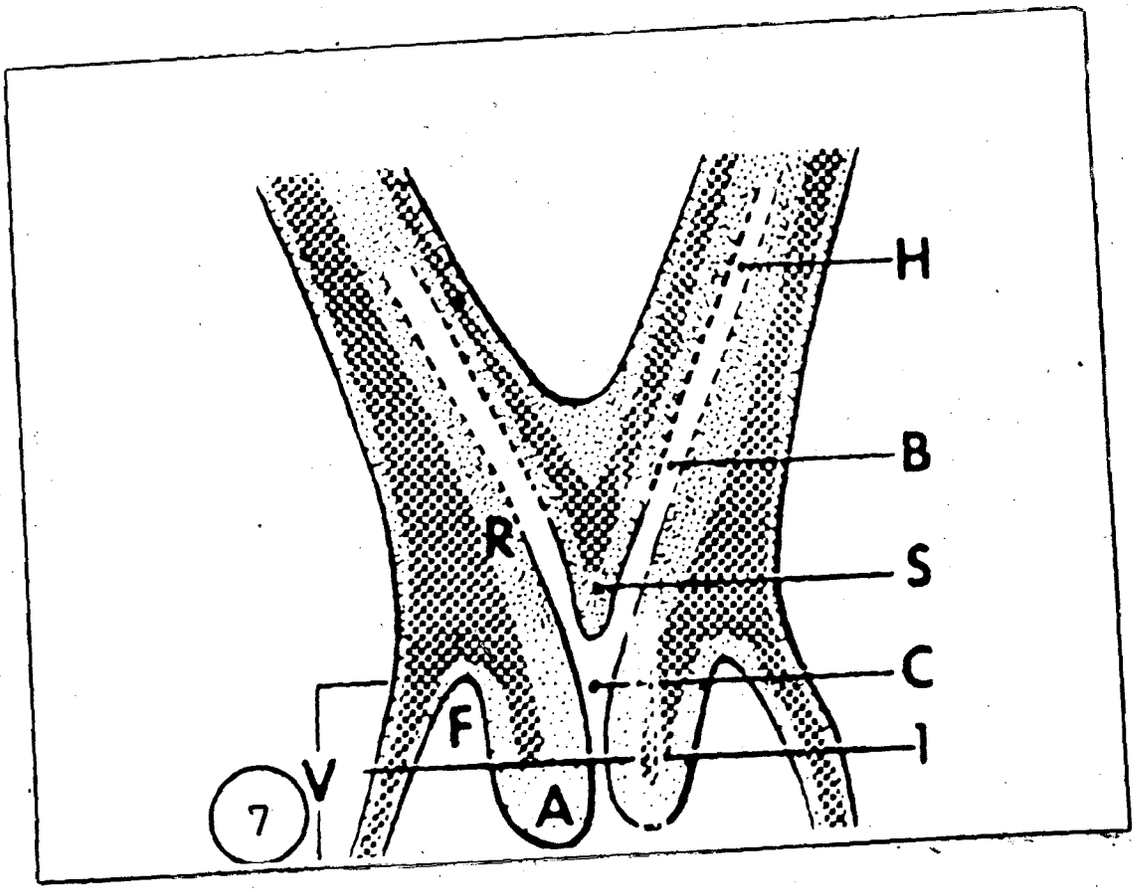
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further caudally (Fig. 7). Grossly and externally the cervical limit of the corpus was determined to be of the level of a transverse line drawn across the point where the medial surface of each horn fused to form the corpus while the reproductive tract was intact and in situ (Leppi, 1964). The luminal epithelium of the uterine horns and cranial one half of the corpus was simple columnar with simple branched tubular, uterine glands projecting down from the lumen. The remainder of the corpus, the cervical canal and the vaginal investment of the outer cervix had stratified squamous epithelium which varied in extensiveness of stratification and cornification with the estrous cycle. The distance from external os to squamo columnar junction varied from 2.6 to 4.4 mm at the different stages of estrous cycle. And the division of distance of external os to columnar junction by distance from external os to bifurcation varied from 0.57 to 0.73 mm (Iijima et al., 1964). The myometrium surrounding the endometrium consists of circularly arranged smooth muscle fibres. The outer surface of the myometrium is covered by a layer of connective tissue containing large blood and lymphatic vessels called stratum vaculosum. This in turn is covered by longitudinal smooth muscle fibres. At the point of fusion of the uterine horns the longitudinal muscle layer and stratum vasculosum disappear. Lateral on each side of the corpus uteri the lumen of the

vagina forms a high a deep fornix (i.e. the dorsal and ventral wall of the corpus are fused with the vagina). The vagina is covered in its entireity by squamous epithelium.

Numerous reports discussing the intraepithelial changes preceding invasive carcinoma of the cervix in rodents, particularly in mice subjected to local or systemic treatment with hormones and/or carcinogens have appeared in the literature (Murphy, 1961; Kaminetzky, 1966; Kehar and Wahi, 1967; Reagan et al., 1955; Gardner, 1959; Koprowska and Bogacz, 1959). Since the cervical neoplastic transformation is accompanied by a decreased mutual adhesiveness of malignant cells, the rate of cellular exfoliation is increased and the vagina becomes "a sac containing free cells" providing a steady supply of malignant cells. Scarpelli and Von Haam (1957) extensively studied the cervical carcinomas and on the basis of the cytologic findings, four distinct lesions could be differentiated:

1. Acute inflammation - The early response of cervical epithelium to a twice weekly application of ^{3}H-benzpyrene in acetone was reflected in the vaginal smear by a large number of polymorphonuclear leucocytes, many of which showed clumping around necrotic squamous cells. The tissue sections showed a focal polymorphonuclear infiltration of

the squamous epithelium forming small micro abscesses. In more severe cases there were in addition varying numbers of polymorphonuclear leucocytes accompained by edema in the submucosa. The subsidence of inflammatory response after 2 to 3 weeks suggests a possible adaptation of the mucous membranes to these physical and chemical irritants.

2. Dysplasia - Epithelial dysplasia of the uterine cervix is characterised by a gross disturbance in the differentiations and keratinization of the basal cell layer with disorderly arrangement of the cells without either mitotic figures or cell crowding indicative of increased cell proliferation. There was also some loss in the polarity of basal cells with small focal intraepithelial accumulation of leucocytes forming microabscesses. An interesting cytoplasmic alteration which appeared to originate in the para basal layer consisted of an intense eosinophilia. This appeared to be keratin and was associated with an intact though frequently damaged nucleus. In the tissues, these cells were present in small cyst- like spaces in the epithelium (Von Haam and Scarpelli, 1955). And in many instances masses of such precociously kertinized cells were seen exfoliating in the vaginal lumen.

Kehar (1967) splits dysplasia into three stages i.e. mild dysplasia, moderate dysplasia and marked dysplasia. In

mild dysplasia cellular abnormalities were mainly seen in parabasal and intermediate cells and were absent in superficial cells. Fluctuating cellular dysplastic changes were characteristic. Histologically the lesions showed varying degrees of basal cell hyperplasia, epithelial hyperplasia and focal areas of epithelial dysplasia.

In moderate dysplasia parabasal and intermediate cells revealed more cellular changes than were evident in the superficial cells. The cytoplasm of the exfoliated cells exhibited both fine and large vacuoles. Nuclear abnormalities were relatively a permanent feature. Compared to those observed in mild dysplasia groups of intermediate cells showed perinuclear halos. Spindle-shaped squamoid cells varied from 2 to 10%. Histologically epithelial hyperplasia, hyperkeratosis and dyskeratosis involving a wide area of epithelium were seen. Isolated cell keratinization and leucocytic infiltration of the epithelium with formation of microabscesses were also noted.

Marked dysplasia exhibited along with the involvement of parabasal and intermediate cells, a moderate number of superficial cells revealing all dysplastic changes. Nuclei had thickened membranes and were hyperchromatic with prominent nucleoli. Squamoid cells such as spindle, tadpole, fibre and snake forms were encountered in fair number. Histologically the lesions presented the picture of the intraepithelial

carcinoma except that there was a variable amount of differentiation of the superficial layer of the epithelium.

Rubio (1974) identified these above mentioned dysplastic changes as Atypia I, II and III. According to him the intraepithelial atypia bifurcates into two types i.e. one with a smooth epithelial stromal border and the other with an epithelial bud projecting into the underlying stroma. There was a close association between epithelial buds, the degree of cellular atypia and progression to invasive carcinoma. Histological evidence suggested that advanced epithelial atypia with buds are severe lesions which proceeded mostly to invasive carcinoma.

3. Carcinoma in situ or non invasive carcinoma - Carcinoma in situ originates some times by progression in an area of dysplasia but probably more often it emerges from a region of incipient neoplasia as a carcinoma in situ from the beginning. Exfoliated cells from the carcinoma in situ stage showed coarse chromatin pattern and nuclei with multiple nucleoli. Malignant transformation was primarily typified by alterations of the nucleus and nucleolus i.e. hyperchromasia, nuclear enlargement and multiple macronucleoli. Nuclear pyknosis, cytoplasmic basophilia, precociously

cornified basal cells, leucocytes and red blood cells were so constantly found in animals with invasive and noninvasive carcinoma that they could be spoken as representing a malignant pattern.

4. Invasive carcinoma - At this stage the carcinomatous cells of the in situ lesion invades in to the stromal region forming many patterns like palisades, whorls, rosettes etc. Exfoliated cells showed the typical malignant characteristics like the in situ stage. Invasive and noninvasive carcinomas were differentiable on the basis of histopathologic criteria, i.e., non-invasive carcinoma is a lesion contained within an intact basement membrane.

Reagen and Wentz (1959) reported on the changes antedating the development of carcinoma and failed to find an in situ stage. In their study dysplasia was followed by epithelial infiltration indicative of early invasive carcinoma. According to Scarpelli and Von Haam (1960) this may be due to the high doses of carcinogen applied and the short latent period of cancer induction by the carcinogen impregnated string. Thus, the duration of the intraepithelial phase appears to depend on the potency of the carcinogen as well as the dosage and the method of application.

These studies and discussions were mainly based on the observations of exfoliated cells during different stages of cervical carcinogenesis. However, no elaborate study is available on the different stages of cervical cancer development based on mainly the histopathological observations which may shed more light on the knowledge of the neoplastic growth. Present investigation makes an attempt to fill up this lacuna.

MATERIALS AND METHODS

Animals:

Randombred Swiss albino mice procured from Moulana Azad Medical College, New Delhi, were used for all the experiments. The mice were maintained in an air-conditioned room providing rat food (Hindustan Lever Ltd., India) and water ad libitum.

Chemicals:

The carcinogen 3-methylcholanthrene was purchased from Sigma Chemical Company, U.S.A. Bees wax was bought from Mysore and was melted and filtered twice to remove particulate dust.

Tumor Induction Technique:

Murphy's string method (Murphy, 1953; Forsberg, 1972) was followed for the induction of cervical cancer. A double cotton thread was cut into suitable lengths. They were immersed in distilled water for 10 hours and then washed in 96% alcohol and finally in ether. A knot was formed at the end of each thread by superimposing three simple over hand knots. A bees wax-carcinogen mixture was prepared in a ratio of 3:1 (w/v) respectively and was heated just above the melting point care being taken to avoid a brown tinge in the normal yellow colour

of the mixture. In each thread, the average content of carcinogen was approximately 600 µg as determined by weight.

The mice were leparatomixed under ether anaesthesia. A blunt microtip (Eppenderf cofortips - West Germany), cut smoothly at the narrow end for the purpose was inserted into the vagina of the mice. Though the microtip a blunted 1.5 cm long straight needle bearing the carcinogen impregnated thread was guided to the external os of the cervix. By observation through an abdominal insertion the tip of the needle was guided through the endocervical canal past the squamocolumnar junction at the internal os into one of the uterine horns. At a point just above the external bifurcation of the uterus the tip of the needle was forced medially through the uterine wall. The thread was drawn into the vagina and cervix until resistance was felt. When the knotted end reached the orifice of the cervical canal, the free end of the impregnated thread was tied loosely around the uterine horn. In this way the thread remained in situ. Since the knotted vaginal end of the thread was also impregnated with bees wax MCA mixture the carcinogen could be released continuously over the squamous epithelium on the portio and also the epithelium inside

the cervical canal. The impregnated part of the thread was of such a length that bees wax or bees wax-MCA impregnation ended a short distance before the thread passed through the uterine wall. Test figure (Fig. 8) illustrates the thread in place in the cervix of a mouse.

Experimental Design:

Seven to eight weeks old mice weighing about 18 to 20 g were used for all the groups. A total number of 105 mice were divided into three groups as follows :

Groups	Number of Animals	Treatments
Group A	15	No treatment (control)
Group B	30	Insertion of wax impregnated threads.
Group C	60	Insertion of carcinogen impregnated threads

The group 'A' mice were autopsied at two intervals i.e. 60 days and 90 days after the commencement of the experiment the number at each interval being 7 and 8 respectively.

The group 'B' mice were autopsied at five intervals, i.e. 15, 30, 60, 75 and 90 days after the thread insertion

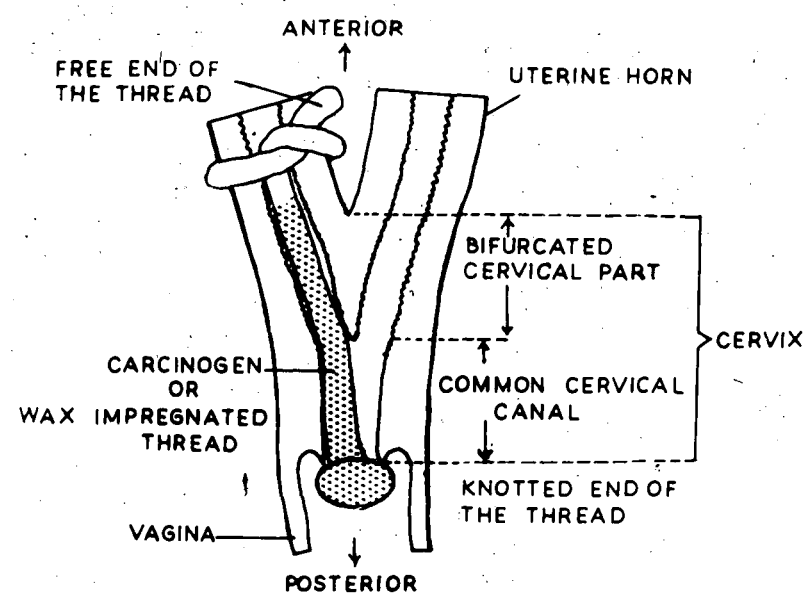


FIG.8 SHEME OF THE CARCINOGEN/WAX IMPREGNATED COTTON THREAD INSERTION IN TO THE CERVIX OF A MOUSE.

and the number of mice sacrificed at each interval being 5, 5, 5, 5 and 10 respectively.

The group 'C' mice were autopsied at seven intervals i.e. 10, 20, 30, 40, 50, 70 and 90 days after the thread insertion. The number of mice sacrificed at each interval were 5, 5, 6, 7, 7, 8 and 20 respectively.

At autopsy a recheck was made that whether the thread was in place in group A and B mice and those exceptional cases where it was not present were excluded. The upper part of the vagina, the uterine cervix and the posterior part of the uterine horns were always removed en bloc and fixed in Bouin's solution. Enlarged lymph nodes, liver and lungs were inspected for metastases. When metastasis was suspected, they were removed and fixed for histological study. All preparations were embedded in the paraplast paraffin wax (Sherwood Medical Industries, St. Louis, Missouri, U.S.A.). Serial sections of 7 μ thickness cut transversely were stained with harris haematoxylin and eosin to study the pathological changes. The following criteria were used for identifying certain salient pathological features:

A. Dysplasia :

Varying degrees of differentiation in the cervical epithelium. In general the more differentiated cell types correspond to the milder forms of dysplasia. The cell nuclei of the dysplastic epithelium are always enlarged and hyperchromatic.

1. Mild dysplasia- Varying degree of basal cell hyperplasia, epithelial hyperplasia and focal areas of epithelial dysplasia.
2. Moderate dysplasia - Epithelial hyperplasia, hyperkeratosis, dyskeratosis involving a wider area of epithelium. Some areas of the epithelium may show leucocytic infiltration forming microabscesses.
3. Marked dysplasia- Variable amount of differentiation in the superficial layer of epithelium also. The chromatin of the epithelial cells are dense and uniform with many darkly stained, slightly enlarged chromocentres with no visible nucleoli.

B. Carcinoma :

Poorly differentiated and undifferentiated squamous epithelial cells, exhibiting pronounced nuclear and cytoplasmic abnormalities i.e. an increased nuclear/cytoplasmic ratio, an enlargement of the nucleus, hyperchromasia and a coarsely

granular chromatin network; mostly invisible nucleoli; presence of binucleation and multinucleation. Cyanophilic cytoplasm with occasional vacuolations. In keratinizing carcinoma the macronucleoli are not prominent whereas in nonkeratinizing carcinoma they are prominent. Large cell nonkeratinizing carcinoma show relatively large cyanophilic carcinomatous cells, while small cell nonkeratinizing carcinoma show predominence of uniform small cyanophilic cells with a high nuclear/cytoplasmic ratio.

1. Early carcinoma - Infiltration of carcinomatous cells to a small region in the stroma.
2. Advanced carcinoma - Wide area of stromal region filled up with carcinomatous cells, forming many patterns like palisades, whorls, rosettes etc.

C. Adenocarcinoma :

Endocervix show malignant glandular epithelial cells. Together with malignant squamous carcinomatous cells it may be named as Adeno() squamous carcinoma.

D. Sarcoma :

The myometrium show spindle shaped poorly preserved cells with elongated hyperchromatic nuclei, indicating malignancy.

GROUP 'A': Control (Mice with no treatment):

Fifteen animals were kept without any treatment to serve for the control group. The cervical epithelium did not show any histopathological changes during the observation period. The stratified columnar and squamous epithelial cells were seen clearly and the exudating cells from the epithelium could also be observed (Fig. 15).

GROUP 'B': Insertion of Wax Impregnated Threads:

Irritational changes like epidermization and slight mild dysplastic changes could be noted in many animals of this group. Infection in the cervical canal was also a common occurrence. The changes in the cervix at five intervals of wax threaded animals are illustrated in Table I. Two animals at 45 days interval showed some abnormalities in the cervix. In certain sites the keratinized cells could be seen in the basal and the para basal layers. They also have many infectious reactions like infiltrations of leucocytes etc. (Fig. 16). At 90 days interval, out of the ten animals autopsied one had moderate dysplastic changes. Keratinization and hyperchromasia could be seen in a wider area of epithelium (Fig. 17). No tumor was found in this group. The vaginal epithelium did not have any notable changes.

GROUP 'C' : Insertion of Carcinogen Impregnated Threads:

10 days:

Five animals were autopsied in the first interval of ten days after the carcinogen thread insertion. Almost all of them showed immediate inflammatory reaction in the squamous and columnar epithelial regions of the cervix. Three animals had mild dysplastic changes. The following pathological features were found in some focal areas of the dysplastic epithelium (Fig. 18). A gross disturbance in the differentiation and keratinization of the basal cell layer was found. There was some disorderly arrangement of the cells with either mitotic figures or cell crowding indicative of increased cell proliferation. Some loss in the polarity of the basal cells was also seen. An interesting cytoplasmic alteration which appeared to originate in the parabasal layer consisted of an intense eosinophilia. Many abnormal big cells with condensed chromatin were found in clusters. The vaginal regions nearer the external os in one ofthese animals showed the same mild dysplastic changes (Fig. 19). Uterine epithelia were found normal without any significant change. One mouse in the group did not show any appreciable change. Another autopsied animal

showed distinct pathological changes with certain sites towards moderate dysplasia. In these regions the atypical changes were more pronounced, involving a wide area of epithelium (Fig. 20). The vaginal epithelium showed only mild dysplastic changes.

20 days:

Out of the five animals autopsied in this interval none had normal epithelium. Mild dysplastic changes were seen in only one animal. Two had pronounced moderate dysplastic changes (Figs. 21 & 22). The nuclear abnormalities were a prominent feature compared to those observed in mild dysplasia. Eosonophilic cells were more widely distributed. There were many spindle shaped squamous cells. The nuclei were polymorphic and hyperchromatic. Often irregular buds of squamous epithelium were observed. Hyperplasia, hyperkeratosis and dyskeratosis involved a wider area of epithelium. Many isolated cells were keratinized. leucocytic infiltration and appearance of micro abscesses were more common. Intercellular bridges were distinct. The remaining two animals had marked dysplasia without any indication of infection. Along with the involvement of basal and intermediate cell layers a moderate amount of superficial cells also showed dysplastic changes (Figs. 23, 24 & 25). The cell margins were distinct

and disturbed polarity could beobserved. Nuclear changes were still more prominent. Fair number of cells showed increase in the nucleocytoplasmic ratio. Nuclei had thickened membranes and were hyperchromatic with prominent nucleoli. Coarse and clumped chromatin pattern were observed. There was variable amount of differentiation in the superficial layer of epithelium. No carcinoma was found in this interval.

30 days :

Six animals were autopsied at this interval. None of them showed normal cervix. Dysplastic changes were mild in the cervix of one of the mice. Two had moderate dysplasia without infection. Another two animals showed marked dysplastic changes. Both had some inflammatory and infectious features (Figs. 26 & 27). There was evidence of increased cell growth. This was characterised by the appearance of hyperchromatic cells in the basal layer. And there was extensive papillomatous over growth consisting of moderately disordered arrangements of hyperchromatic cells in the basal and the parabasal layer. The presence of papillomatosis and plump hyperchromatic cells near the mucosal surface were felt to be indicative of rapid cellular growth.

One of the animals in this group had early invasive carcinomatous changes in the squamous epithelial region

(Figs. 28 & 29). Neoplastic cells had invaded to a small portion of stroma. This invasion was found in only rare sites in the epithelium. Otherwise the whole epithelium was invaded in the neoplastic process with the intact basement membrane (Fig. 30). The cells showed varied shapes and sizes and large nuclei with a definite increase in the nucleocytoplasmic ratio. The chromatin pattern was coarse and nuclei contained multiple nucleoli (Fig. 31). The cytoplasmic changes were almost similar to those in the marked dysplastic stage as explained previously except for atypical keratinization which was found much more frequently. Malignant transformation was primarily typified by alterations of the nucleus and nucleolus i.e. hyperchromasia, nuclear enlargement, and large macronucleoli. In many sites the columnar epithelium was completely replaced by squamous cells.

The vaginal squamous region showed moderate dysplastic changes without any evidence of carcinoma.

<u>40 days</u>:

Seven mice were sacrificed at this interval. The squamous epithelial region of almost all the cervices displayed histopathological changes. Two animals had severe invasive carcinoma (Figs. 32, 33, 34 & 35), the neoplastic cells extending through out the stromal region.

Out of these two, one preparation had infection besides the carcinomatous changes (Fig. 35). Both of them had the carcinoma spread in the common cervical canal and the bifurcated region (Table IV). The islands of neoplastic cells showed numerous keratinized pearly bodies (Fig. 36). There was no distinct epithelial lining either in the common cervical canal or in the lower bifurcated region and that it had completely merged with the invasive growth. The columnar epithelium was also severely affected in them. In many sites it was completely replaced by neoplastic squamous cells.

Apart from these two animals with severe invasive carcinomas, one animal showed early invasion with the neoplastic cells invading to a small portion of the stromal region (Figs. 37 & 38).

Three animals had marked dysplastic changes with a good number of hyperchromatic atypical cells. Very mild infection was noted in one of them (Fig. 39). One preparation had moderate dysplastic changes with most of the cells having coarse chromatin.

During this interval one preparation had marked dysplasia in the vaginal squamous epithelium (Fig. 40). Two animals had mild and another one had moderate

dysplastic changes. Three animals had normal stratified squamous epithelial region without any disturbance.

50 days :

Out of seven animals sacrificed at the 50 days inverval four had invasive carcinomas, i.e. two in the early stage and the other two in the advanced stage. Three of them were nonkeratinizing large cell carcinomas (Fig. 41), and one was keratinizing small cell type (Fig. 42). Notably in this interval, one preparation had a severely affected adenocarcinoma (Fig. 43) nearer to the squamocolumnar junction. It is identified as differentiated infiltrating adenocarcinoma type retaining the distinctive features of cervical epithelium in varying degrees. It was some what a mucinous tumor made up of closely aggregated glandular elements of atypical configuration and presents irregularly tubular (Fig. 44), papilliform (Fig. 45) or convoluted (Fig. 46) patterns or a mixture of these designs. Commonly the lining epithelium was multilayered. There was cellular crowding and cells had a decreased amount of cytoplasm in distinct broders and a comparatively large nucleus that had lost its polarity.

Other two preparations had marked dysplasia. Hyperkeratosis and dyskeratosis were involved in a wider

area of epithelium. In many sites irregular buds were seen (Fig. 47). One preparation showed mild dysplastic changes. Among the seven preparations in this interval one showed severe well differentiated squamous cell carcinoma in the vaginal region (Figs. 48 & 49). There was an extensive atrophy of the vaginal epithelium. The vagina was completely filled up with necrotic squamous material. Surprisingly the cervix of this preparation had only marked dysplastic changes.

70 days :

Seventy days after the carcinogen thread insertions eight animals were sacrificed. Among them one preparation had extensive sarcoma as well as adenocarcinoma. Histologically the tumor was composed of small vaguely fusiform cells with poorly discerniable boundries resembling connective tissue cells (Figs. 50 & 51). Neither cellular abnormalities nor abundant or abnormal mitotic figures were found. There was no apparent pattern of cell arrangement except for a perinecrotic palisading (Fig. 50). The adenocarcinomatous region exhibited an extremely labile microscopic structure generally lacking in constancy and distinctive features. Some times one could however distinguish elements morphologically suggestive of a derivation from endocervical epithelium and infrequently an origin from

cervical epithelium could be traced (Fig. 50). The patterns vary in different parts of the tumor (Figs. 53, 54 & 55) and occasionally transitions occur from an endometrial like structure to a more typically endocervical architecture. The cervical adenocarcinoma vary from well differentiated through moderately differentiated to undifferentiated variants. But these differences in structure do not represent tumor entities since they are frequently seen in the same neoplasm. The adenocarcinoma consisted of round, oval and irregular, tubular, branching glandular elements closely packed together and separated by a scanty stroma (Fig. 56). These glandular structures were composed of tall columnar epithelium easily identified as cervical derivation and penetrated sinuously in all directions. The glands varied in size and their wall might be infolded into papillae. The glandular lining was formed of fairly uniform cells, arranged in one or more layers. The cells (Fig. 57) had distinct borders and an abundant, lightly staining vacuolated or finely granular cytoplasm which contained basal or central deeply staining and relatively large nuclei. Mitoses were infrequent.

Four animals had advanced invasive carcinoma (Fig. 58 & 59). They were very weak and the tumors could be identified externally by the bulging lower abdomen.

The pathological features were very distinct. The cells in the tumors were completely disposed in linguate processes (Fig. 60), oblong or club shaped masses or more loosely in irregular networks (Fig. 61). The stratified cells were large, polygonal or rounded and contain nuclei frequently of large size (Figs. 62, 63 & 64). Occasionally small dark staining, apparently compressed tumor cells arranged in widely dispersed clumps were present (Fig. 65). The fibrovascular stroma was sometimes abundant but often scanty. It may contain inflammatory cells including polymorphonuclear leucocytes which invade the degenerating tumor cells (Fig. 66). Out of the four carcinomas three were of non-keratinizing squamous cell type and one was keratinizing small cell type. Two preparations also showed severe infections along with tumorous changes.

Another two preparations had early invasive squamous carcinoma. There were many sites in which the limiting membrane of the squamous epithelium was broken and the hyperchromatic keratinizing neoplastic cells were seen invaded into the stromal region (Fig. 67). Notably the epithelium was completely atrophied in those sites. One preparation at this interval showed marked dysplastic changes. Wider area of epithelium showed the keratinizing cells.

90 days:

At this interval 20 animals were sacrificed. Ten of them had advanced squamous cell carcinomas (Fig. 68 & 69) consisting of proliferating, round, oval, or spindle shaped small cells (Figs.70, 71 & 72) with basophilic cytoplasm and a disproportionately large nuclei. The cells were crowded together and the axes of the nuclei often lie perpendicular to the surface (Fig. 72). Very often, the cells exhibited varying degrees of pleomorphism. Occasionally there were polyhedral cells with a acidophilic cytoplasms and hyperchromatic nuclei which varied in size (Fig. 74).

Disorganization and loss of normal cellular stratification and the replacement of the entire thickness of the epithelium by abnormal cells were characteristic (Fig. 75). Other pathological changes of the carcinoma include disturbance in the orderly arrangement of cells which show nuclear pleomorphism, cytoplasmic basophilia and loss of cellular differentiation.

Two adenocarcinomas were observed at this interval. Both of them arose within the cervical canal. One adenocarcinoma eroded the whole cervical cross section except the rim area. As the prototype of the tumor depicts, the neoplastic cells included epithelial and glandular structures

with many pathological changes (Figs. 76 & 77). Various shapes of cells like round, columnar to bizarre shapes were found (Fig. 78 & 79). The nuclei were often large and hyperchromatic. Attempted glandular formation was sometimes discerned in these poorly differentiated adenocarcinomas (Fig. 80) but the predominating feature was the disposition of cells singly, in small clusters or in different sheets. The cells may be mucinous goblet or signet ring forms and at the same time resembling the cervical epithelium to some extent. In these tumors the lack of differentiation was so pronounced that the glandular and sometimes also the epithelial nature of the neoplasm may be difficult to recognize. It was evident from the histological appearance that the endocervical adenocarcinomas had the capacity to secrete mucin (Fig. 79) and imparted the gelatinous portion of the tumor growth.

One preparation showed typical sarcoma in the endocervical canal (Fig. 81). Histologically there were many spindle shaped smooth muscle cells (Figs. 82 & 83). The fibroblastic tissue was the other mesodermal derivative found in the sarcoma. Apart from these, five preparations had early invasive carcinomas with limited extensions of epithelial neoplastic cells into the stroma. Most of the pathological features resembled the earlier descriptions. Four of them were of differentiated, nonkeratinizing type

and one was of undifferentiated nonkeratinizing type. Then in addition two preparations had marked dysplastic changes. The characteristic dysplastic changes were found in a wider area of epithelium without invasion into the stroma.

Out of the 20 animals in this interval three had invasive squamous cell carcinoma in the vaginal region (Figs. 84 & 85). The pathological features resembled those of the cervix. Other changes in the squamous epithelium of the vagina at different intervals of autopsy are depicted in the Table III.

Major Sequential Histopathological Events During Experimental Cervical Carcinogenesis:

The detailed pathological changes occurred during the observation period have been described above and the major sequential events have been represented in the histogram (Fig. 86).

All the types of dysplastic changes could be found upto 30 days interval and thereafter mostly marked dysplasia was observed. It is to be noted that the proportion of animals showing marked dysplastic changes decreased from 30 days to 90 days interval. The tumor appearance started

at the 30 days interval. A steady increase of the percentage of tumor incidence was noted with the increase in the passage of time. At the autopsy period of 90 days after the carcinogen thread insertion 90% of the animals showed tumorous changes.

TABLE I

CHANGES IN THE CERVICES OF WAX THREADED ANIMALS (GROUP B)

Interval time after thread insertion	Total number of animals sacrificed	Normal	Mild dysplasia	Moderate dysplasia	Marked dysplasia	Tumors
15 days	5	3	2*	0	0	0
30 days	5	4	0	1*	0	0
45 days	5	2	1	2**	0	0
60 days	5	3	2*	0	0	0
90 days	10	5	4**	1	0	0

Note : The number of '*' denotes the number of preparations which showed infection.

TABLE II

PATHOLOGICAL CHANGES OF CERVICES IN MCA THREADED ANIMALS (GROUP C)

Interval time after thread insertion	No.of mice sacri-ficed	Normal	Mild dysplasia	Moderate dysplasia	Marked dysplasia	Carcinoma Early	Carcinoma Advanced	Other tumors (ACM & SCM)
10 days	5	1	3	1	0	0	0	0
20 days	5	0	1	2*	2	0	0	0
30 days	6	0	1	2	2**	1*	0	0
40 days	7	0	0	1	3	1	2*	0
50 days	7	0	1	0	2	2	2	1
70 days	8	0	0	0	1	2	4**	1
90 days	20	0	0	0	2*	5	10***	3

Note : The number of '*' denotes the number of animals with infection.
ACM = Adenocarcinoma; SCM = Sarcoma.

TABLE III

PATHOLOGICAL CHANGES OF VAGINA IN MCA THREADED ANIMALS (GROUP C)

Interval time after thread insertion	Total anumber of animals sacrificed	Normal	Mild dysplasia	Moderate dysplasia	Marked dysplasia	Tumors
10 days	5	3	2	0	0	0
20 days	5	2	2	1	0	0
30 days	6	3*	2*	1	0	0
40 days	7	4	2	1	0	0
50 days	7	4*	1	1	1	1
70 days	8	2	2	1	1	1
90 days	20	10	5**	0	2	3

Note: The number of '*' denotes the number of preparations which showed infection.

TABLE IV

THE NUMBER AND LOCALIZATION OF TUMORS IN THE CERVICES OF 'GROUP C' ANIMALS

Specific sites	Duration of MCA string application in days					Total number of tumors
	30	40	50	70	90	
I. Number of tumors in squamous areas:						
Vagina	0	0	1	0	2	3
Portio vaginalis and common cervical canal	1	0	0	1	3	5
Bifucated canal	0	1	3	2	3	9
Vagina and portio vaginalis	0	0	0	1	2	3
Common and lower bifurcated cervical canal	0	2	1	0	4	7
Vagina, Portio vaginalis, Common and lower bifurcated cervical canal	0	0	0	2	2	4
Total (%age incidence)	1 (20%)	3 (60%)	5 (83%)	6 (75%)	16 (80%)	31

Contd..../-

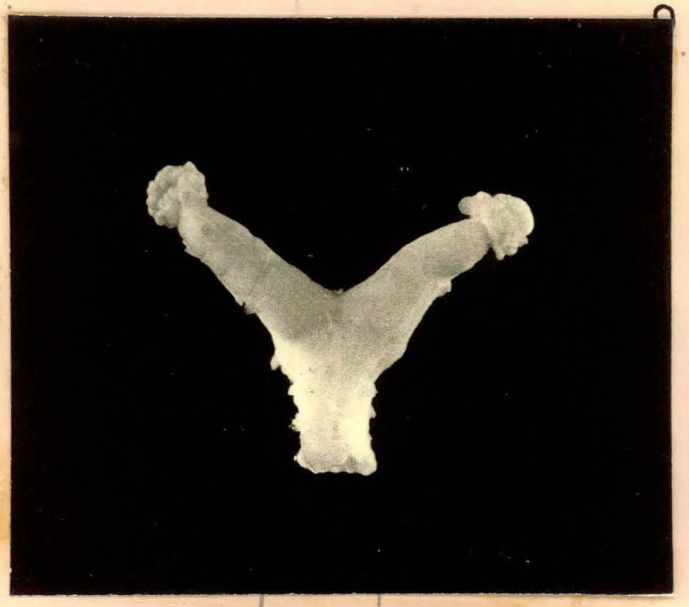
TABLE IV (contd.)

Specific sites	Duration of MCA string application in days					Total number of tumors
	30	40	50	70	90	
II. Number of tumors in columnar areas:	1	2	3	3	10	19
III. Number of tumors in squamous and columnar areas	0	0	0	1	2	3
Total number of tumors in all the regions	2	5	8	10	28	53
Number of tumor bearing animals	1/6	3/7	5/7	7/8	18/20	
(%age of incidence)	16.67%	42.9%	71%	87.5%	90%	

Fig. 9 : Female genitalia of a normal Swiss albino mouse. X 3.5.

Fig. 10a: A section at the ectocervix region (nearer to external os) showing the cervical canal as well as the vaginal lumen. X 60.

Fig. 10b: A section at the mid cervix region showing the cervical canal only. X 60.



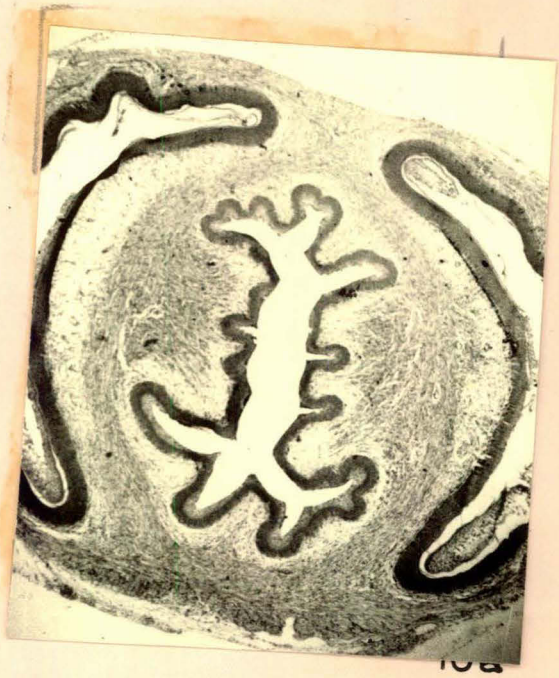
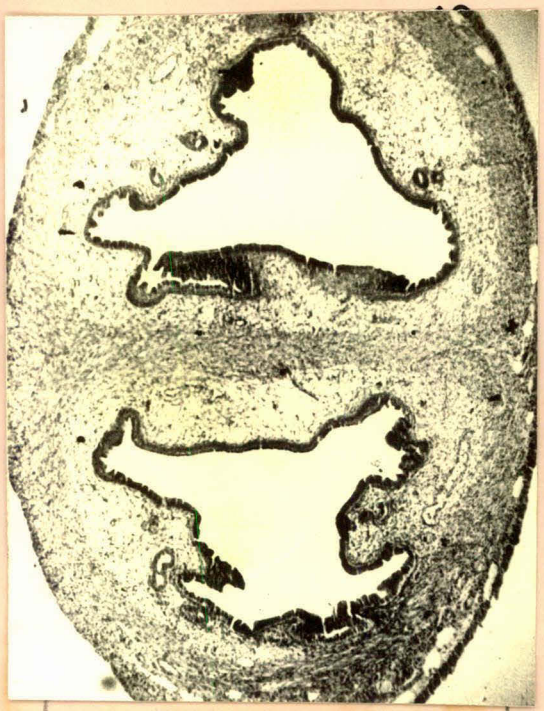


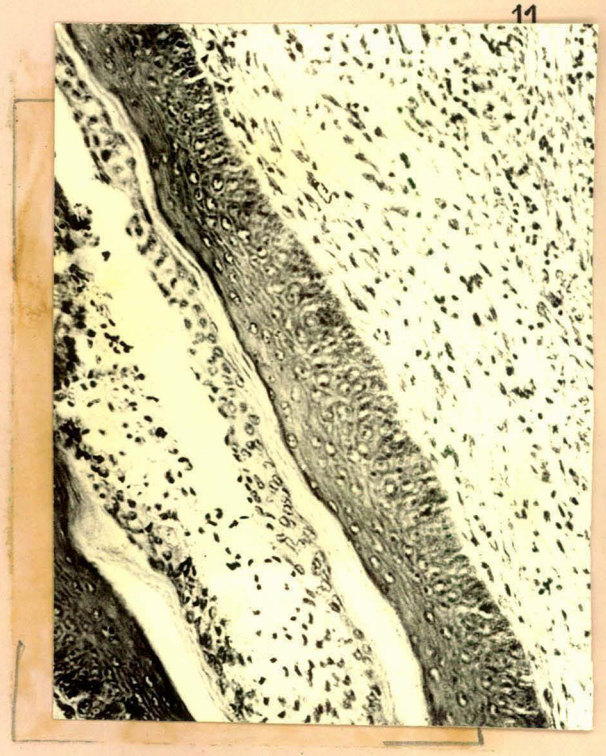
Fig. 10c : A section at the endocervix region showing the bifurcated cervical canal. Few cervical glands can also be noted. X 60.

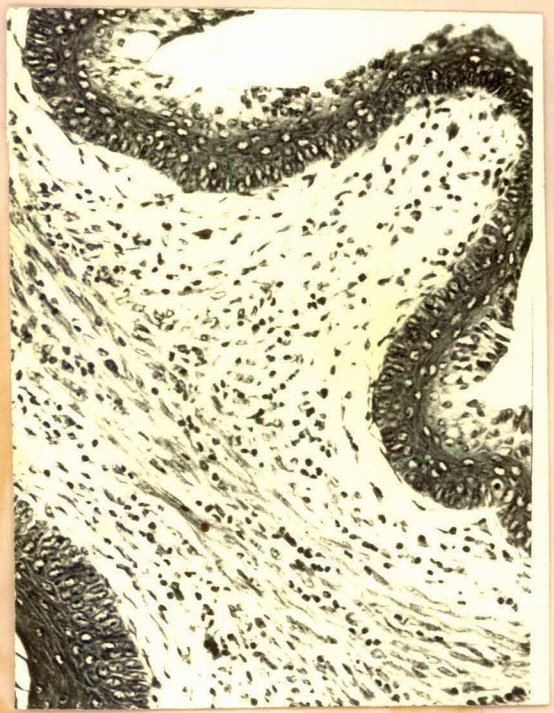
Fig. 11 : Section showing the stratified squamous epithelium of the vagina in a normal Swiss albino mouse (8 weeks old). X 320.

Fig. 12 : Sections showing the stratified squamous epithelium of the cervix in a normal Swiss albino mouse (8 weeks old). X 320.

Fig. 13 : Simple columnar epithelium of the uterine horn in a normal mouse (8 weeks old). X 800.







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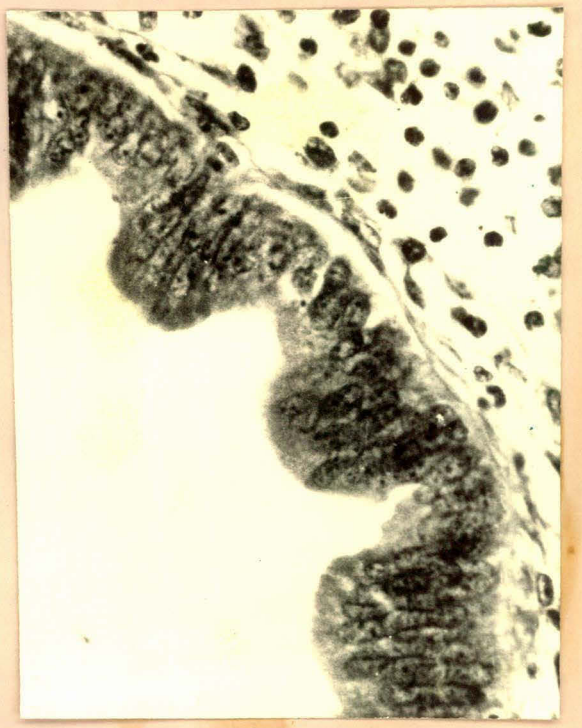


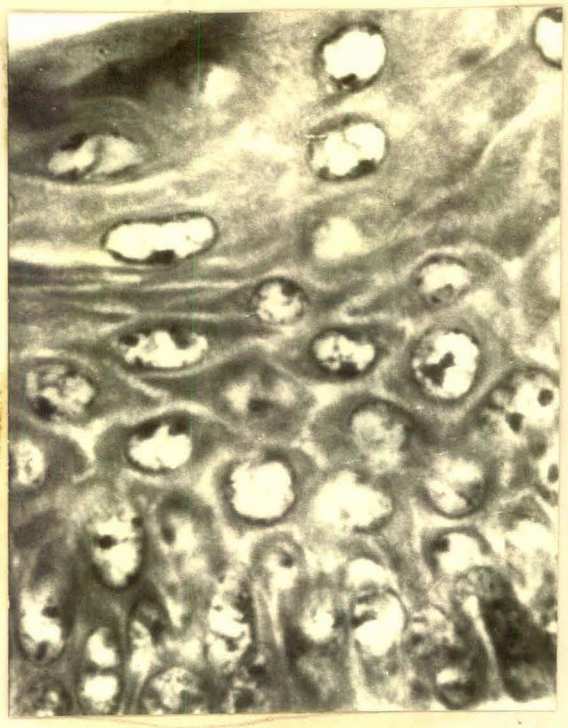
13

Fig. 14 : Higher magnification of the vaginal squamous epithelium showing the stratified squamous cells. X 2000.

Fig. 15 : Higher magnification of the cervical squamous epithelium showing the stratified squamous cells. Some of the exudating squamous cells can be seen clearly. X 2000.

Fig. 16 : Cervical squamous epithelium with infectious reactions in a wax thread inserted mouse autopsied at 45 days interval in group B. X 320.

Fig. 17: Cervical squamous epithelium with moderate dysplastic changes in Group B mouse autopsied at 90 days interval. Hyperchromatic and keratinizing cells are found in wider areas. X 800.



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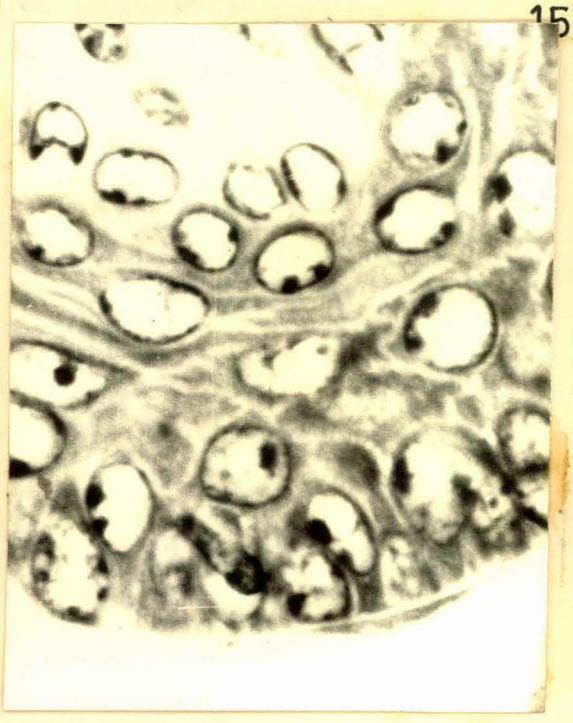

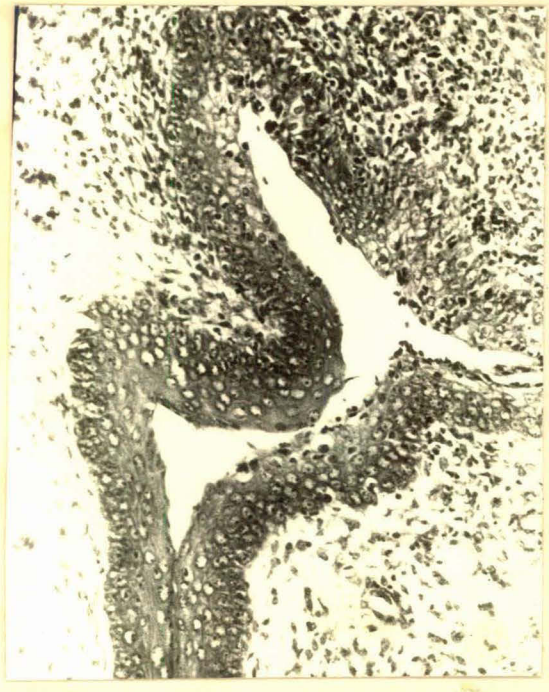

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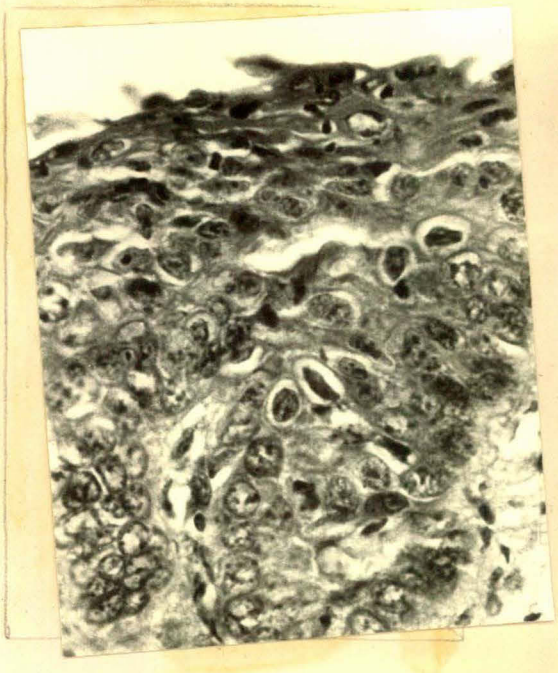


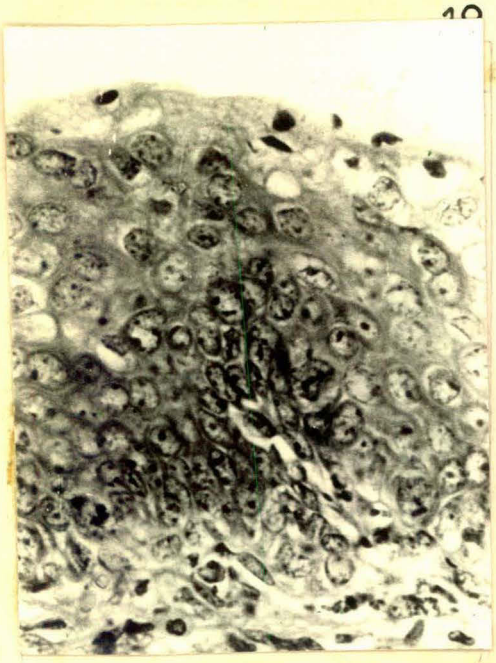
17

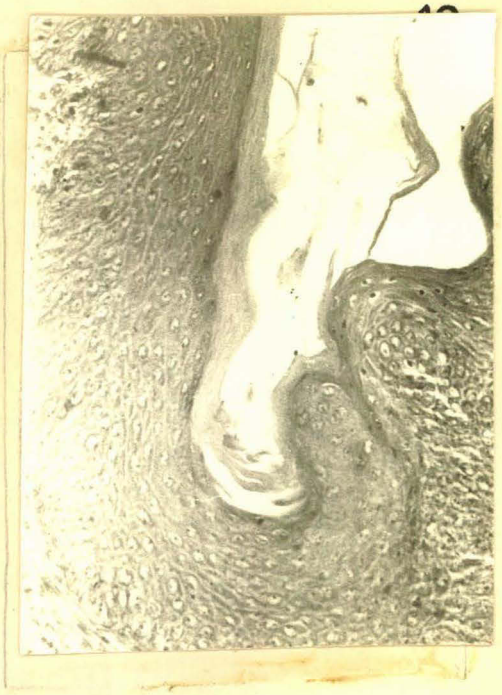
Fig. 18 : A mild dysplastic epithelium from a mouse autopsied at 10 days interval (Group C). Keratinization in the basal cell layer, disorderly arrangement of the cells forming cell crowding and some loss in the polarity of the basal cells are found. X 800.

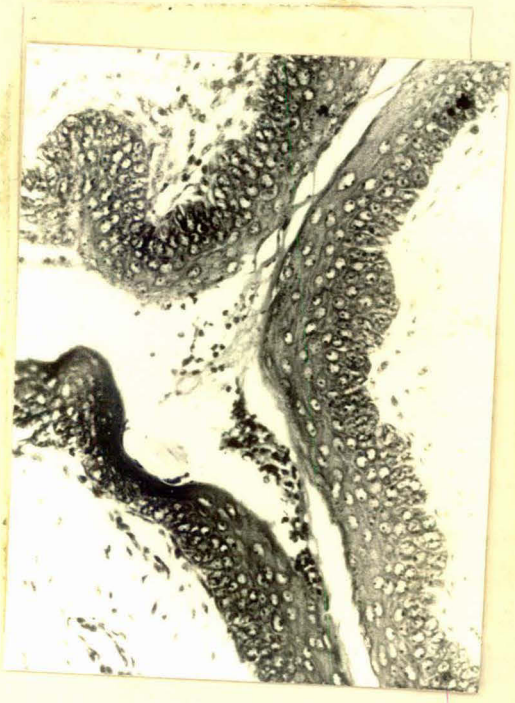
Fig. 19 : Vaginal squamous epithelium of a mouse from 10 days interval in Group C, showing mild dysplastic changes like disturbed polarity and some keratinized cells in the basal cell layer etc. X 320.

Fig. 20 : Wider area of cervical epithelium showing the atypical changes towards moderate dysplasia, in a mouse autopsied at 10 days interval in Group C. X 320.

Fig. 21 : A typical moderate dysplastic cervical epithelium from a mouse autopsied at 20 days interval (Group C). Keratinization, disturbed polarity and disorderly arrangement of cells are seen clearly. X 320.







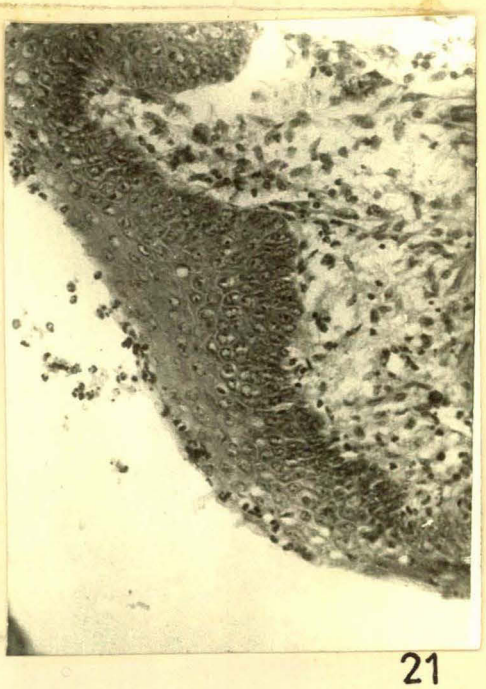


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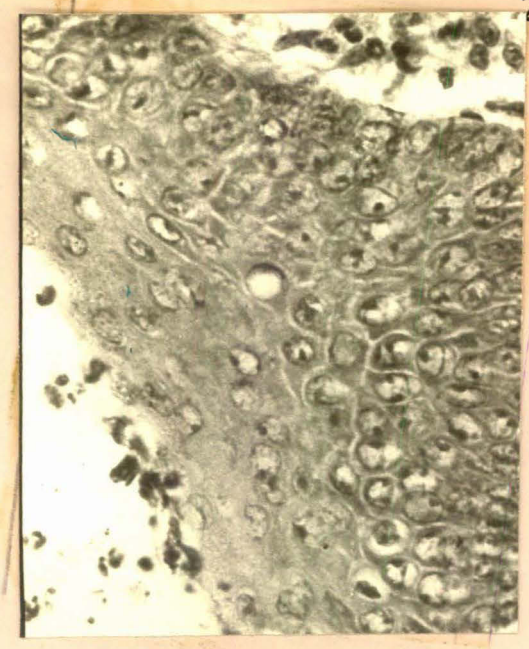
Fig. 22 : Higher magnification of the moderate dysplastic epithelium, showing the nuclear abnormalities and other pathological changes. Keratinized cells with distinct intercellular bridges, polymorphic and hyperchromatic nuclei are the prominent features. X 800.

Fig. 23 : Marked dysplastic cervical epithelium of a mouse autopsied at 20 days interval (Group C). Along with the involvement of basal and intermediate layer cells, a moderate amount of superficial layer cells also show dysplastic changes. Disturbed polarity and nuclear abnormalities are prominent. X 800.

Fig. 24 : Higher magnification of a portion of the marked dysplastic epithelium (20 days interval in Group C). Fair number of cells show increase in the nuclear cytoplasmic ratio. Nuclei have thickened membranes and are hyperchromatic with prominent nucleoli. X 2000

Fig. 25 : A typical marked dysplastic cervical epithelium in a mouse of 20 days interval in Group C. X 800.



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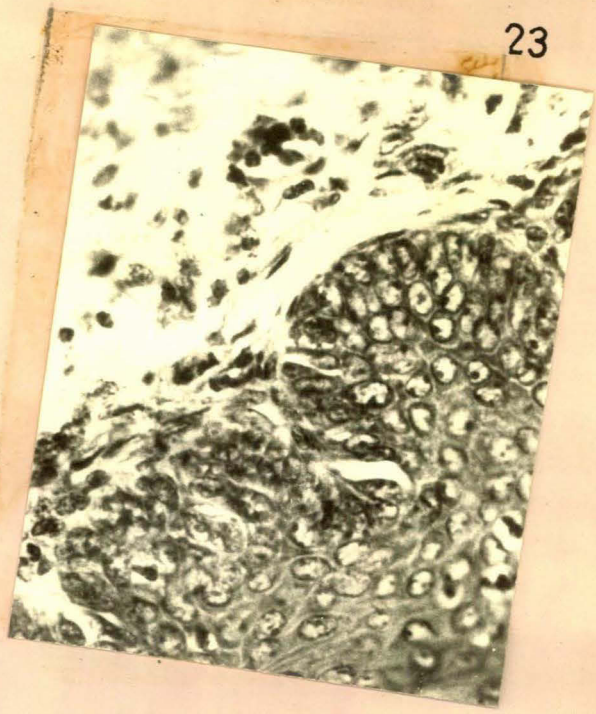

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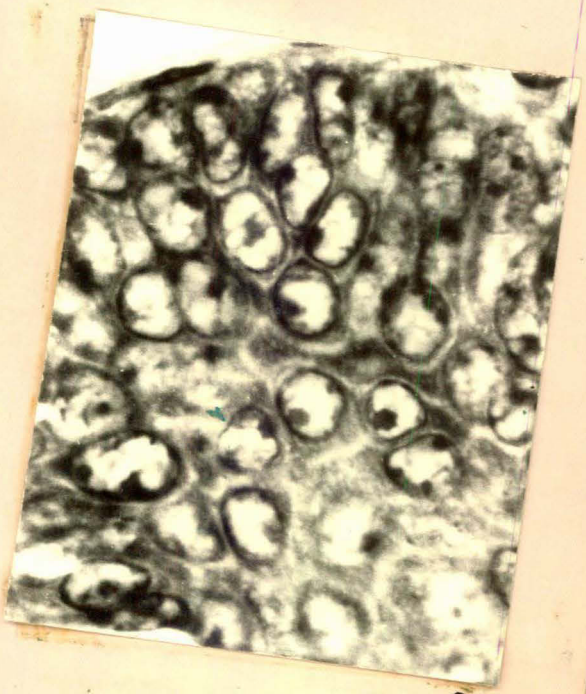

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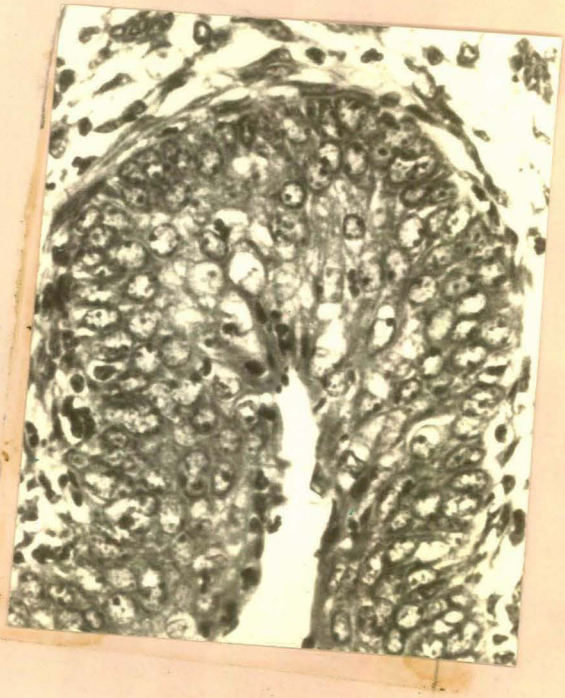

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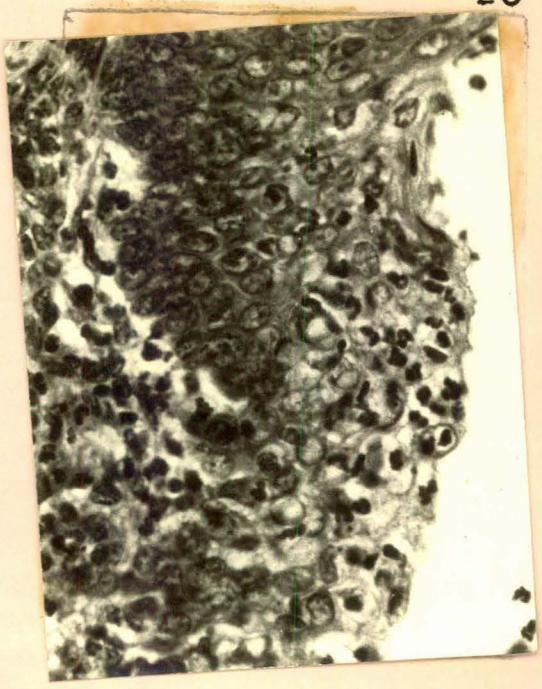
Fig. 26 : Cervical squamous epithelium of a mouse autopsied at 30 days interval (Group C) showing marked dysplastic changes along with inflammatory and infectious features. Groups of leucocytes can be noted. X 800.

Fig. 27 : Extensive papillomatous over growths consisting of disorderly arrangements of hyperchromatic and keratinizing cells in a marked dysplastic cervical epithelium of a mouse from 30 days interval (Group C). X 800.

Fig. 28 : Invasion of carcinomatous cells to a small portion of the stroma in the early carcinomatous cervix of a mouse from 30 days interval (Group C). X 800.

Fig. 29 : Another region from the same (early carcinomatous) cervix (30 days interval in Group C) showing the early invasion of carcinomatous cells. X 800.

26



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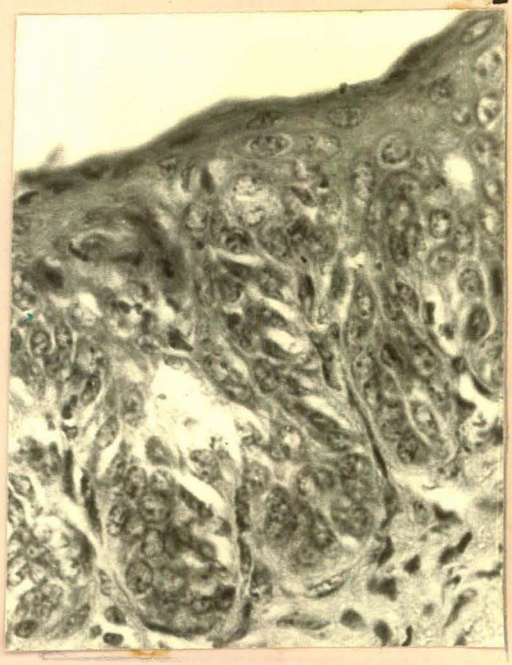

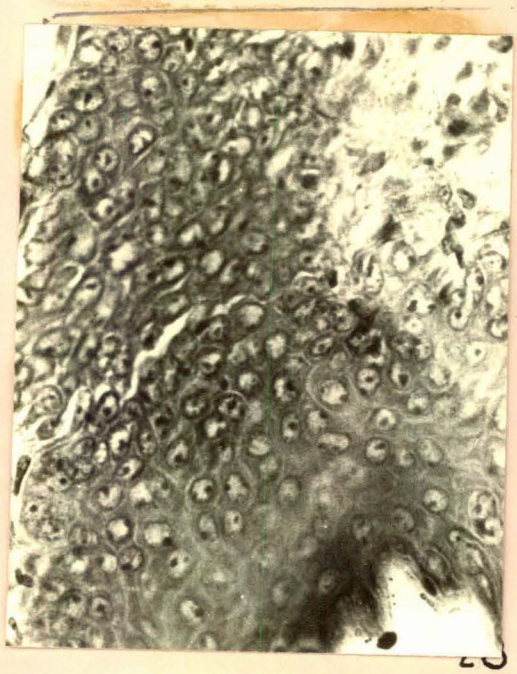

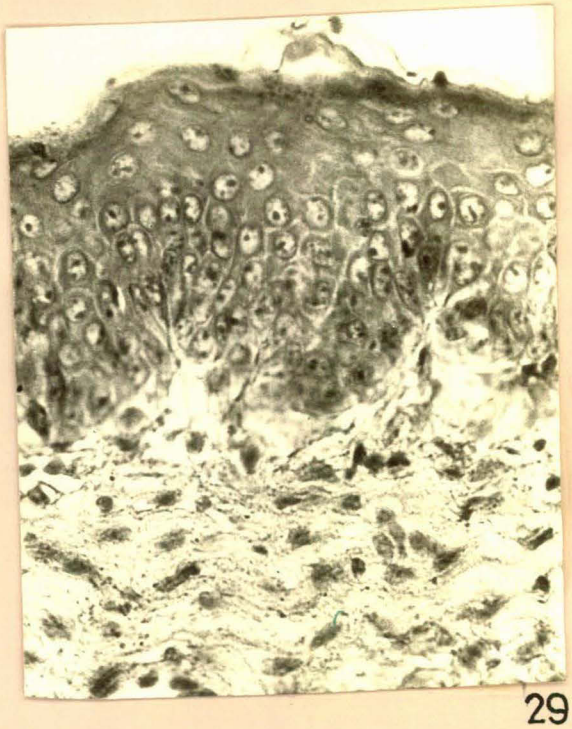

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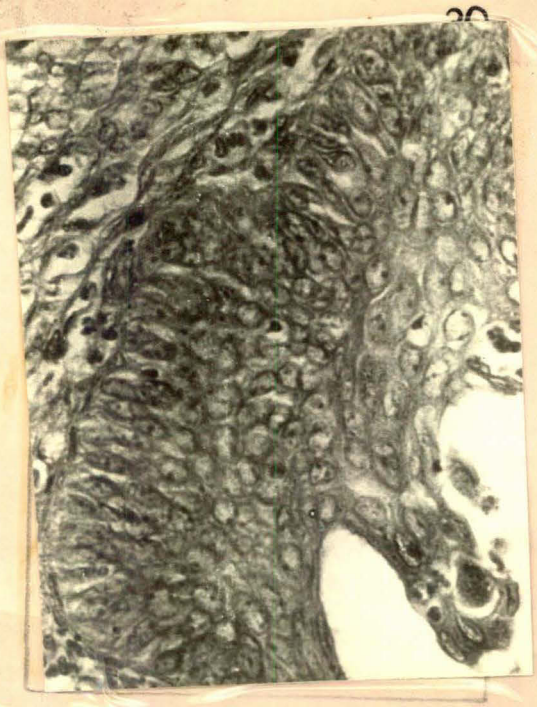
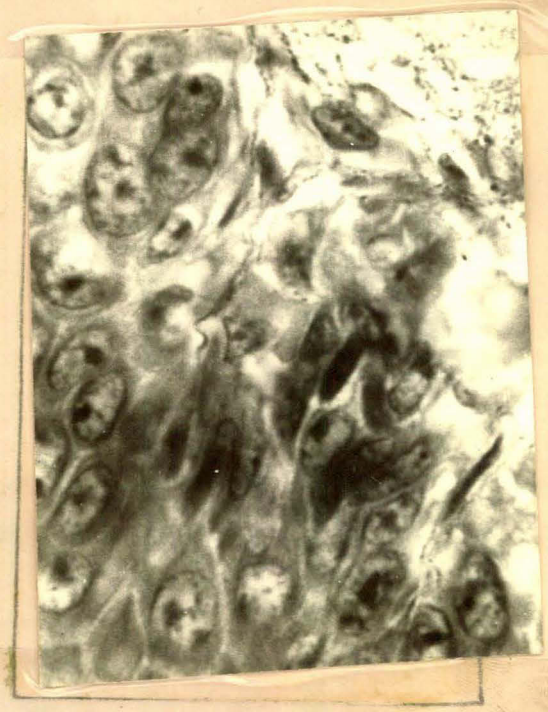
Fig. 30 : Section showing an another region in the same early carcinomatous cervix (30 days interval in Group C) where the early invasion of neoplastic cells is absent. The intact basal membrane of epithelial lining can be noted clearly. X 800.

Fig. 31 : Higher magnification of a portion of the early carcinomatous cervical epithelium (in 30 days interval of Group C) showing the details of neoplastic cells. Varied shapes and sizes of the cells with increase in the nucleocytoplasmic ratio, coarse chromatin patterns, multiple nucleoli and more frequent atypical keratinizations can be noted. X 2000.

Fig. 32 : Photograph showing the external appearance of an advanced invasive carcinoma in the cervix of a mouse autopsied at 40 days interval in Group C. X 6.





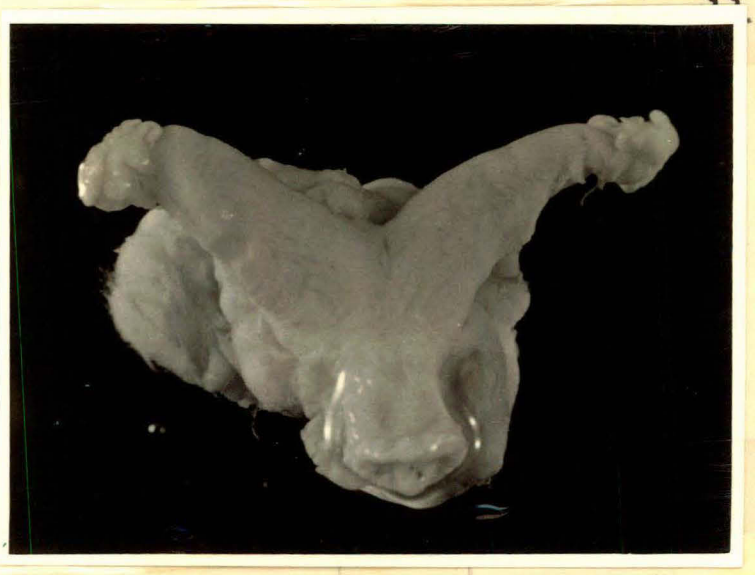
32

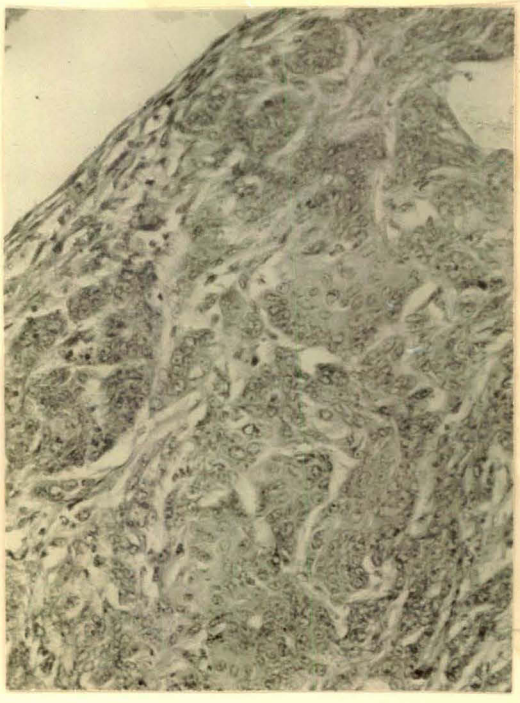
Fig. 33 : Photograph showing the external appearance of another advanced invasive carcinoma in the cervix of a mouse autopsied at 40 days interval in Group C. The carcinoma also had infectious reactions. X 6.

Fig. 34 : A section from an advanced carcinomatous cervix (40 days interval in Group C) where the squamous epithelium was completely eroded and the stromal region was filled up with the extensions of neoplastic cells. X 320.

Fig. 35 : Section from another advanced carcinomatous cervix (40 days interval in Group C) which had infections. An island of neoplastic cells on the stromal region and erosion of squamous epithelium can be noted. X 320.

33





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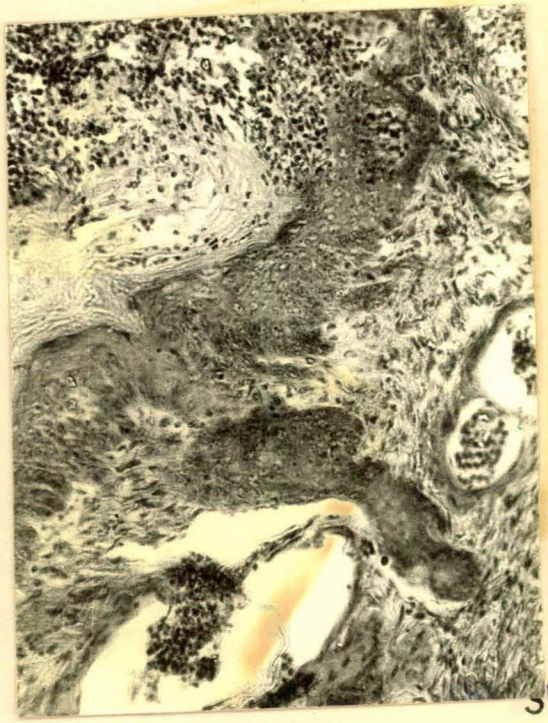


35

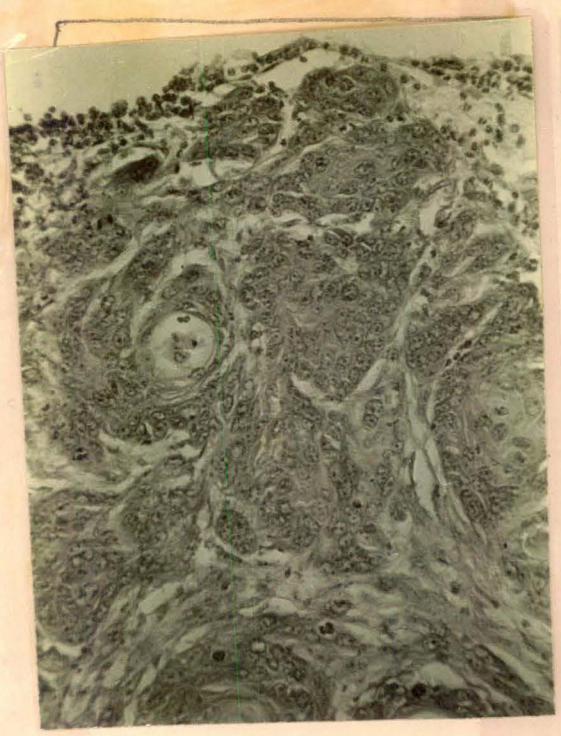
Fig. 36 : A section from the advanced carcinoma with infections (40 days interval in Group C) showing islands of neoplastic cells and pearly bodies. The squamous epithelial lining is completely eroded. X 320.

Fig. 37 : Section from an early invasive carcinoma (40 days interval in Group C) showing the limited intrusion of neoplastic cells into the stromal region. X126.

Fig. 38 : High power magnification of the early invasive carcinoma (40 days interval in Group C) showing patches of keratinized neoplastic cells. X 320.

Fig. 39 : A typical marked dysplastic squamous epithelium with infection in a cervix of a mouse autopsied at 40 days interval in Group C. X 800.

36



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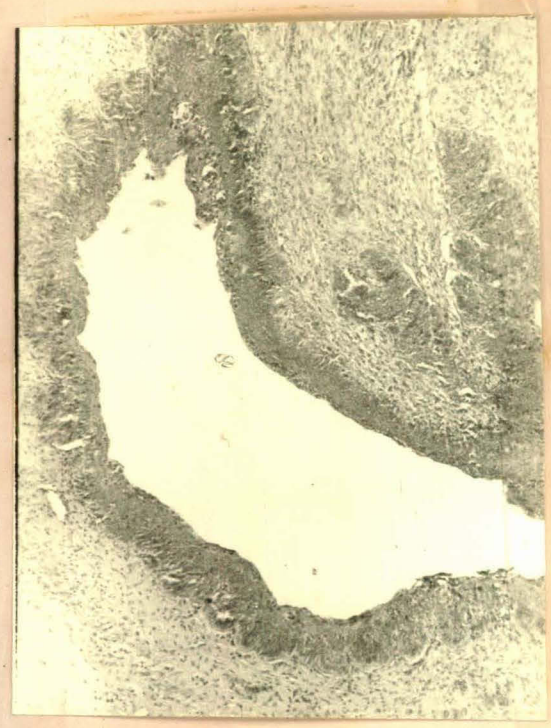

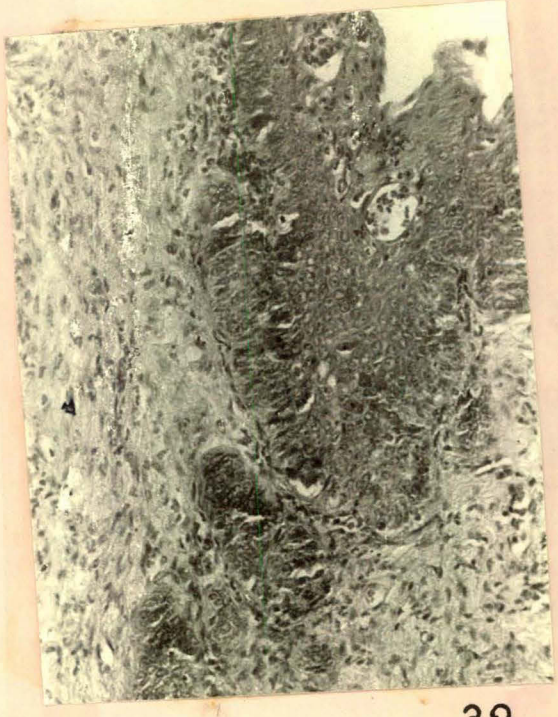

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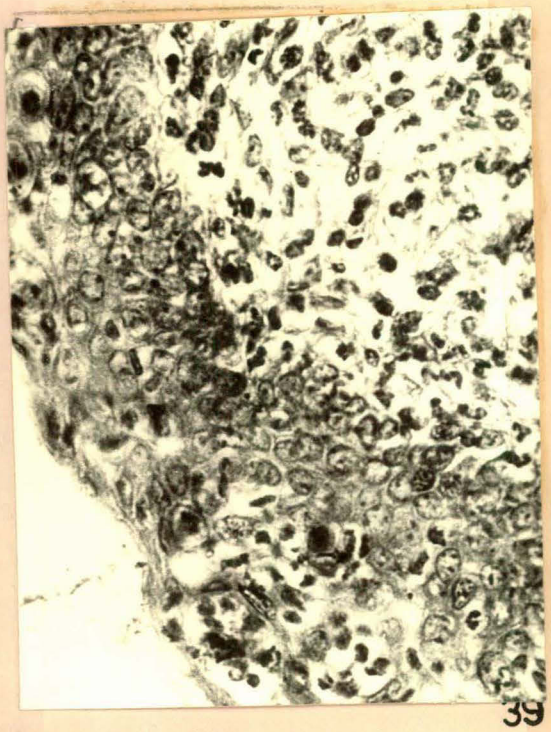

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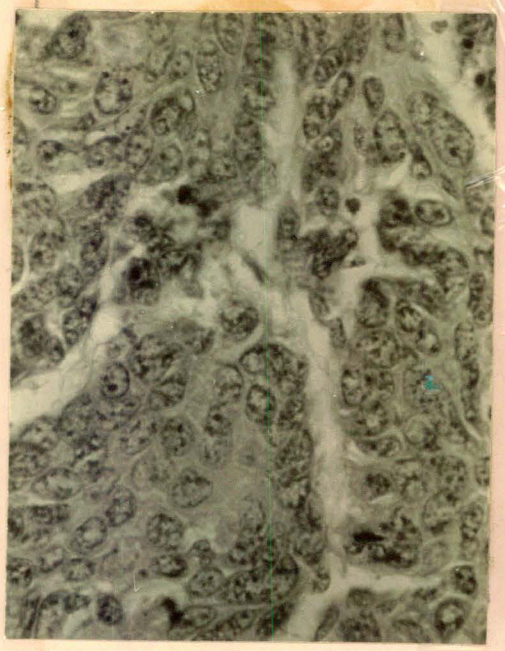
Fig. 40 : A section showing the marked dysplatic changes in the vaginal squamous epithelium (40 days interval in Group C). X 800.

Fig. 41 : Section from a nonkeratinizing squamous cells carcinoma of the cervix of a mouse autopsied at 50 days interval in Group C. Typical neoplastic cells with prominent nucleoli can be seen. X 800.

Fig. 42 : Section from a keratinizing squamous cell carcinoma of the cervix of a mouse autopsied at the same 50 days interval in Group C. The nucleoli are not prominent in the neoplastic cells. X 800.

Fig. 43 : External appearance of an adenocarcinoma of the cervix procured at the 50 days interval in Group C. X 6.

40



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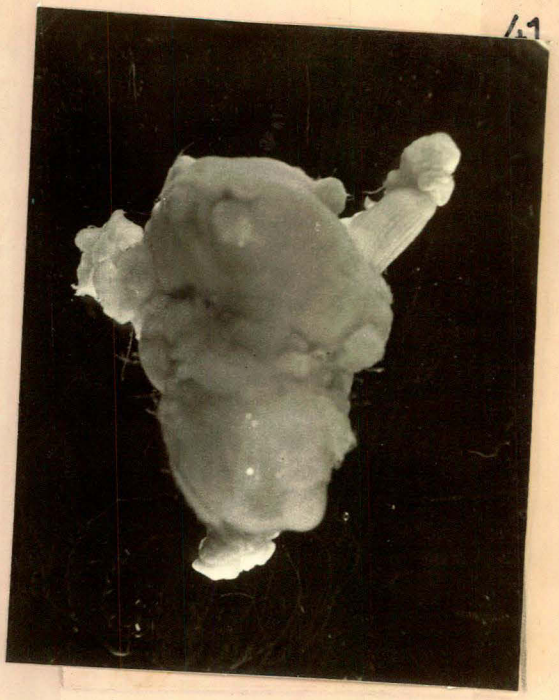

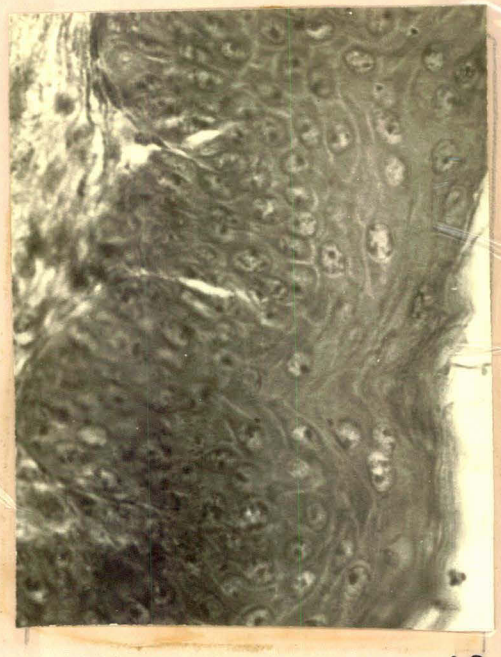

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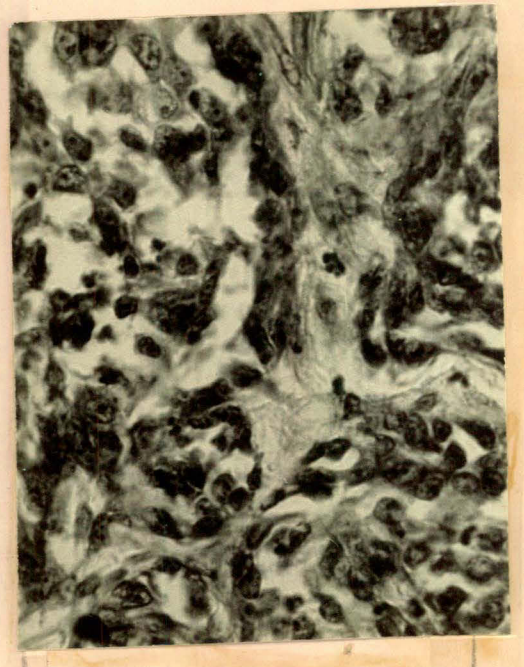


43

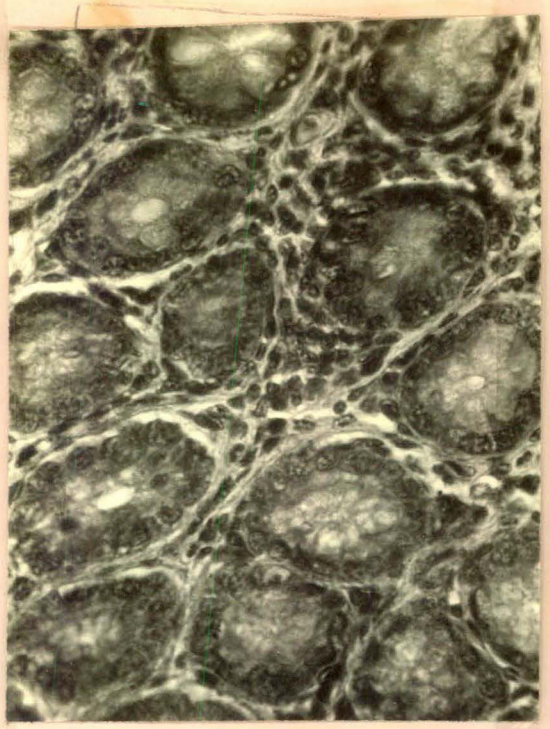
Fig. 44 : A section of the adenocarcinoma of the cervix (50 days interval in Group C) showing 'tubular' pattern of the neoplastic cells. X 800.

Fig. 45 : A section of the same adenocarcinoma of the cervix (50 days interval in Group C) showing the 'papilliform' pattern of the neoplastic cells. X 800.

Fig. 46 : Another section from the adenocarcinoma of the cervix (50 days interval in Group C) showing the arrangement of neoplastic cells in a 'convoluted' pattern. X 800.

Fig. 47 : Cervical squamous epithelium showing marked dysplastic changes with irregular buds in a mouse autopsied at the 50 days interval in Group C. X 800.

44



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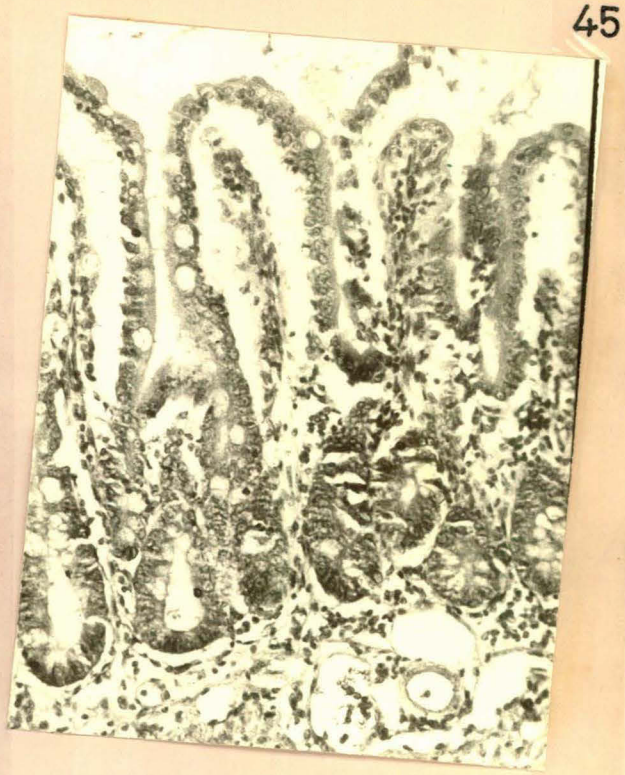

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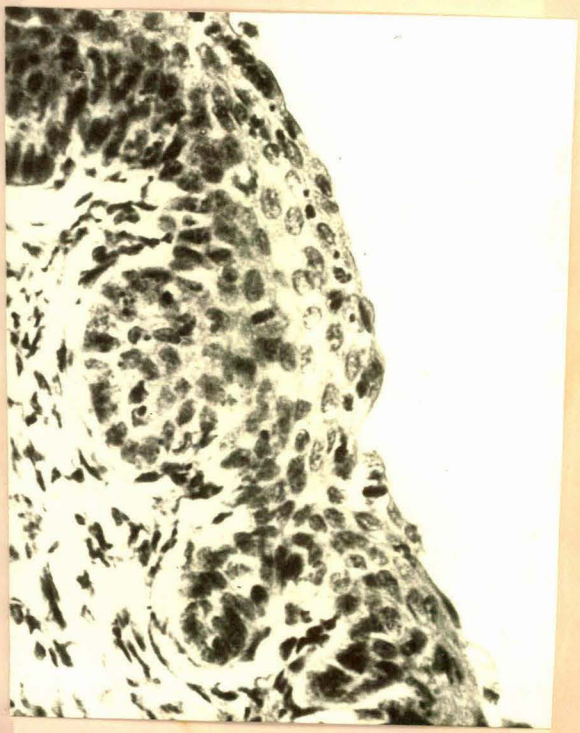

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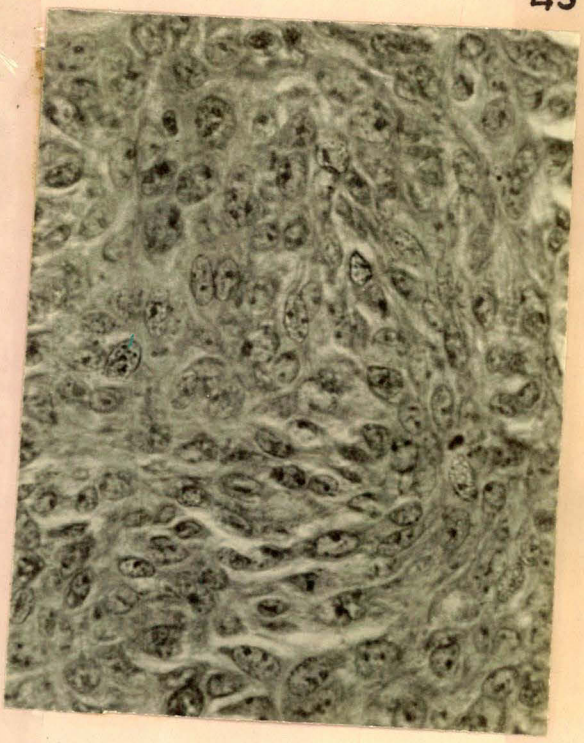
Fig. 48 : A section of the vaginal squamous cell carcinoma in a Group C mouse autopsied at the 50 days interval. Complete atrophy of vaginal epithelium by the neoplastic process can be noted. X 126.

Fig. 49 : Higher magnification of the vaginal carcinoma (50 days interval in Group C) showing details of the neoplastic cells. X 800.

Fig. 50 : Sarcomatous region of a cervix (70 days interval in Group C) which has adenocarcinoma as well as sarcoma . Palisading pattern of sarcomatous cells can be noted. X 320.

Fig. 51 : Higher magnification of the sarcomatous region showing the fusiform neoplastic cells. X 2000 .

3



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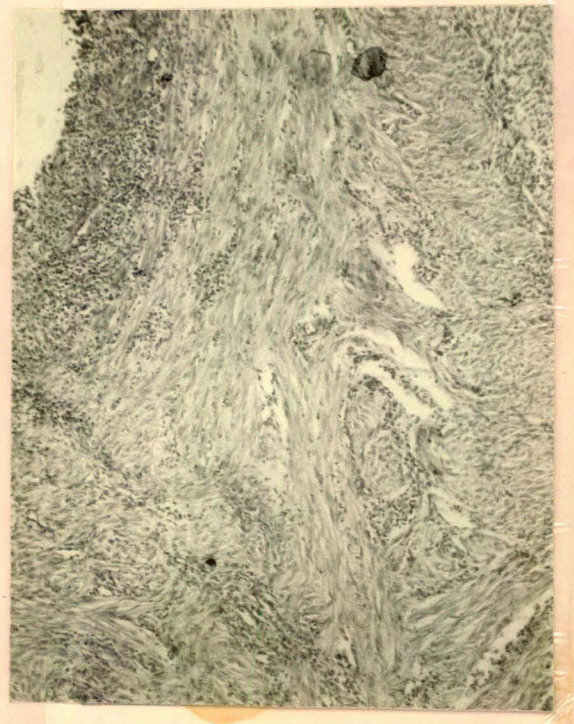

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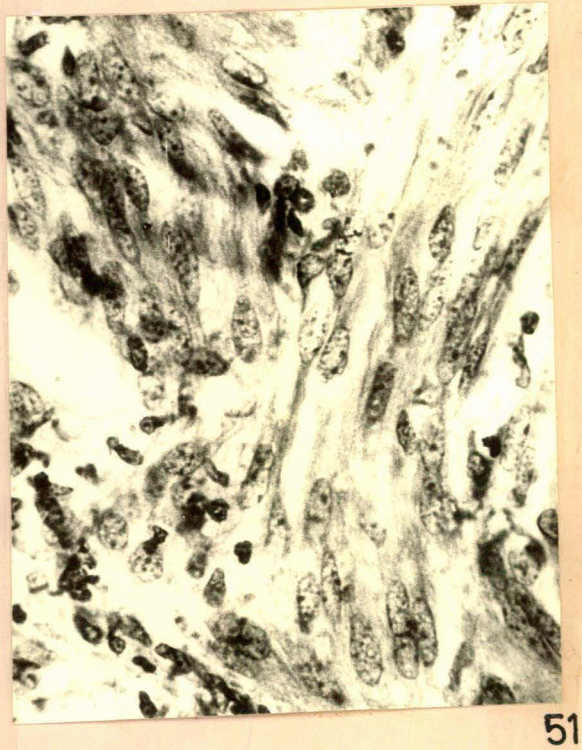
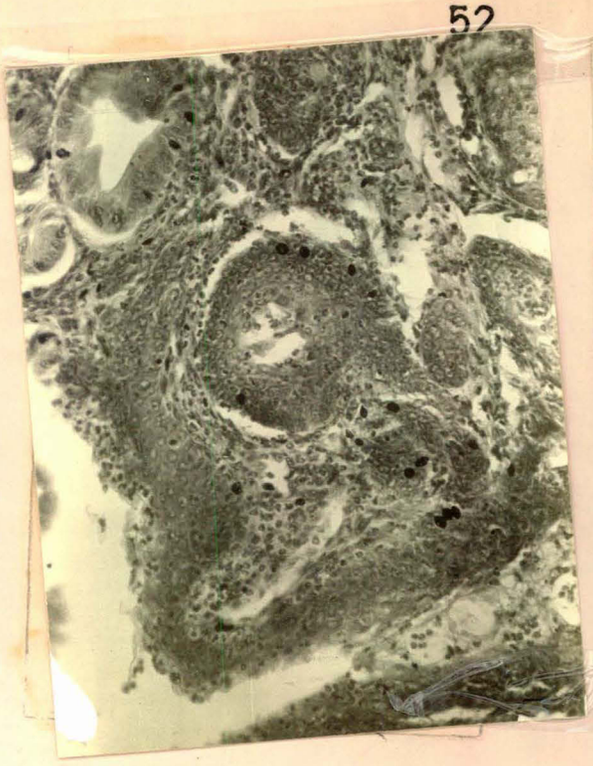


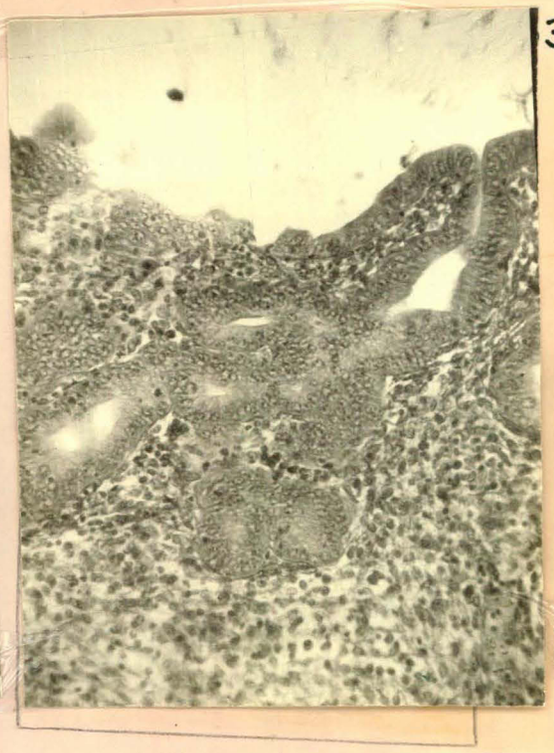
51

Fig. 52 : The adenocarcinomatous region of the cervix (70 days interval in Group C) which had both adenocarcinoma and sarcoma. The enlarged glands show various types lacking in constancy. X 320.

Figs.53,54 & 55: Higher magnifications of the adenocarcinomatous regions (70 days interval in Group C) showing variant patterns in different parts of the tumor. Transition from an endometrial like structure to a typical endocervical architecture can be noted. X 800; X 320; X 800 respectively.

52





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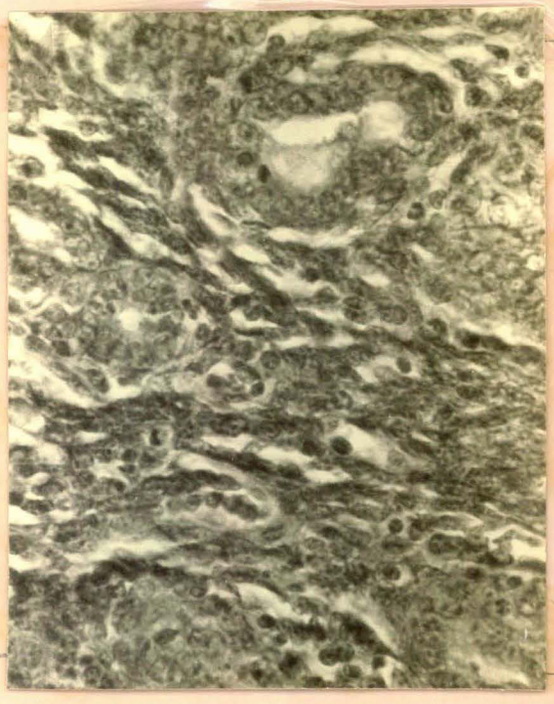

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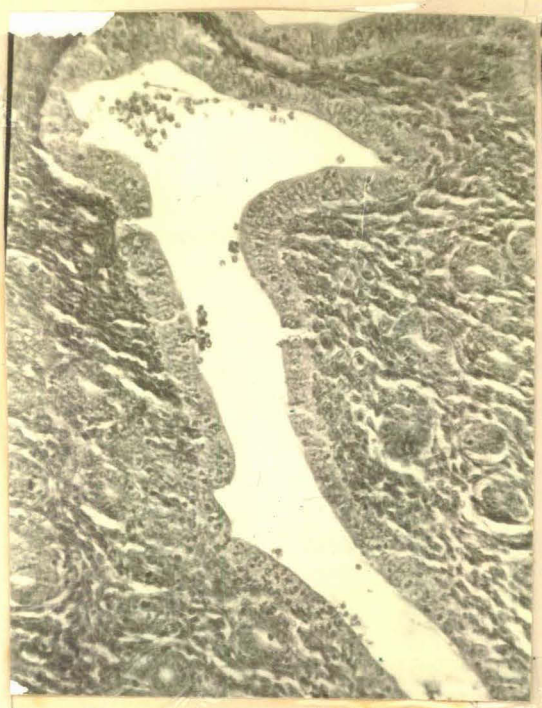
Fig. 56 : A section showing closely packed tubular, oval and irregular patterns of the adenocarcinomatous (70 days interval in Group C) cells separated by a scanty stroma. X 800.

Fig. 57 : Higher magnification of a portion of the adenocarcinoma showing the details of the neoplastic cells. The secretory activity of the adenocarcinomatous cells is prominent. X 2000.

Fig. 58 : External appearance of an advanced invasive carcinoma of the cervix in a mouse autopsied at 70 days interval in Group C. X 6.



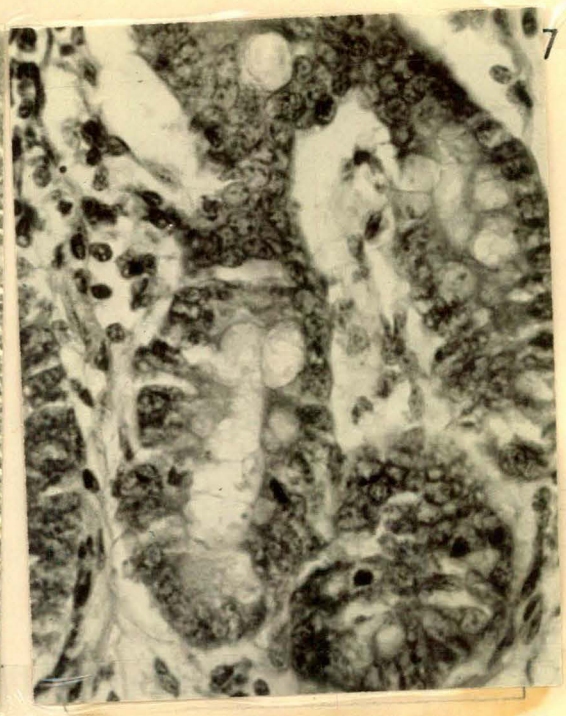


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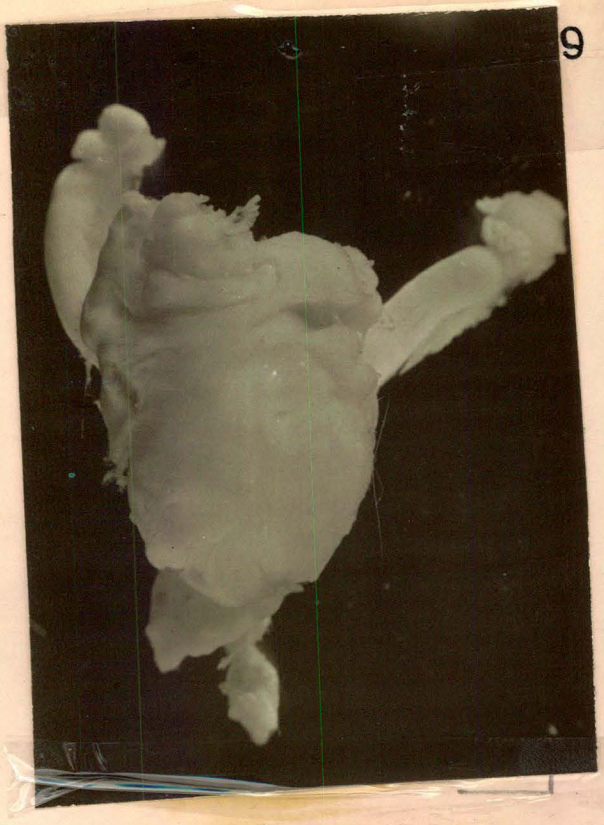
Fig. 59 : External appearance of another advanced invasive carcinoma of the cervix in the same interval time (70 days interval in Group C). X 6.

Fig. 60 : Section of an advanced invasive carcinoma of the cervix (70 days interval in Group C) showing the linguate processes and club shaped masses formed by the arrangement of neoplastic cells. X 126.

Fig. 61 : Section of an advanced invasive carcinoma of the cervix (70 days interval in Group C) showing the irregular networks formed by the loosely arranged neoplastic cells. X 320.

Fig. 62 : Higher magnification of a portion of the advanced invasive carcinoma (70 days interval in Group C) showing more details. Varied shapes and sizes of the neoplastic cells with increased nuclear cytoplasmic ratio can be seen. A giant cell with a big nucleus can be noted at the center of the photomicrograph. X 800.



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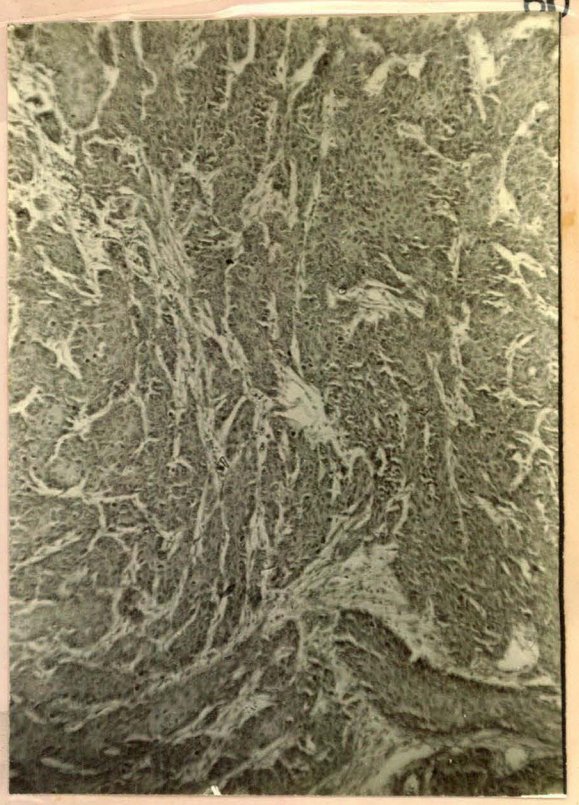

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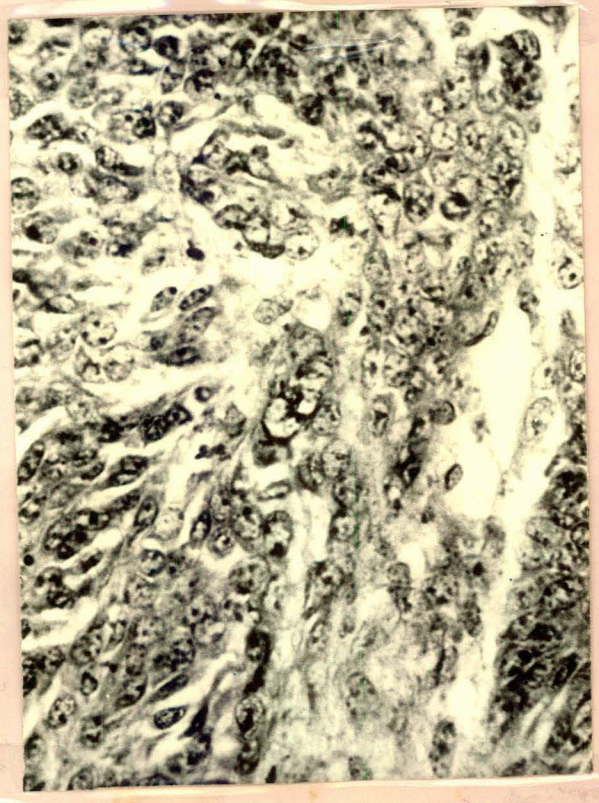

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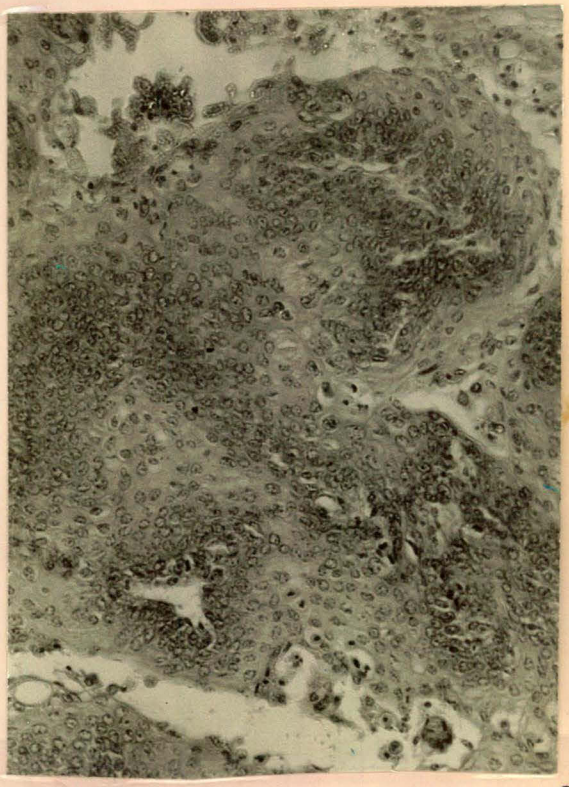

62

Fig. 63 and 64 : Higher magnifications of some regions of an advanced invasive carcinoma (70 days interval in Group C) showing the large, polygonal or rounded kertinizing and non keratinizing neoplastic cells containing large nuclei. X 800; X 800 respectively.

Fig. 65: Section from an advanced invasive carcinoma of the cervix (70 days interval in Group C) showing the widely dispersed cell clumps formed by the compressed, and darkly stained tumor cells. X 320.

Fig. 66: Section from an advanced invasive carcinoma of the cervix (70 days interval in Group C) showing the scanty stroma containing inflammatory cells which invade the degenerating tumor cells. X 126.

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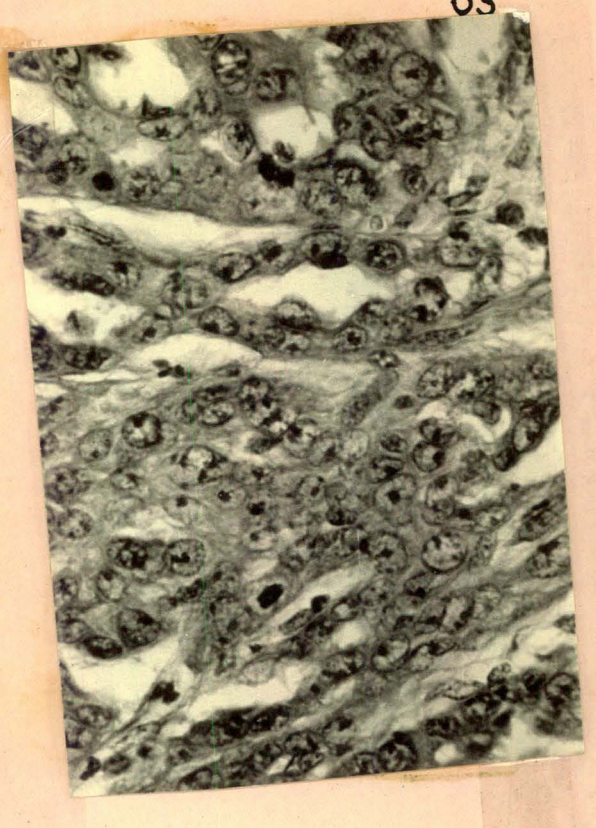

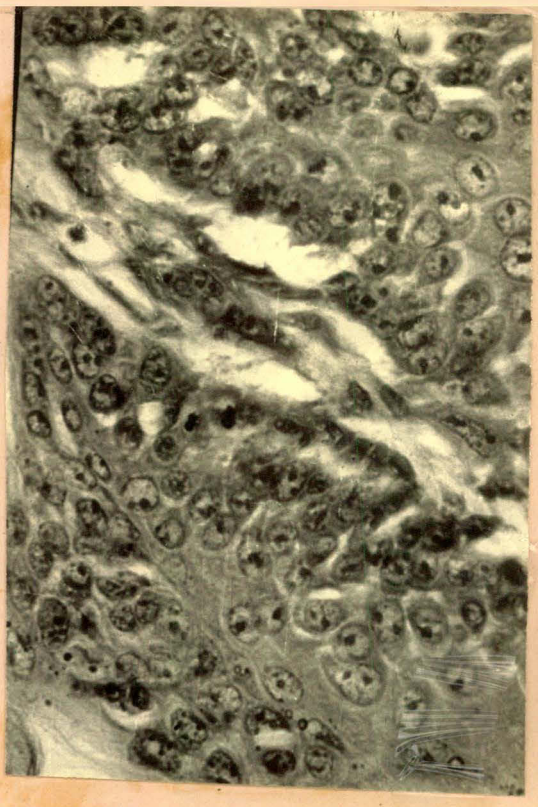

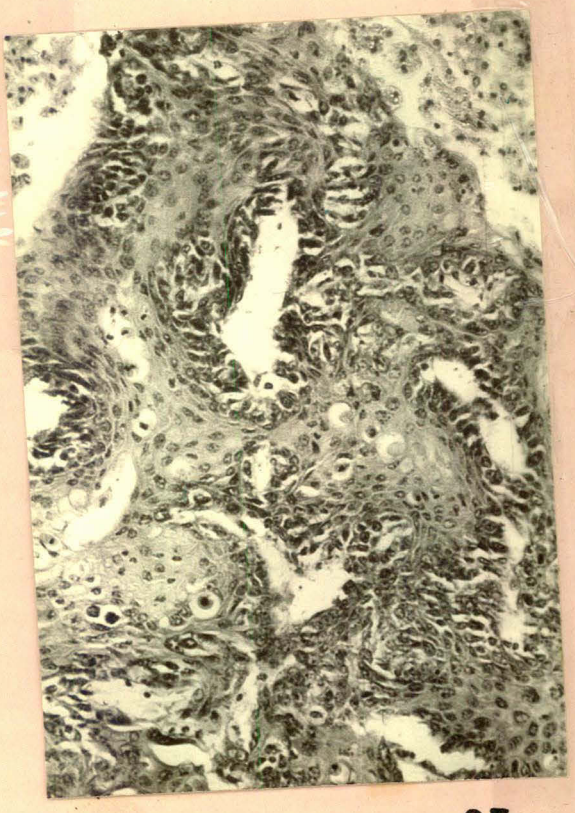

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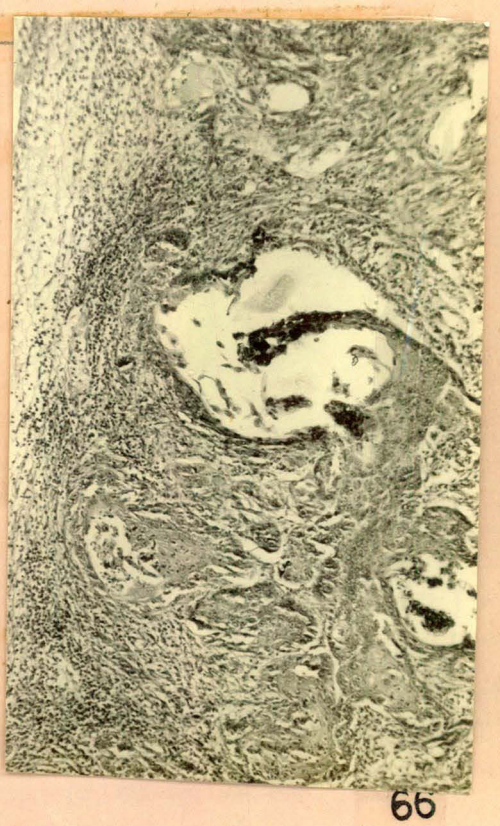

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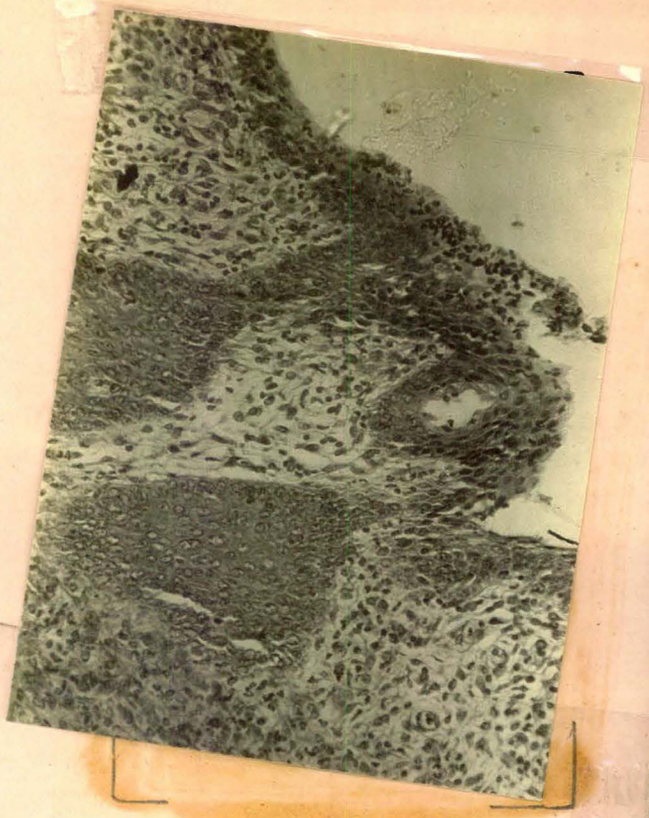
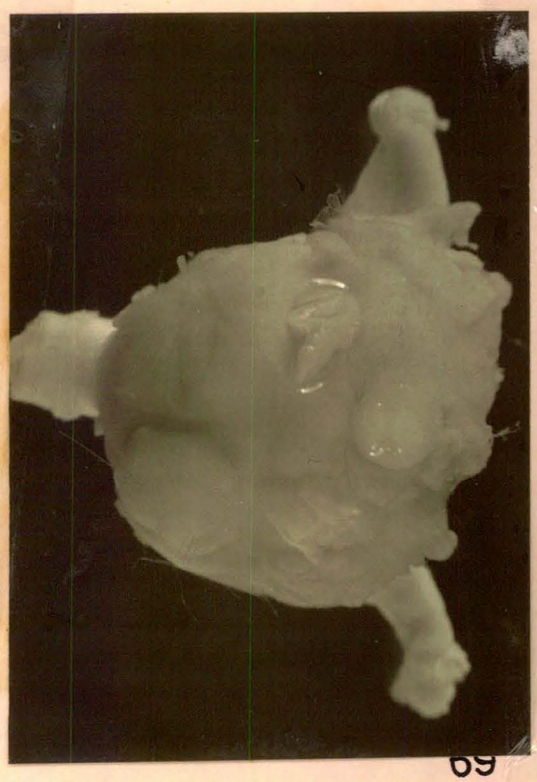
Fig. 67 : Section showing the early invasion of the carcinomatous cells in patches (70 days interval in Group C). Atrophy of the cervical epithelium can be noted. X 320.

Fig. 68 & 69 : External appearance of two big advanced carcinomas of the cervix (90 days interval in Group C). X 6; X 6, respectively.

Fig. 70.: Section showing the details of the neoplastic cells in an advanced carcinoma from the 90 days interval in Group C. Darkly stained, keratinized neoplastic cells with big nuclei can be noted. X 600.





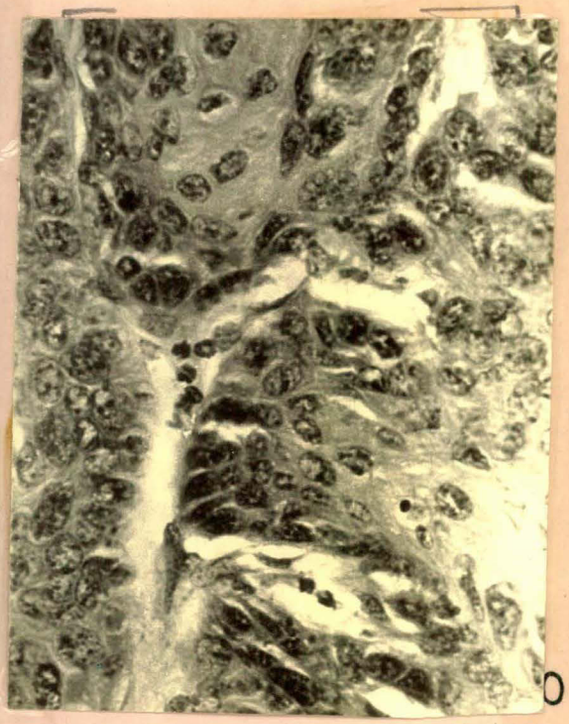


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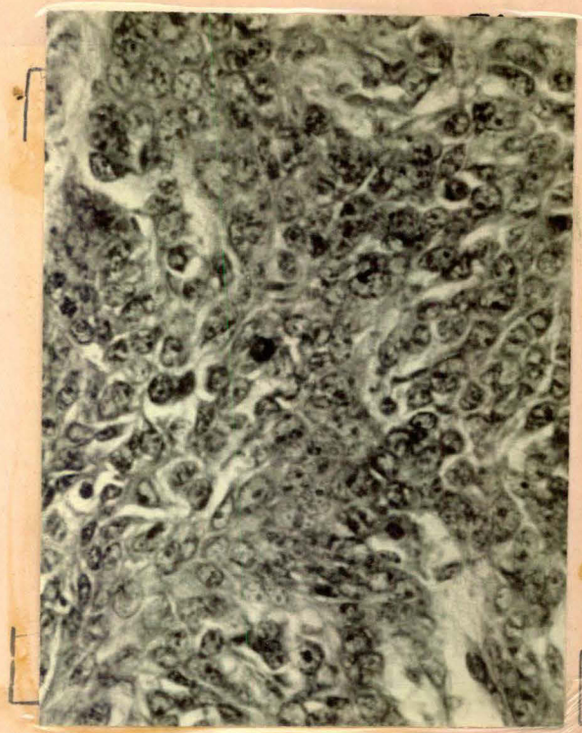
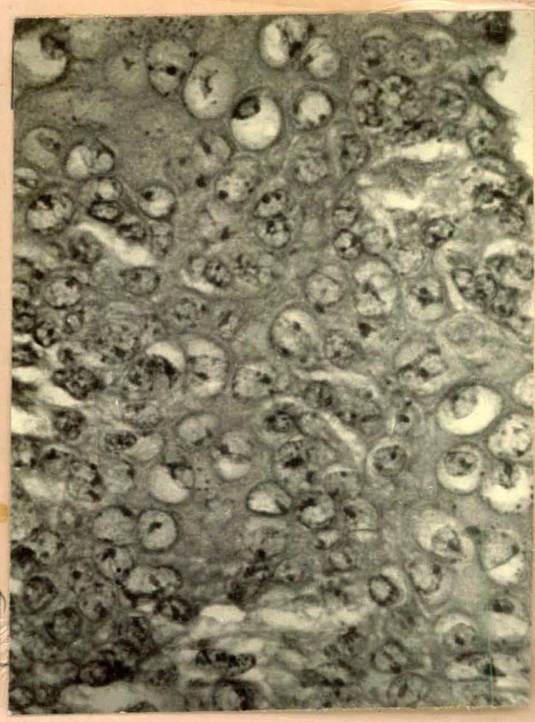
Fig. 71 : Section from an advanced carcinoma of the cervix (90 days interval in Group C) showing some interesting features of the neoplastic cells. Note the halos round the nuclei. X 800.

Figs.72,73 & 74: Sections from the advanced squamous cell carcinomas of the 90 days interval (Group C) showing the various sizes, and shapes of neoplastic cells and the different patterns formed by them. X 800; X 800, X 800 respectively.





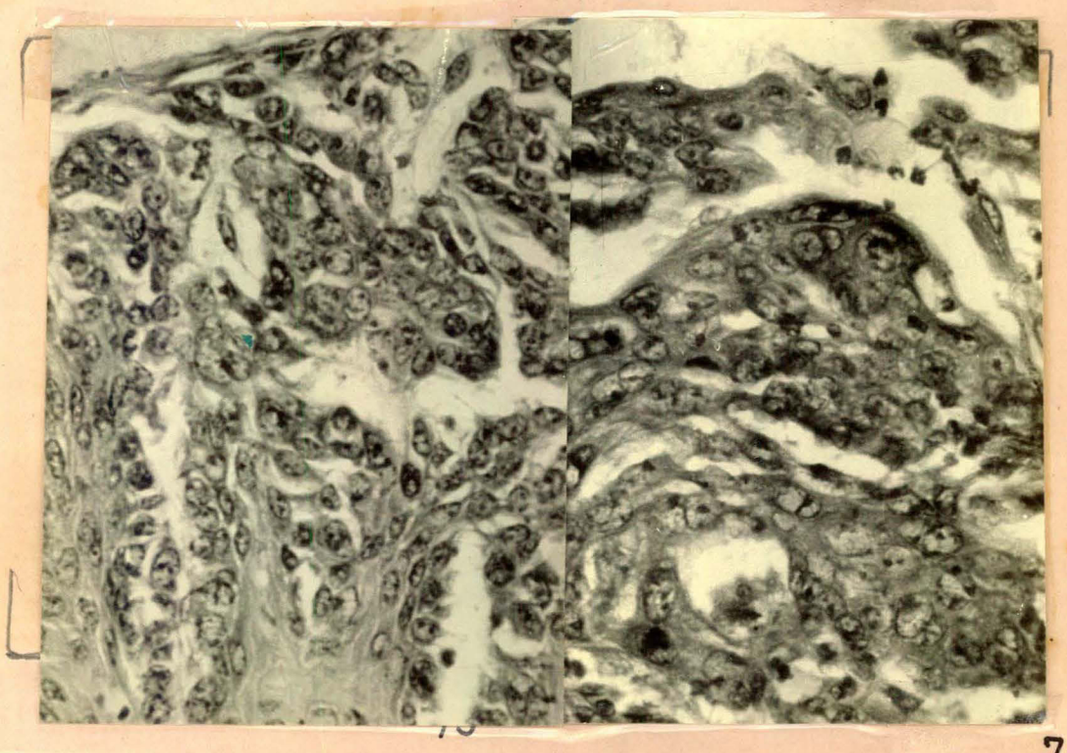
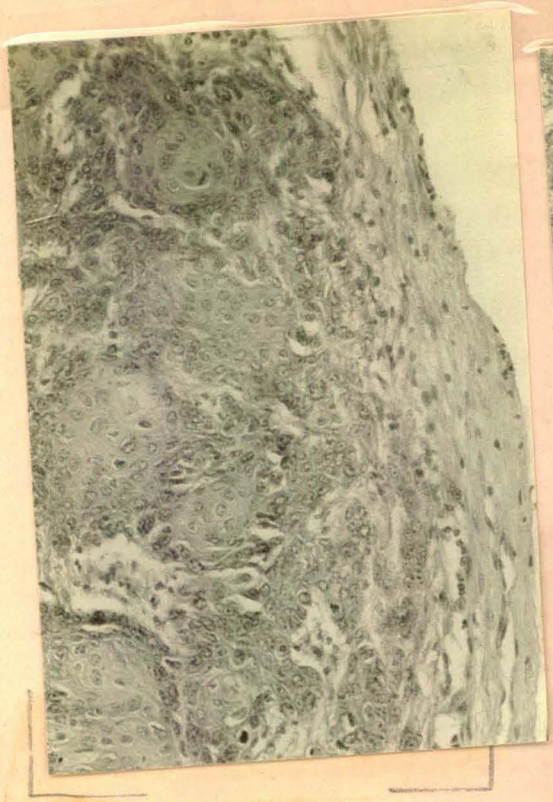


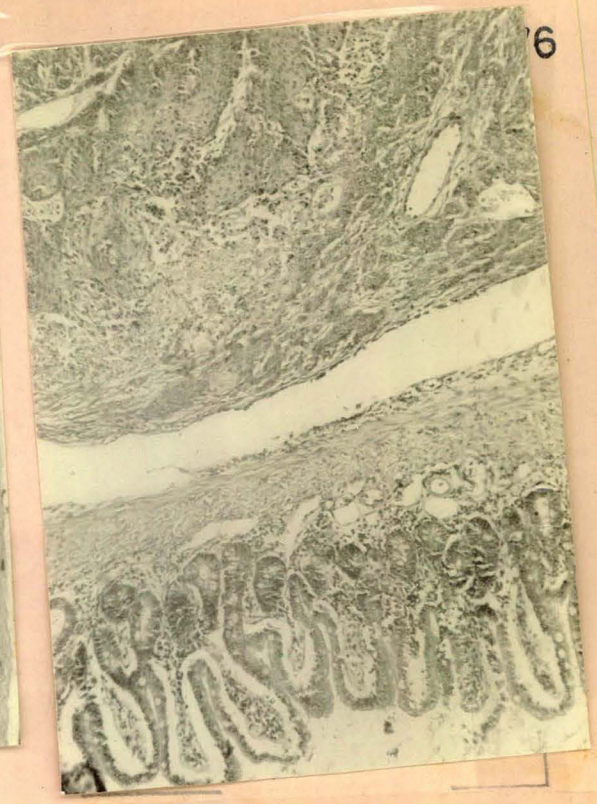
Fig. 75: Section from an advanced carcinoma (90 days interval in Group C) showing the typical nature of malignancy. Disorganisation and loss of normal cellular stratification and the replacement of entire thickness of the epithelium by neoplastic cells can be noted. X 320.

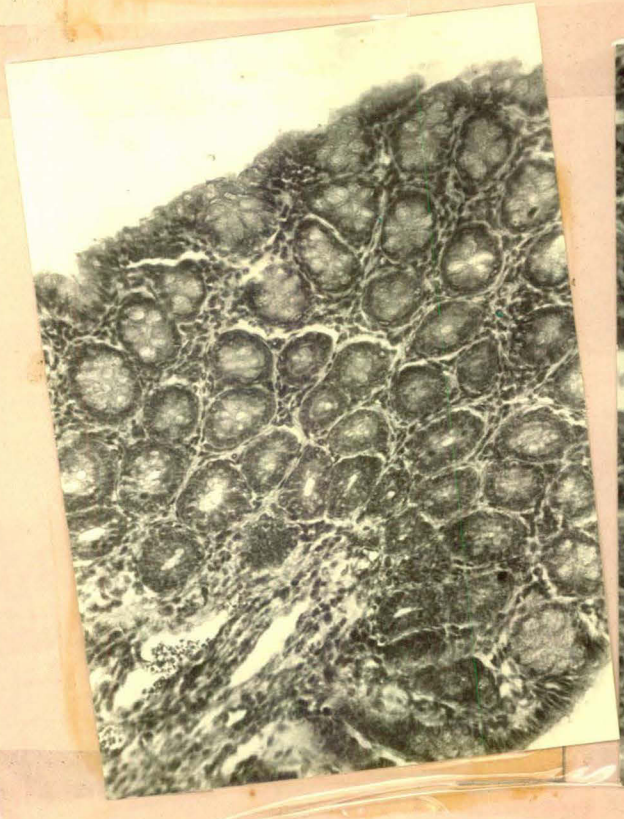
Fig. 76: Section of a cervical tumor (90 days interval in Group C) showing epithelial as well as glandular structures. X 126.

Fig. 77: Higher magnification of the glandular region of the same tumor (90 days interval in Group C) showing compactly arranged oval patterns. X 320.

Fig. 78: Higher magnification of a glandular region of the tumor (90 days interval in Group C) showing a flower like pattern formed by the adenocarcinomatous cells. X 800.







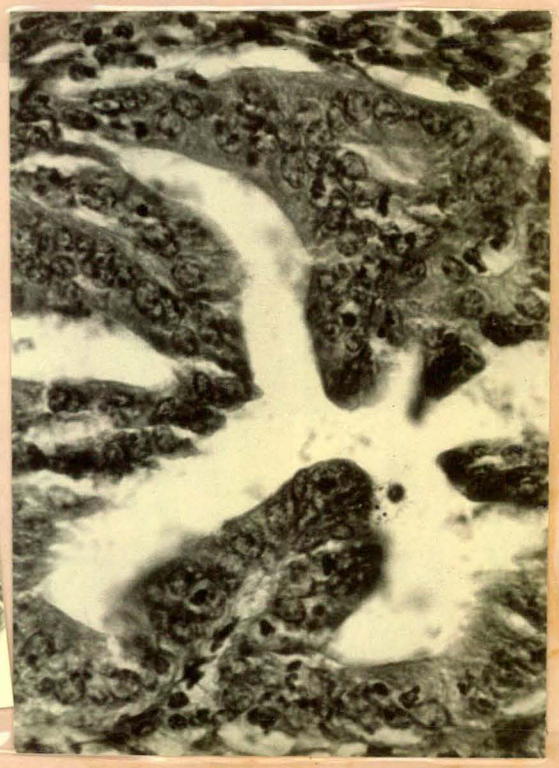


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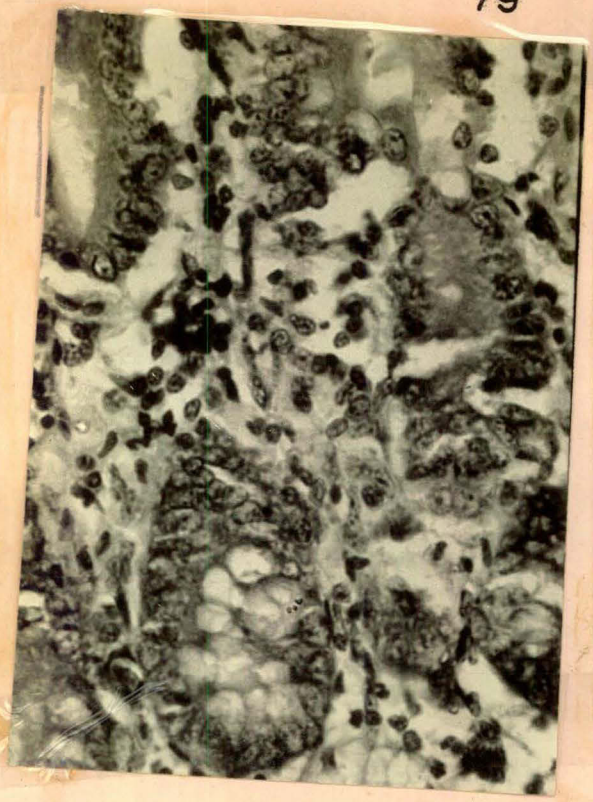
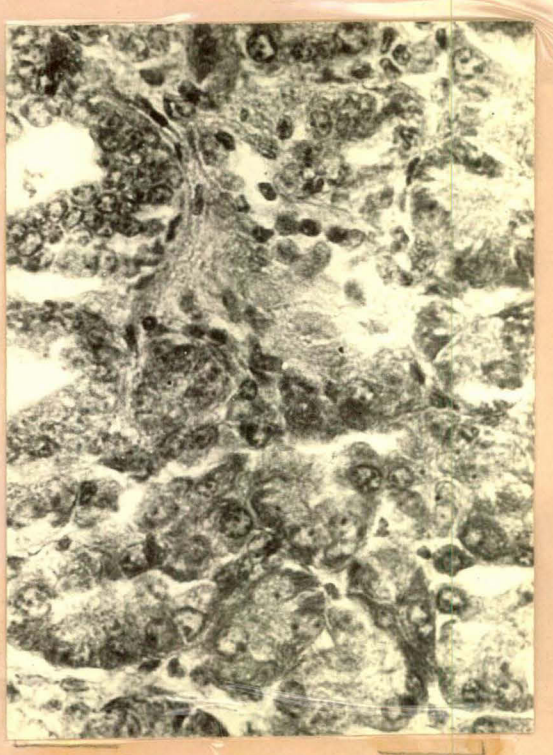
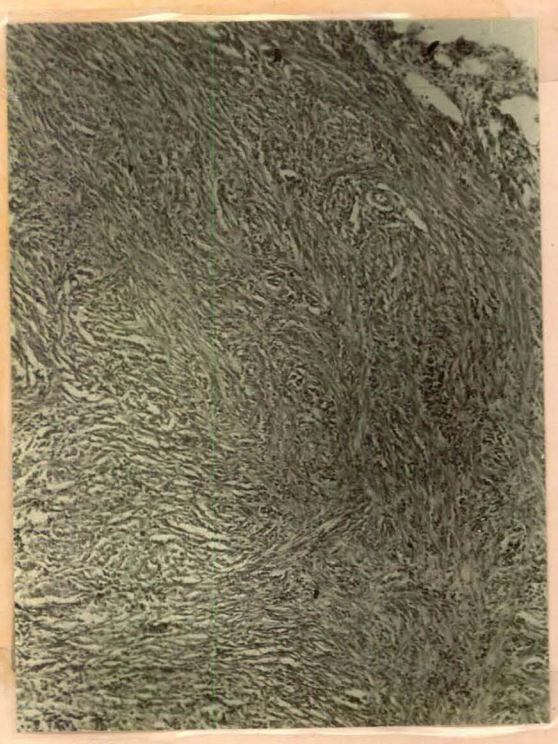
Fig. 79 : Section from the adenocarcinoma (90 days interval in Group C) showing round columnar and bizarre shaped neoplastic cells arranged in irregular patterns. The secretory capacity of the adenocarcinomatous cells is also evident. X 800.

Fig. 80 : Section from another region of the adenocarcinoma (90 days interval in Group C). Attempted glandular formation is discerned and sheets of tumor cells are found singly or in clusters. X 800.

Fig. 81 & 82: Sections from a cervical sarcoma (90 days interval in Grup C) found in the endocervical canal showing the spindle shaped cells forming irregular palisade patterns. X 800; X 800 respectively.







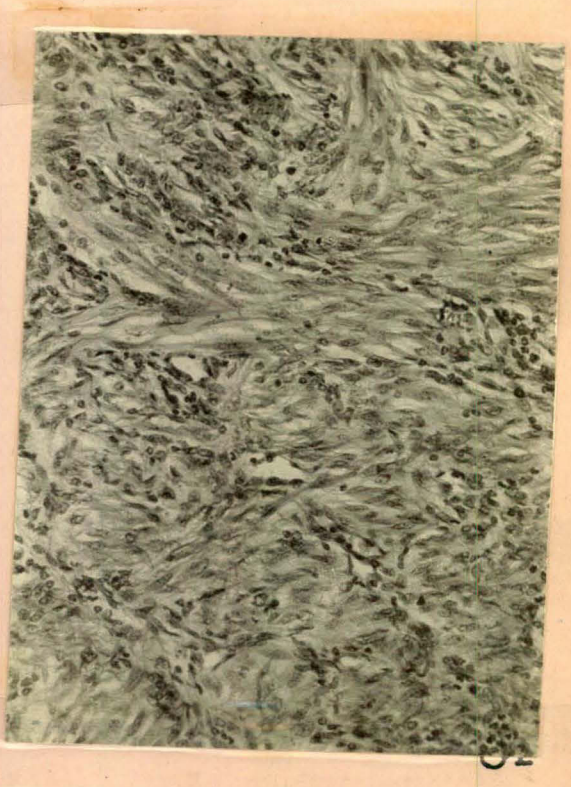


Fig. 83 : Section showing the inflammatory cells along with the spindle shaped cells in an another region of the same sarcoma (90 days interval in Group C). X 800.

Fig. 84 : Section from a vaginal tumor procured from the 90 days interval in Group C. Complete erosion of the squamous epithelium in one side of the lumen can be noted. X 126.

Fig. 85 : Higher magnification of a portion of the vaginal tumor (90 days interval in Group C) showing the over crowded neoplastic cells in varied shapes and sizes with big nuclei. X 800.

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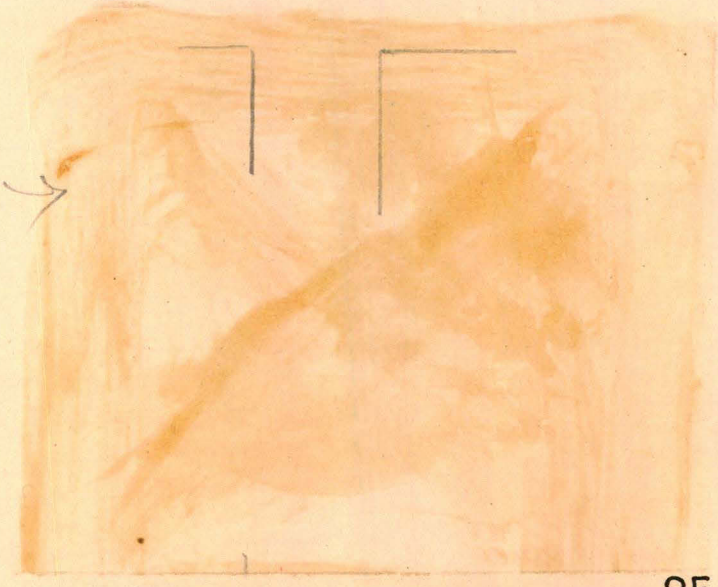

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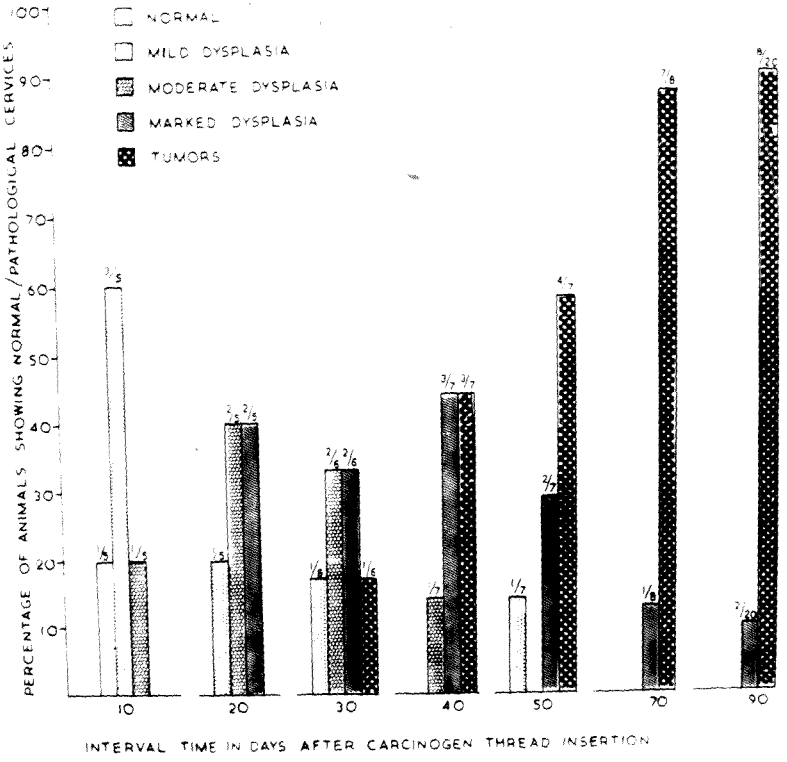
Fig. 86 : Histogram showing the pathological changes of cervices in carcinogen threaded animals (Group C).


NORMAL
MILD DYSPLASIA
MODERATE DYSPLASIA
MARKED DYSPLASIA
TUMORS
PERCENTAGE OF ANIMALS SHOWING NORMAL/PATHOLOGICAL CERVICES
100
90
80
70
60
50
40
30
20
10
10
20
30
40
50
70
90
INTERVAL TIME IN DAYS AFTER CARCINOGEN THREAD INSERTION

DISCUSSION

Mice have been widely used for the experimental induction of cervical carcinomas. Cotchin and Marchant (1977) have discussed the literature in detail on this subject. Various methods of induction of cervical cancer have been devised using hormones like estrogen, testosterone, chemical carcinogens like dimethyl benz(a)-anthracene, benzo(a)pyrene, methylcholanthrene and viruses like herpes virus type 2 etc. Among these the highest incidence of cervical tumors within a shortest span of time was obtained by the methylcholanthrene impregnated string method. Other methods involving benzo(a)pyrene string insertion, methylcholanthrene or benzo(a)pyrene painting came in order. Scarpelli and Von Haam (1960) induced cervical carcinoma by using MCA impregnated strings. The incidence was 80% in about 77 to 230 days. String method is the best of the available methods, because carcinogen paintings often result in contamination of the vagina leading to many vaginal lesions. Present investigation with a slightly modified string method of Murphy (1953) using Swiss albino mice reveals that a maximum of 90% incidence can be obtained in 70 to 90 days after the thread insertion.

As against Murphy (1953) and Scarpelli (1957) the highest incidence of the cervical carcinomas by the present

method is because of the increased dose of carcinogen (approximately 600 µg/animal) in the thread and also the facilitation of continual release of the same by the in situ threads in the cervical region.

It should be mentioned that the release of carcinogen 3-methylcholanthrene at different interval times could not be estimated. However, it is suggested that by using labelled methylcholanthrene (^{3}H-MCA) impregnated threads the release of carcinogen at different intervals and the average amount of carcinogen required for cervical cancer induction can be estimated.

Also the changes in the cervical epithelium during the different stages of estrous cycle was not accounted because of the continued exposure of carcinogen over the epithelium.

The beeswax used for the impregnation of the carcinogen on the cotton threads could not elicit any tumorous changes. However, because of infections and the irritation of the thread in the cervical canal, slight epidermization and mild dysplasias were noted in some animals (Table I).

3-methyl cholanthrene is the chemical carcinogen used in the present investigation. The general aspects of the metabolic activation of chemical carcinogens to reactive

debatable theories. Meyer (1941) proposed that the lesion results from the healing of erosions and arises from the basal layer of cells of the portio vaginalis. Carmichae and Jefferson (1941) considered it to result from the subcylinderical basal cells of the endocervix. The present investigation enabled to study dysplasia of the endocervical epithelium and portio vaginalis in different stages of development. Most of the earlier stages of dysplasia with the pathological changes like, keratinization, hyperchromasia are found first in the basal layer of the endocervix than in the portio vaginalis. So with the limitation of the experimental system it may be suggested that the histogenesis of the dysplasia proposed by Carmichae and Jefferson is correct and that this lesion develops as a result of proliferation of the reserve or basal cell of the endocervical epithelium. It is possible that these reserve cells are remnants of the primitive cervical lining epithelium and that they have a latent potentiality for proliferation and differentiation when the proper stimulus is present.

It should be noted that in the case of humans, the carcinoma in situ or the epidermoid carcinoma is very common and remains for a longer period of time in the patient's life. For example in the studies of Peterson (1956) it was found that carcinoma in situ continued to

remain for almost 1 to 20 years before the invasion started. But in the experimental studies the epidermoid carcinoma is very rare. If there is any lesion like that of carcinoma in situ in some sites or other, there are few neoplastic cells invading into the stromal region breaking away the basement membrane of the epithelium. And they are usually included in the category of early invasive carcinoma.

According to Kehar (1967) dysplastic changes confined primarily to the cytoplasm of cells are more frequently reversible than when the nucleus shows marked dysplastic changes. And regression to normalcy occurs upto the stage of moderate dysplasia only. Finding of marked dysplastic cells should always be looked upon with suspicion as they invariably antedate cancer. Since marked dysplasia closely resembles the epidermoid carcinoma it may be stated there may be a carcinoma in situ stage in experimental animals also but the duration of its occurrence is very minimal. Reagan and Wentz (1965) also could not find the carcinoma in situ stage in their experimental animals and they could not explain this phenomenon.

Rubio (1974) focussed much attention between the possible association between epithelial buds and invasive

carcinoma. He induced irregular epithelial buds by the local application of benzo(a)pyrene apparently similar to those characterizing carcinoma in situ with buds (CISB) of the human cervix. According to him there is a close association among epithelial buds, the degree of cellular atypia and progression to carcinoma. The same type of epithelial buds comparable to the carcinoma in situ with buds in humans could be seen in almost all the preprarations of marked dysplastic stages but with less severe neoplastic features. Induction of cervical lesions in mice does not show a carcinoma in situ simplex (CISS) of the human cervix. Mostly carcinoma in situ simplex indicates the epidermoid carcinoma and the carcinoma in situ with buds to the invasive carcinoma (Coppleson, 1967) even at the earliest stages. Almost all the neoplastic atypia in the epithelia of mice remain with buds. So when they progress to a stage like carcinoma in situ with severe atypia in the whole epithelium some of them get their way easy to the stromal region. Ultimately they are classified as early invasive carcinoma in the present investigation. This discussion may possibly explain the apparent absence of carcinoma in situ and epidermoid carcinomatous stages. Nevertheless their presence during the sequential development of cervical cancer can not be discarded.

The differences between the cervices of humans and of mice were discussed earlier in the distribution of squamous and columnar epithelium. The significant difference is that the location of squamocolumnar junction is at a greater depth in the mouse compared to the humans. Table IV illustrates the localization of tumors in the cervices of carcinogen threaded animals. It can be noted that the bifurcated canal occupies the maximum number of tumors and the common and the lower bifucated cervical canal occupying the next position. In mice since the position of squamocolumnar junction itself differs from humans obviously the occurrence of the cervical cancer also differs. It is well known that in humans, squamocolumnar junction is the most susceptible region for the cervical cancer development. So even in experimental conditions the squamocolumnar junction plays a vital role in place of origin of the cervical cancer.

Compared to the human situations where most of the cervical cancers occurs at the portio vaginalis it is to be noted that in experimental conditions it is not so. And it was found that the cervical cancer in the portio vaginalis region in rare situation is due to the spread from the other sites.

Many clinical and experimental studies were made on the biological progression of cervical cancer (Richart, 1973;

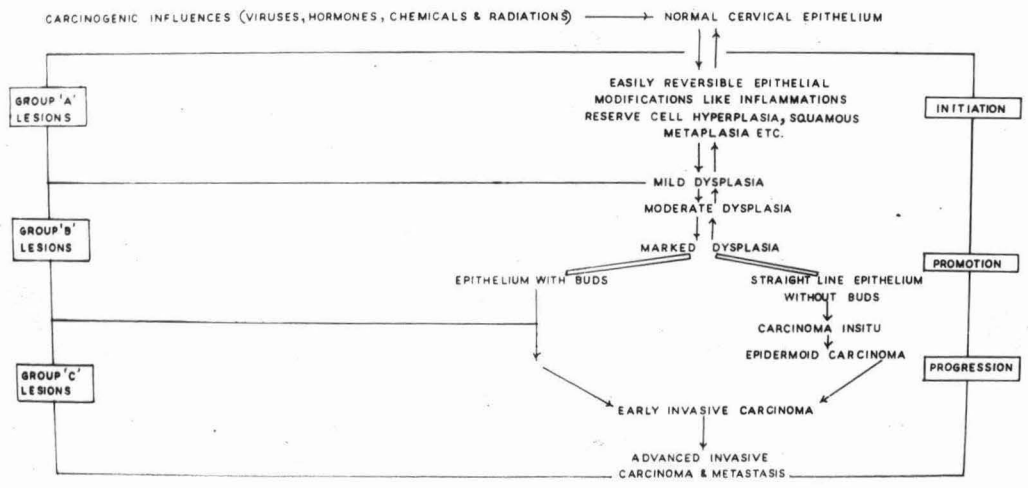
Christopherson, 1977; Koss, 1978). But a very clear model for different steps of the carcinogenesis had not emerged, because of the overlapping of different stages. Considering the previous literature and the present study the following model is proposed (Fig. 87).

A normal cervical epithelium is easily accessible and susceptible to the varied stimuli like infections, hormonal levels, carcinogens etc. The usual primary responses for these stimuli like inflammations, leucocyte infiltrations, hyperplasia, and metaplasia, are easily reversible back to normalcy. It is a very common phenomenon. If the stimuli persists for a longer time or if the intensity is more the chances of reversibility decreases and the epithelium passes to the dysplastic changes, which itself can be divided into three stages according to the severity of the lesion, i.e. mild, moderate, and marked. There are possibilities for reversion to earlier stages, but the probability decreases with the advancement of the lesion.

At the stage of marked dysplasia two deviations in the progression of the lesion can be suspected according to the nature of the lesion. These are atypia with buds the importance of which was first propounded by Rubio and the atypia with straight line borders (Fig. 87). In the first category with buds the neoplastic cell easily invades

Fig. 87: Model for the multiple steps in cervical carcinogenesis.

A MODEL FOR THE MULTIPLE STEPS IN CERVICAL CARCINOGENESIS.



into the stromal region. Evidence of these atypical buds and their early invasion was often reported in clinical and experimental studies (Rubio, 1974).

The second category with straight line epithelial border passes through the stage of carcinoma in situ, or epidermoid carcinoma with more severity in the neoplastic characters. The epidermoid carcinoma may remain for a longer period before the invasion starts.

Fould's concept of A,B,C schema (see Introduction and Review of Literature for details) can be conveniently accomodated in the proposed model. The 'A' phase comprises a variety of hyperplastic and metaplastic lesions of dubious relevance to neoplastic development and therefore classified as group 'A_1' lesions and some of the smallest and abnormal dysplasias can be possibly assignable to the 'A_2' group. The lesions moderate dysplasia and marked dysplasia can be regarded as group 'B' lesions since the four developmental fates of group 'B' lesions (Foulds, 1975) are expressed by them i.e. i) they may give origin to invasive carcinoma by progression; ii) they may grow steadily without progression; iii) they may persist indolently without progression or iv) they may regress.

The advanced phase 'C' of neoplastic development includes the lesions nearly and advanced invasive carcinomas

in the present model. Here it should be noted that compared to the human situations the experimental carcinomas in mice lack the vigorous metastastic properties. The highly invasive carcinomas invade upto urinary bladder or ureter, whereas in the human situations the highly invasive carcinomas metastasize to distant sites like liver, lungs etc. through lymphatic and vascular systems.

Also the present model is not inconsistent with the concept of two stage carcinogenesis proposed by Berenblum (1947). The initiating events by carcinogens and other unknown agents occur upto the stage of mild dysplasia. The factors for promotion persists until the stage of carcinoma in situ and preinvasive carcinomatous changes develop. Invasion and metastasis may be included as the steps in progression.

The nature of earliest events and the subtle cellular changes to reach the cancerous stage is difficult to explain in the present model because of the limitation of the microscopical examination and the conventional methods of tissue preparation. Conceivably, histochemical and immunocytologicalmethods coupled with appropriate *in vitro* approaches may ultimately reveal such subtle early changes if they exist.

CHAPTER - II

AGING AND CERVICAL CANCEROGENESIS

INTRODUCTION AND REVIEW OF LITERATURE

One of the strong determinants of cancer incidence rates* in man appears to be age. The probability that a man of Birmingham in United Kingdom will develop cancer in the next five years is 1 in 14 if he is 65, but only 1 in 100 if he is 25 (Doll, et al., 1970). The differences in cancer incidence rates between young adults and old adults of this order of magnitude are found in many species other than man. In countries with adequate cancer registries total cancer incidence can be separated into age-specific incidence rates for each site and between ages of 25 and 64 many of these separate rates increase approximately as fourth, fifth or sixth power of age. The rapid and continuous rise in incidence with age occurs characteristically in cancer of skin, in most of the epithelial cancers of respiratory, digestive and urinary tracts and in chronic lymphatic leukemia and myelomatosis which according Burnett (1962) may also be of epithelial origin.

Representative data for mortality rates for different types of cancers show that always the risk increases with

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*The incidence rate of a cancer at a particular age is the proportion at unit time of people of that age who develop the cancer of the interest (Doll, 1970).

the increase in age. This type of relationship has been reported several times and has been made the basis for several different models on the mechanisms of carcinogenesis. Nordling and Stock (Nordling, 1953; Stock, 1953) accounted it by the postulation that the production of cancer required between five and seven independent events or mutations. Fisher and Hollomon (1951) suggested that it might mean that seven contiguous cells had to be altered before the altered cells could break loose from control and appear as cancer. Later Armitage and Doll (1959) postulated that only two or three changes were needed but that each change gave the daughters of affected cell an advantage over normal cells in that they multiplied more rapidly. Whatever may be the hypothesis, construction of the detailed mechanism of carcinogenesis based on only the epidemiological grounds is definitely faulty. Nevertheless these studies and hypotheses indicate the possible link between aging processes and the development of cancers.

Following these epidemiological observations several reports on experimental animals have appeared concerning the effect of aging on the development of cancers. A variety of carcinogenic stimuli were given to mice and rats by different administration routes to study the influence of aging on carcinogenesis.

Van Durren et al. (1975) reported that skin cells are more susceptible to cancer with increasing age and there is general decrease in skin tumor production with increasing age at the time of promotion. Ebbesen (1974) also pointed out that aging increases the susceptibility of mouse skin to DMBA induced carcinogenesis in which skin grafting was used in syngeneic hosts of various ages. Contrary to the above mentioned reports Forbes (1965) found that in the Rhino strain, younger mice were more suseeptible to skin carcinogenesis than older mice. However, the interpretation of the results is complicated by the fact that Rhino mice show marked unusual pathological changes in the skin with aging.

Meranze et al. (1969) reported that among the neoplasms in Wistar rats by DMBA, youngest females (8-15 days old) showed more incidence of fibroadenoma of the breast and the subcutaneous sarcoma was more frequent in both the sexes of the same age group. And young adult females had the mammary carcinoma predominance than the older females.

Bela Toth et al. (1963) reported that the incidence of subcutaneous sarcomas at the site of injection increased with larger dose and advancing age. A most interesting observation was made in C57 BL/6N x $C_3H/HCNF_1(B_6C_3F_1)$ mice

(Ward et al., 1979). This strain of mice is used for the National Cancer Institute's Carcinogenesis Testing Program and was found to have several neoplasms like hepatocellular adenomas and carcinomas, lymphomas, leukemias and pulmonary adenomas and carcinomas in their normal untreated life span. The important observation made was that the risk of developing most neoplasms increased with the age of the mouse.

Age at the time of carcinogen administration had been demonstrated to be an important modifying factor of mammary carcinogenesis with regard to susceptibility to tumor development (Huggins et al., 1961; Dao, 1969). Haslam (1979) reported recently that in Lewis rats specifically age at carcinogen administration affected the number of tumors induced, the latent period of tumor development and the number of tumors which regressed spontaneously.

According to Pike (1966), when a constant dose of carcinogen is applied to experimental animals the incidence rate of carcinoma is approximately proportional to

$$(t-w)^{k-1}$$ where

't' is the age of the animal and

'w' and 'k' are constants.

This means that a constant dose of carcinogen produces a

much higher incidence rate of carcinomas in later life than in early life. Influence of aging of the neoplastic development is thus well documented in the literature.

So it is well known that aging affects the development of cancers in some way or other. In the case of uterine cervical cancer the incidence rate varies in different ages. The report from Cancer Institute, Madras by Shanta and Krishnamoorthy (1969) stated that women aged 40-60 were mostly affected by the cervical carcinoma. According to Bhargawa (1981) out of 1594 cervical cancer patients in Kidwai Memorial Institute of Oncology, Bangalore, for the period 1979 and 1980, 64.80% occurred during the fourth and fifth decades while as high as 91.15% were found between 21 to 60 years.

Shyamala et al. (1978) reported that in S.V. Medical College Hospital, Tirupati 24.5% of the cancer patients were of the age group 41-50 years and 40.5% of the cancer patients were above 51 years. According to Wahi et al. (1964) in North India age group 45-64 years showed the maximal incidence of cervical cancer. In their studies the incidence of 175.4/1000 was found in the age between 50-54 years. Such clinical studies on the aging and cervical cancer incidence were reported many times. But there has been no report from experimental studies about the influence of aging in cervical cancer.

The mouse cervical epithelium undergoes dramatic changes in its structure and function at different times of life (Leppi, 1964). Also the well known changes in the endocrine and immunologic system by the aging process are suspected to influence the induction of cervical cancer in them. So the present investigation was taken up to find out the variances of cervical cancer development in different age groups.

MATERIALS AND METHODS

Randombred Swiss albino mice from Maulana Azad Medical College, New Delhi were used for the present set of experiments. The animals were maintained in an air-conditioned room. They were fed with rat food (Hindustan Lever Ltd., India) and tap water adlibitum.

The carcinogen, 3-methylcholanthrene was procured from Sigma Chemical Company, U.S.A. and the pure beeswax locally from Mysore. The carcinogen thread preparations and the intracervical thread insertions were done as explained previously in the I Chapter.

A total number of 133 animals of different ages were divided into five main groups. The number of animals, the age and the type of inserted threads are illustrated in the Table V. In each group apart from carcinogen threaded animals six to ten mice were inserted with wax threads to serve as control.

The animals were sacrificed three months after the thread insertions. At autopsy a recheck was made for the presence of threads and those exceptional cases where they were not present were excluded. The upper part of the vagina, the uterine cervix and the posterior part of

TABLE V

Experimental Design to Study the Influence of Aging on Cervical Carcinogenesis

No.	Age Group	Type of thread insertion	Number of mice
1	8 weeks	Carcinogen	20
		Wax	10
2	25 weeks	Carcinogen	20
		Wax	6
3	42 weeks	Carcinogen	20
		Wax	6
4	65 weeks	Carcinogen	20
		Wax	5
5	80 weeks	Carcinogen	20
		Wax	6

the uterine horns were always removed en bloc and fixed in Bouins solution. Serial sections of 7 µ thickness were cut transversely and stained with Harris haematoxylin and eosin.

Whenever an animal died during the observation period its cervix was also removed, processed and inspected for tumorous changes.

RESULTS

8-Week Age Group:

All the carcinogen threaded animals were alive till the autopsy period of three months time. Ten animals had advanced squamous cell carcinoma. Most of them were well differentiated large cell non-keratinizing type. The tumorous lesions exhibited varying degrees of pleomorphism. Disorganization, loss of normal cellular stratification and the replacement of entire thickness of the epithelium by abnormal cells were some of the characteristic features. Five preparations had early stages of squamous cell carcinoma with minimal invasion into the stromal region. Apart from them two adenocarcinomas were observed. The neoplastic cells include epithelial and glandular structures with many pathological changes. It is evident from the histological appearance that the endocervical adenocarcinomas have the capacity to secrete mucus. One preparation had typical sarcoma in the endocervical canal. Histologically many giant cells and spindle-shaped, enlarged smooth muscle cells were predominant. Totally out of 20 animals 18 were bearing tumors and 22 tumors were found in all.

The wax threaded animals served as control for this group. At the three months autopsy period none of them showed tumors. Nevertheless, four preparations showed mild dysplasias and one had moderate dysplastic changes. Infection was also a common occurrence in the wax threaded animals.

25-Week Age Group:

The animals of this group were 25 weeks old when the carcinogen thread insertion was made. Two animals died because of advanced tumors before the autopsy period, one at 20 days and another at 52 days after the carcinogen thread insertion. Histological sections of lymph glands and lungs did not show any metastatic infiltration in them. One of them had severe infection along with the carcinoma. Six preparations of this group showed early carcinomatous changes with minimal invasion of neoplastic cells. Nine preparations had advanced well differentiated squamous pearls, keratinization and pleomorphism were often observed in them. One preparation was found to be affected with typical adenocarcinoma with neoplastic glandular and epithelial cells. Abundant mucus secretion was the typical feature found in that preparation (Fig. 88). Totally this group showed twenty six tumors in eighteen tumor bearing animals. Thus though the number of tumor

bearing animals are same for both the 8-week and 25-week age groups, the average number of tumors per tumor-bearing animal was more in the animals of the latter group.

Some of the wax threaded animals of this age group showed lesions similar to mild and moderate dysplastic changes with slight inflammations (Table VII).

42-Week Age Group:

The carcinogen threaded animals of this age group showed more mortality than the previous groups. Five animals died before the autopsy period because of tumorous lesions. Four of them were severely affected by the squamous cell carcinomas and one with adenocarcinoma. Among the autopsied animals at three months time, 11 showed advanced carcinomatous changes. Pathological observations were characteristic in all these lesions. Two preparations were found to have early squamous cell carcinomas, one with adenocarcinoma and another one with sarcoma. Many swollen, spindle-shaped smooth muscle cells could be found. The average number of tumors per tumor-bearing animal and the total number of tumor-bearing animals exceeded those in the previous groups.

The control wax threaded animals of this group showed more irritational changes with three preparations

having mild dysplasia and one having moderate dysplasia. One other animal showed typical cystic hyperplasia in the glandular endocervical region (Fig. 89).

65-Week Age Group :

The mortality number with tumorous lesions was seven in the carcinogen threaded animals of this group. This was the maximum mortality among all the groups in the present investigation. Out of them, six preparations showed squamous cell carcinomas and one adenocarcinoma. All the carcinomas were well differentiated and advanced types. The typical nature of this group is that twelve autopsied preparations had advanced squamous cell carcinomatous changes. There was no early carcinoma or any other type of tumors like adenocarcinoma, sarcoma etc. This group showed maximum number of tumors per tumor-bearing animal (Table VI) among all the groups.

Interestingly parallel to this, the wax threaded control animals also had more pronounced changes. Out of the five controls one preparation had marked dysplastic changes with changed polarity and loosely arranged cells (Fig. 90). One preparation showed moderate dysplasia and the other two mild dysplasia.

80-Week Age Group :

The carcinogen threaded animals of this age group showed similar tumor incidence like that of the previous group. Out of the six animals died with tumors before the autopsy period four had well differentiated squamous cell carcinomas and two with adenocarcinomas. Ten preparations showed advanced carcinomas, at the autopsy period. Two preparations had early carcinomas. One animal in this group was found to have severe sarcoma and another with adenocarcinoma. A total number of 28 tumors were found among the twenty tumor bearing animals. All the carcinogen threaded animals were found to have tumors like that of the animal from 42-week age group.

The control wax threaded 80-week age group animals showed two marked dysplasia which is maximal number among all the groups. Three preparations were found to have mild dysplasia and one with moderate dysplasia-like lesions.

TABLE VI

TUMORS IN DIFFERENT AGE GROUPS OF CARCINOGEN THREADED ANIMALS

Age groups	Mice at risk	No. of deaths with tumors before the autopsy period		No.of tumor-bearing animals at the autopsy period			No. of animals with tumors in the cervical epithelium only (ex.ACM)	Total No. of tumors	No. of tumors per tumor bearing animal
		Carcinoma	Other types (ACM & SCM)	Carcinoma Early	Advanced	Other types (ACM & SCM)			
I. 8 weeks	20	0	0	5	10	3	15/20(75%)	22	1.22
II.25 weeks	20	2	0	6	9	1	17/20(85%)	26	1.44
III.42 weeks	20	4	1	2	12	2	17/20(85%)	32	1.6
IV.65 weeks	20	6	1	0	12	0	18/20(90%)	38	2
V.80 weeks	20	4	2	2	10	2	16/20(80%)	29	1.45

Note : Figures in parantheses indicate the percentage of animals with tumors in the cervical epithelium only.

ACM = Adenocarcinoma; SCM = Sarcoma.
ex.ACM = Excluding adenocarcinoma.

TABLE VII

PATHOLOGICAL CHANGES IN THE CERVICES OF WAX THREADED ANIMALS

Age groups	Mice at risk	Normal	Mild dysplasia	Moderate dysplasia	Marked dysplasia	Tumors and other changes
I. 8 weeks	10	5	4**	1	0	0
II.25 weeks	6	3	1	2*	0	0
III.42 weeks	6	1	3*	1	0	1@
IV. 65 weeks	5	1	2	1	1	0
V. 80 weeks	6	0	3**	1	2	0

Note : The number of '*' denotes the number of preparations which showed infection.

@ = Cystic glandular epithelium.

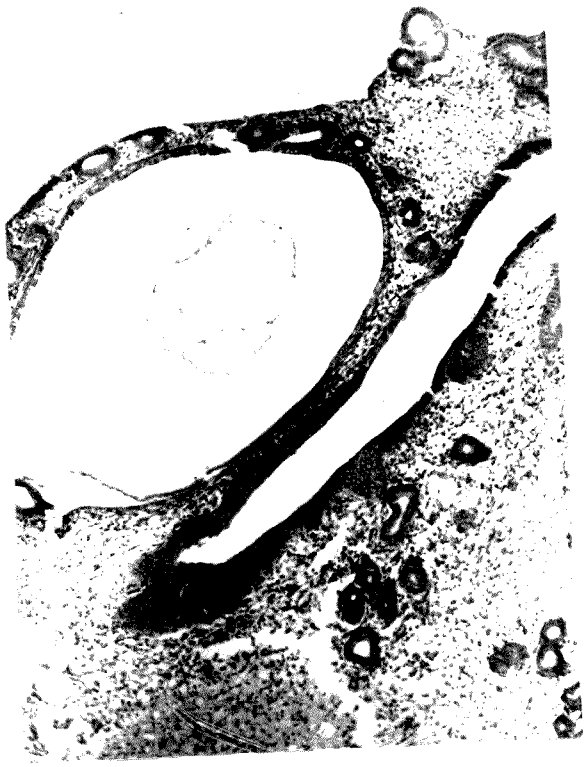
Fig. 88 : Section of a cervical adenocarcinoma in a 25 weeks age group mouse autopsied after 3 months of carcinogen thread insertion. Abundant mucus secretion in the glandular neoplastic cells can be noted. X 320.

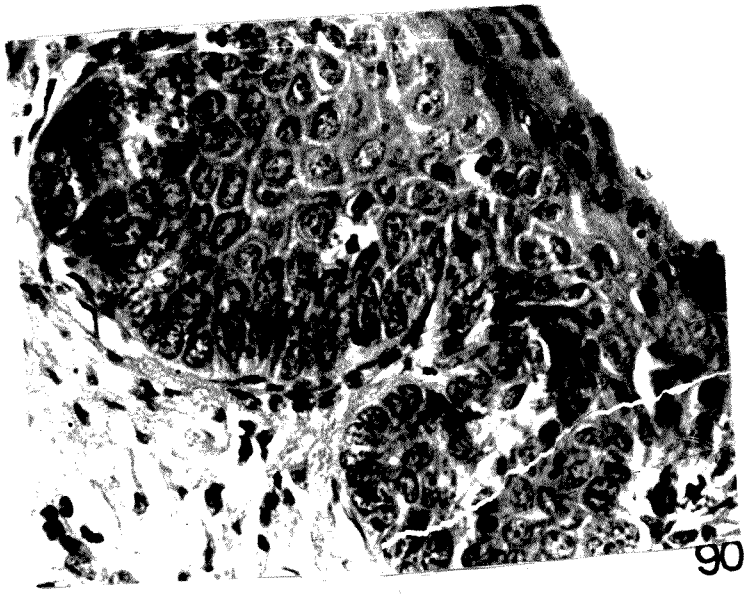
Fig. 89: A section from the cervix of a 42 weeks age group mouse autopsied after 3 months of carcinogen thread insertion showing the typical cystic hyperplasia in the glandular region. X 126.

Fig. 90: Section from the cervix of a 65 weeks age group mouse autopsied after 3 months of wax thread insertion showing the marked dysplastic changes. Loosely arranged cells with maximal nucleo-cytoplasmic ratio can be noted. X 800.

88

89





90

The present investigation reveals interesting findings on the influence of age on the experimental cervical carcinogenesis elicited by 3-MCA in virgin Swiss albino mice. When all the tumors (tumors appearing both in epithelial and extra epithelial areas of cervix) are taken into account, the tumor incidence oscillated from 90% (in 8 weeks age group) to 100% (80 weeks age group). However, when the tumors recorded only in the cervical epithelial area (excluding extra epithelial and glandular epithelial regions) are taken into account, the incidence swings from 75% (in 8 weeks age group to 90% (80 weeks age group). The tumor incidence per tumor bearing animal seems also to vary with age. Thus in 8 weeks age group, the average tumor incidence was 1.22 per animal, and this incidence gradually increased with age reaching a maximum of 2 per animal. Then in 80 weeks age group the incidence dropped to 1.45 per animal. Looking into the Table VII it is clear that the incidence of dysplasia increases with age in wax threaded animals. The dysplastic changes can be reasoned to be elicited by the wax and it seems this irritational response increases with age. So it may be possible that the age-related increase in irritational changes due to wax could predispose to murine cervical epithelium to

neoplastic changes by the carcinogen. However, progressive age dependant depletion of follicular population in the ovary (Eckstein, 1977) could alter the gonadal hormonal level in the system and thereby alter the tumor incidence in the aging animals. The observed decline in the average number of tumors per tumor bearing animal in 80 weeks age group could possibly be due to this change in the hormonal levels.

As far as we know there are no reports of experiments involving the aging and cervical cancer in animals. However, a number of experimental studies have been made on the influence of age in the development of other cancers (Peto, 1970; Peto, 1974) as reported earlier. Almost all of them indicate the increase in the incidence of cancers with advancing age. Epidemiological evidences also confirm this phenomenon. So the results of the present investigation are in confirmity with earlier studies involving other types of cancers.

Various hypotheses and suggestions have been put forward by many to explain the increase in cancer incidence with age. Peto *et al.* (1975) have recently reviewed this aspect extensively. One of the hypotheses, the local multistage model for the development of cancer is noteworthy.

It has been shown in the case of mouse skin that exposure to a tumor-initiating stimulus (which may be a subcarcinogenic dose of a skin carcinogen or one or more doses of an incomplete carcinogen i.e. an agent by itself is incapable of giving rise to skin cancer) may result in skin cancer formation if there is subsequent exposure to a tumor promoting agent, whereas exposure to the same two agents in the reverse order does not result in skin cancer (Berenblum and Shubik, 1947; Roe, 1959). Although there may be some loss of effect of exposure to a tumor initiating agent if the promotor is given after a long time after the initiator either because of repair or because of selective destruction or shedding of altered cells (Roe et al., 1970), there is an abundant evidence from studies of experimental animals or of humans exposed to occupational or other carcinogens that some increased risk of cancer development among exposed groups may persist for a longer period after the exposure ceases. So it is evident that the change from the normal to cancerous state can take place in distinct 'stages' each with its own causes. In the multistage model, the changes (i.e. the stages) once they have occurred in a particular cell are not repaired but remain forever. Also the changes are transferred to all its descendants, so the proportion of the cells in the tissue

with one particular change will increase with the passage of time as further exposure to carcinogens continues.

If a number of specific changes are necessary before a cell can proliferate into recognizable cancer, then the proportion of cells which have suffered all those changes will even if the changes are not entirely independant of each other increase as time passes rather like the product of several things that themselves increase with age and will therefore increase very sharply with age. The simplest assumption is that the rate constant for each stage is either constant or alternatively negligible until certain other stages have occurred, and constant thereafter. Therefore, the local multistage model is capable of explaining the strong dependence of cancer incidence rate on age by the postulation that several heritable cellular alterations are necessary to produce a cell which can proliferate into a cancer.

However, the alternative hypothesis is that some other intrinsic mechanism of aging might have greater relevance than that suggested by the mere postulate of a local multistage model. For example Burnet (1970) has suggested that in young people immunological surveillance mechanisms

might bemuch more efficient at eliminating changed cells before they manage the final stage which is proliferation to form cancer.

There are many reports of the influence of aging on the immune system in animals. Old (1960) found that phagocytic activity of old mice is lesser than the young mice and that of older mice is less susceptible to stimulation. According to Aoki (1964) high titers of antibody were produced in young mice and to a significantly lesser degree in old mice. Baumgartner (1934) found that rabbits over two years of age produced a lower agglutinin titer to Bacillus entiritidis than animals of six to 13 months age. Two months old guinea pigs were reported by Baer and Bowser (1963) to have a higher serum antibody titres and greater contact skin sensitivity against pentadecylcatechol than those that had reached the average life span of two to three years.

Dilman (1921) has suggested that tumorigenesis might be promoted by hormonal changes due to age related changes in the hypothalamus. Thus age related changes in the hypothalamus, hypophysis and ovaries could alter the hormonal milieu in the animal and thereby change the response of cervical epithelium to carcinogenic stimulus.

According to Kahn (1966) epidemiological studies of lung cancer showed that the death rate increased by fourth power proportional to the duration of smoking rather than by the influence of age. It is an indirect evidence for the local multistage model explained earlier.

According to Peto et al. (1975) during normal life random heritable changes may occur by accident in particular stem cells and that when certain changes happen in a single stem cell the result may be proliferation into a recognizable cancer. However, of all the heritable changes that might occur in a stem cell the majority will presumably be irrelevant to carcinogenesis and so, ignoring lethal changes which would eliminate a cell line in old age several stem cells must have suffered many non-lethal heritable changes by the same general mechanism that are involved in carcinogenesis. Burnett (1974) has suggested that this accumulation of somatic changes in the fundamental process of aging and that the rate at which they occur is subjected to evolutionary control (perhaps by interspecies differences in the efficiency of DNA repair enzymes) and largely determines the typical life span of each species. There evolved controls on the rates at which random heritable changes arise in the stem cells of an organism would presumably affect not only the time span of aging but also that of carcinogenesis.

If this is so, then the rate of somatic mutations determines both the rate of aging and the age specific incidence rate of cancer. This postulated common cause for aging rates and for cancer incidence rates might be the reason why most species suffer some cancer of old age whether old age occurs at 90 weeks or 90 years even though aging itself does not affect oncogenesis.

CHAPTER - III

INFLUENCE OF CHEMICAL MODIFIERS ON THE CERVICAL CANCEROGENESIS

INTRODUCTION AND REVIEW OF LITERATURE

Current epidemiological data suggests that chemical carcinogens have a significant role in the etiology of cancer in man. That is why extensive studies are made on the mechanisms of chemical carcinogenesis in recent years. Further evidences are piling up to demonstrate that several natural as well as synthetic chemical modifiers can alter the tumor incidence elicited by known chemical carcinogens. These modifiers either enhance or inhibit the tumor incidence depending upon the role they play during initiational or promotional events. When cancer cure is a remote possibility, one should certainly probe into its preventive aspects. Thus if suitable chemicals which could inhibit the neoplastic transformation and/or its progression are discovered it would certainly help in the prevention of human cancer by chemical carcinogens.

Experiments on the inhibition of chemical carcinogenesis date back to 1929. At that time it was shown that dichloroethylsulfide inhibits skin tumor formation (Berenblum, 1929) resulting from repeated paintings of mouse skin with carcinogenic tar. Inhibition of epidermal neoplasia is an unusual situation in that it allows for high local concentrations of inhibitors. In addition toxicity problems are considerably less than those with

systemic administration. Early studies of inhibition of chemical carcinogenesis in which highly toxic materials were employed were followed by a more diverse groups of experiments based on an increasing amount of information concerning the chemistry and metabolism of chemical carcinogens and biochemistry of cancers in general (Wattenberg, 1966).

There are many chemical compounds that have the capacity to modify or inhibit the effects of chemical carcinogens with low toxicity administered either prior to or after the exposure of the carcinogen. The following are the potential mechanisms of modifications of chemical carcinogenesis :

i. Reversal of early carcinogenic processes.

ii. Alteration of metabolism of the carcinogen,
   a) decreased activation;
   b) increased detoxification;
   c) combination of (a) and (b).

iii. Scavenging of active molecular species of carcinogens to prevent their reaching critical target sites in the cells.

iv. Competitive inhibition.

Overall the modification entails the prevention of active form of the carcinogen from reaching or reacting

with the target sites. The modification involving the reversal of early phases of the carcinogenic process has been achieved with retinoids and has been reviewed by Sporn et al. (1976). The use of antioxidants as possible inhibitors of the chemical carcinogens has been based in general on the concept that the antioxidants may exert a scavenging effect on the reactive species of carcinogens thus protecting cell constituents from attack. In early studies, wheat germ oil and α-tocopherol were employed. Experiments showing positive and negative results have been published. These investigations have been summarized recently by Wattenberg (1972).

During the past several years, studies have been performed with other antioxidants. The most extensive work of this type has been done with phenolic compounds like BHA (butylated hydroxyanisole) and BHT (butylated hydroxy toluene). Inhibitory modulation occurs under a variety of experimental conditions and with a broad range of chemical carcinogens (Wattenberg, 1978). Also several non phenolic antioxidants were found to inhibit chemical carcinogenesis. One of these is ethoxyquin a widely used antioxidant commonly added to commercial animal feed.

Experimental studies of the capacity of disulphiram and some selected compounds to inhibit chemical carcinogenesis have been done. These sulphur-containing compounds are potent inhibitors of BP induced neoplasia of the forestomach and large intestine (Wattenberg, 1976).

When added to the diet disulphiram & diethyldithiocarbamate profoundly inhibit large bowel neoplasia (Wattenberg, 1975). Also benzylthiocyanate, phenethylisothiocyanate and benzylthiocyanate the three naturally occurring constituents of edible cruciferous plants were found to inhibit the development of forestomach and breast neoplasms (Wattenberg, 1977).

Inhibition of chemical carcinogenesis by Selenium salts has been reported. Sodium selenide added to the croton oil suppressed the development of skin tumors (Shamberger, 1966). In a subsequent work MCA was repeatedly applied to the skin and the addition of sodium selenide inhibited epidermal neoplasia (Shamberger, 1970).

Several studies have demonstrated that protection against chemical carcinogens by the administration of inducers of increased microsomal mixed function oxidase activity is possible. The inducer employed varied from compounds such as polycyclic hydrocarbons which are

noxious agents to chemicals such as flavones which have little toxicity (Wattenberg, 1970).

There is no detailed study on the modifiers of cervical carcinogenesis although such reports are available for many other types of cancers. In the present investigation three selected modifiers were chosen to study the influence on the cervical cancerogenesis. They are BHA (butylated hydroxy anisole - a phenolic antioxidant), retinoic acid (a synthetic analogue of vitamin A) and MPG (2-mercapto propionyl-glycine - a detoxifying sulphhydryl compound).

1. BHA (butylated hydroxyanisole):

As noted earlier BHA is one of the phenolic anti-oxidants - a group of compounds widely used in the food consumed by human populations (Chipault, 1966). The modulation of carcinogenesis by BHA occurred under a number of experimental conditions. It has been found in the situation where the route of administration results in direct contact of carcinogen with the target tissue, i.e. neoplasia of the forestomach in mice fed benzo(a)pyrene or 7,12 dimethylbenz(a)anthracene (Wattenberg, 1972). It was found when rats were fed with PAH (polycyclic aromatic hydrocarbon) carcinogens containing diet, most of them developed gastric neoplasia.

But when BHA or BHT was added to the diet, the incidence was significantly lesser. Comparative suppression of neoplasia was also obtained in experiments in which the carcinogen is acting at a site remote from that of administration i.e. inhibition of mammary tumor formation in rats given DMBA orally.

BHA and BHT are of primary interest because of their extensive use as food additives. Of these two compounds BHA is preferable, since it is less toxic than BHT. However, both of them can be employed at very high doses before the evidence of toxicity appears (Hathaway, 1966).

In studies where BHA or BHT were added to the diet along with BP (1 mg/gm of diet) it was found that at a concentration of the antioxidant 5 mg/gm of diet, inhibition of the carcinogenic effect on the forestomach of the mouse occurred. The human consumption of these phenolic antioxidants is of the order of magnitude of several milligrams a day. Assuming that the results of the animal experiments hold for man, this amount of antioxidants could be of importance in inhibiting the effects of chronic exposure to low doses of carcinogens, the type of exposure which is most likely to occur in human populations.

The mechanism by which the antioxidants inhibit neoplasia has not been established and may differ for various antioxidants. Several possibilities exist which can be divided into the major categories. The first involves some type of direct interaction between antioxidant and reactive species of carcinogen. The second possibility is that the antioxidant is acting in an indirect manner of primary interest in this regard is alteration of enzyme activity. A series of studies have been initiated to determine the mechanism of inhibition of B(a)P carcinogenesis by BHA. B(a)P is metabolized by the microsomal mixed function oxidase system which acts upon a wide variety of xenobiotic compounds including polycyclic aromatic hydrocarbons. Reactive metabolites as well as detoxification products are produced (Mannering, 1971). The phenolic metabolites of individual PAHs can be quantitated as a group. This category of reaction is designated as AHH (Aryl hydrocarbon hydroxylase) system. Some phenolic antioxidants induce increased activity of microsomal mixed function oxidase activity towards several of the substrates metabolized by the microsomal mixed function oxidase system.

According to Wattenberg (1975) consumption of a diet containing BHA results in an increased cytochrome P-450 in

the liver and an alteration in the microsomal mixed function oxidase system of that organ. Four parameters reflected this alteration. (1) the microsomal AHH system shows an increased sensitivity to inhibition by ANF (Alpha naphthoflavone) although the level of AHH activity is not altered; (2) the ethyl isocyanide difference spectrum shows significant change; (3) cytochrome P-450 reductase activity in the presence of ethylmorphine differs from that found in control mice; (4) microsomal incubation of BP in the presence of added DNA shows a decreased binding of BP metabolites to the DNA.

At the present time BHA probably is the most versatile inhibitor of chemical carcinogenesis that has been identified. In the present investigation, the modulatory action of BHA on the cervical carcinogenesis induced by 3-methylcholanthrene has been explored.

2. Retinoic Acid (Vitamin A):

Several in vivo and in vitro experiments have suggested that analogues of vitamin A (retinoids) may be useful as agents that can prevent or limit carcinogenesis. Sporn et al. (1976) call vitamin A and its derivatives as chemopreventive agents since they have been found to interfere with tumor induction by carcinogens.

The theory that a lowered level of vitamin A in tissues results in higher tumor incidence has been supported by the experiments of Nettestein and Williams (1976). Groups of rats were maintained on a diet designed to be low but not deficient in vitamin A. Their liver vitamin A was reduced by 50% and their growth was normal. Nonetheless following an intratracheal injection of the carcinogen methylcholanthene, the depleted rats developed precancerous lung nodules (shown to develop into invasive lung tumors later) to the extent of about four times the total nodular volume of control rats receiving a moderate amount of vitamin A in their diet (2.5 µg retinyl acetate per gram diet).

The work of Newborne and Rogers (1973) on increased colon cancer incidence induced by aflatoxin in vitamin A depleted rats points in the same direction; so do the epidemiological studies of Bjelke (1975) suggesting that among cigarette smokers low dietary vitamin A leads to higher lung cancer incidence.

Sporn and his team (1977) has shown that high amounts of retinoids administered during the later period of tumor development can drastically lower tumor incidence. Here the vitamin acts more like a pharmacodynamic than

like a nutritional substance. This idea originated with Saffioti and his group (1967) who gave an excess of vitamin A to hamsters after treatment with a lung carcinogen and found a greatly reduced number of tumors. The rationale for the treatment was that both vitamin A deficiency and epithelial carcinogenesis in its early stages result in the condition of squamous metaplasia. Hence one might expect to overcome the early effect of the carcinogen by an excess of vitamin A. Since excess of vitamin A is toxic, Sporn et al. (1976) developed a series of synthetic vitamin A analogues which had lower toxicity because they were rapidly metabolized and not stored in the liver.

Although a report has appeared indicating that vitamin A deficiency led to enhanced binding of benzopyrene to DNA to tracheal epithelium, present evidence favours the mechanism whereby the vitamin A counteracts the carcinogen after initiation stage. Thus Sporn et al. (1977) concluded with respect to bladder cancer induction that vitamin A probably reversed the effect of carcinogen at the preneoplastic rather than the initiation stage. Nettestein et al. (1976) lowered the incidence of induced preneoplastic lung nodules from 42 percent to 3 percent even if vitamin A was given as long as ten weeks

after the carcinogen. The most convincing evidence to demonstrate that vitamin A acts during the tumor promotion rather than initiation phase of carcinogenesis however was recently provided by Boutwell's group (Varma et al., 1979). They applied the promoter TPA (12-0 tetradecanoylphorphol 13-acetate) to the skin of mice and assayed ODC (Ornithine Decarboxylase) activity at various times in supernatant fractions of seperated epidermis homogenate. Maximum activity appeared four hours after the TPA applications. Since retinoic acid is an inhibitor of skin tumor induction (Bollaj, 1972) it would counteract the ODC induction ability of the promoter. They found a severe inhibitory effect by the retinoid on the induction when 0.5 µg retinoic acid was applied in a single dose to the skin of mice one hour before the promoter treatment. The response was dose dependant and became decreasingly effective if applied longer times prior to TPA.

They checked to see that under their conditions of treatment retinnic acid inhibited the incidence of skin tumors (papillomas); the initiator being DMBA applied to mouse skin in acetone. Ten days later TPA was administered twice weekly as promoter to the control group for 14 weeks. The experimental group received retinoic acid in acetone applied to the skin one hour before each TPA treatment. The retinoid inhibited skin papillomas by 55 percent

compared to controls at the same time as inhibiting epidermal ODC induction by the promoter. The degree of inhibition of ODC induction correlated with the inhibition of the skin tumors for a number of retinoids (retinoic acid, retinol, retinal, retinyl esters).

Even when given by mouth (150 mg/kg body weight by stomach tube, one hour before TPA application) instead of directly to the skin, the retinoids were active in approximately the same order of effectiveness when applied directly to the skin and with dose dependence.

It was also demonstrated (Varma et al., 1979) that in dimethylbenzanthracene-initiated mice, retinoic acid treatment of the skin one hour before each promoter treatment (twice weekly for 16 weeks) reduced skin tumor incidence per mouse by 75 percent. On the other hand, if retinoic acid was applied before, during or just after carcinogen initiation, followed by regular promoter treatment, no reduction in tumor incidence occured. This demonstrated that retinoic acid exerts its anti tumor activity during the promotion and not the initiation phase of tumor induction, most likely as a result of the inhibition of ODC induction. When the retinoid was applied 24 hours after TPA, when ODC induction was no

longer evident, skin tumor formation was not inhibited. Further, those synthetic retinoid which inhibited ODC induction also inhibited tumor formation. So according to the Boutwell's group the induction of ODC activity may be one of the essential biochemical events required for skin tumor promotion. Hence interference with this process by retinoids indicates retinoids act as anticarcinogens in the promotion phase. It should be noted that retinoids were also found to inhibit the process of neoplastic transformation of normal cells by radiation (Harisiadis, 1978). Also studies have demonstrated that retinoids can reverse hyperplastic and squamous metaplastic changes that are induced in mouse prostate tissues by hormones, (Chopra and Wilkoff, 1979). Recent studies have shown that retinoids may enhance the specific immune response against tumor cells (Lotan and Dennert, 1979).

However, several experimental models failed to confirm the antitumor properties of retinoids. Some studies have demonstrated cell activating properties of retinoids or enhanced tumorigenic responses to chemicals or UV irradiation by high doses of topically applied retinoids. Specifically 10% retinyl palmitate applied to the hamster cheek pouch enhanced the tumorigenic

activity of DMBA (Levij and Poliack, 1968; Leviz et al., 1969). Also mouse skin treated with 0.3% retinoic acid showed an increased tumorigenic response to ultraviolet rays (Epstein, 1977).

Virtually there is no report on the influence of retinoids on cervical cancers except a single one by Elizabeth (1965). She painted the uterine cervix of syrian hamsters with DMBA in acetone and induced perineal skin and vaginal carcinoma in 22% of the animals. Addition of vitamin A palmitate to the fluid used for painting the cervix prevented the development of the carcinoma of cervix and vagina but did not inhibit the development of the carcinoma of the perineal skin.

3. MPG (2-mercaptopropionylglycine):

Due to the progress in the enzyme chemistry in recent years the requirement of -SH (sulfhydryl) group in many systems has become known. The presence of the -SH group in co-enzymes is especially important. The inactivated enzyme restores the activity by -SH group containing compounds like glutathione. It is presumed that such compounds may play an important role in the detoxifying mechanism and treatment of liver diseases (Shimoyama et al., 1965). MPG, also known

as Thiola, is a new synthetic sulfhydryl compound and is applied to the clinical treatment of hemosiderosis and other hepatic disorders like chronic hepatitis, cirrhoris and toxic effects of antineoplastic agents. It has prophylactic and therapeutic effect on leukopenia as well as antidotal and hepatotonic effect. Protective effects of MPG against radiation hazards are well studied (Koyama et al., 1965; Nagata et al., 1972; Kawasaki, 1977, 1978). A review of the protective action against ionizing radiation has recently been done by Sugahara and Srivastava (1976).

The high redox potential of MPG may be related to its hepatotrophic detoxicating property. It has got a verylow toxicity and also effective at very low dose indicating that MPG is not only useful for practical application but also for elucidating the mechanism of chemical radiation protection (Sugahara and Srivastava, 1976).

The sulfhydryl groups are widely distributed in animals, plants and microorganisms participating in various enzymatic reactions, peptide hormone activities and other biological phenomenon. They also function as antidotes against heavy metal ions by forming stable co-ordination compounds. MPG is one of these compounds and has been reported to enhance the excretion of toxic compounds of mercury, copper and iron (Funae et al. 1971).

In recent years a number of clinical studies on the influence of MPG have been reported. Kosaka and his coworkers (unpublished work) observed a decrease in serum iron concentration after MPG administration in patients suffering from hepatic cirrhosis. However, the effect of MPG on anaplastic anaemic patients and in patients of chronic hepatitis was not confirmed. Montero et al. (1972) also reported a significant improvement in patients suffering from cirrhosis which tends to worsen when treated with radiation therapy. A protective action of MPG against toxic effects of anti-neoplastic agents was noticed by Cacciari and his associates (1972). It has also been observed to have inhibitory effects on cellular immunity (Monna et al., 1972) in guinea pigs where MPG was noticed to reduce the number of lymphocytes and RNA synthesis. These authors also reported that MPG administration to the patients having acute hepatitis, chronic hepatitis and liver cirrhosis resulted in an improvement in the liver function of about 50 percent of chronic hepatitic patients.

There are many recent evidences for the detoxifying effects of MPG. It afforded significant protection against the hepatotoxicity induced by paracetamol (Labadarios et al., 1977) and Ethionine (Chiba et al. 1979). Labadarios (1977) and his co-workers observed a significant

protection against hepatic necrosis, it has little effect on covalent binding of paracetamol with molecules of biological importance. Furthermore, MPG failed to prevent the fall in hepatic glutathione concentration in animals treated with paracetamol. They suggested that MPG acts by preventing deleterious effects on hepatocellular function caused by the binding of the reactive paracetamol metabolite rather than by modifying its production or elimination. Such a mode of action could allow hepatocytes to continue glutathione and protein synthesis at normal rates than those damaged by the reactive paracetamol metabolites.

MPG suppressed increase in serum transaminase, accumulation of liver triglycerides and decrease of glucose-6-phosphatase induced by carbon tetrachloride (CCl_4). It also suppressed the decrease in non-protein thiol induced by CCl_4. In addition to these biochemical findings Horiuchi and his coworkers (1979) also noted that MPG prevented necrosis and decrease of glycogen in liver. However, no such detoxifying action of MPG on carcinogen is demonstrated.

A reduction in transaminase was also induced by MPG in patients suffering from chronic liver disease (Dioguardi et al., 1972). Kaito and his associates (1970)

observed that fatigue, lessitude, anorexia, nausea, abdominal fullness and pruritus decreased after MPG administration in patients suffering from chronic liver diseases. Balsano *et al.* (1972) noticed a significant increase in the activities of glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase and ATP, thereby increasing glycogen synthesis.

However, there is no report available on the influence of MPG on chemical carcinogenesis.

MATERIALS AND METHODS

Randombred Swiss albino mice from Moulana Azad Medical College, New Delhi were used for all the experiments. The animals were maintained in an air-conditioned room and were fed with rat food in pellet form (Hindustan Lever Ltd., India) and tap water ad libitum.

The carcinogen 3-methylcholanthrene was bought from Sigma Chemical Company, U.S.A. and the pure bees-wax from Mysore. The wax and carcinogen thread preparations and their intracervical insertions were done as explained in the I Chapter.

Experiment No. 1: (See Table VII)

The first experiment was set upto find out the influence of BHA on cervical carcinogenesis. BHA in powder form was bought from Sigma Chemical Company, U.S.A. It was mixed with the powdered rat feed (Hindustan Lever Ltd., India) in the ratio of 5 mg/gm of diet and the mixture was stirred for about 30 minutes.

The mixture was prepared freshly every week. All the experimental animals were given either the mixture of BHA or the powdered form of diet. A total number of 71, seven to eight weeks old mice were divided into four

groups. Nineteen animals were inserted with carcinogen threads and were started feeding with BHA mixed diet from the same day. Another group of 15 animals were put on BHA mixed diet to serve as controls. Out of the 15, five of them were inserted with beeswax threads intracervically and the rest ten were left without thread insertions. Further 37 mice were ovariectomized when they were of 6 weeks age. The procedure of ovariectomy was same as that described by Zarrow et al. (1964). After two weeks all the ovariectomized animals were inserted with carcinogen threads intracervically. Eighteen of them were put on powdered normal diet and the rest 19 on BHA added diet mixture.

Experiment No. 2: (See Table ix)

Groups were maintained to find out the influence of retinoic acid (All trans-β-retinoic acid) on the cervical carcinogenesis. Seven to eight weeks old mice were utilised for the study. The retinoic acid was suspended in olive oil (4 mg and 8 mg in 0.05 ml) and injected to mice intraperitoneally. Two doses of administration were followed i.e. 200 mg/kg body weight and 400 mg/kg body weight weekly. Out of the 57 mice, 21 were injected with the first dose of 200 mg/kg. A second dose of 400 mg/kg was injected to another group of 20 animals. Both the group animals were inserted

with MCA carcinogen threads intracervically before the retinoic acid administration. Sixteen animals were given 400 mg/kg of retinoic acid intraperitoneally to serve as controls. Six mice out of these sixteen were inserted with beeswax threads to find out difference in the control results if any.

Experiment No. 3: (See Table X)

The influence of MPG of the cervical carcinogenesis was aimed at in the third experiment. MPG was a gift from Shantan Pharmaceutical Co. Japan.

MPG was dissolved in distilled water in the ratio of 0.5 mg in 0.1 ml. The pH was maintained at 6.4. Intraperitoneally 35 animals of seven to eight weeks of age were given a dose of 25 mg/kg of body weight twice weekly through out the experiment. Out of them 20 were already inserted with the carcinogen threads making the carcinogen plus MPG group. Fifteen MPG injected animals sefved as controls. Five control animals were inserted with beeswax threads to reveal the difference of results if any.

Experiment No. 1 :

There was no mortality among the experimental animals before the autopsy period. Carcinogen threaded animals fed with BHA in their diet showed a decreased tumor incidence of 31.58%. The incidence has lowered to a level of one third when compared to the 90% incidence of the first group treated with carcinogen alone. However, it should be noted that seven out of 19 animals showed marked dysplastic conditions in the carcinogen plus BHA treated group. The neoplastic features of the squamous epithelial cells were prominent. Hyperchromasia, atreisia and pleomorphism were commonly noted. Five preparations had moderate neoplastic conditions and one with mild dysplasia. The ovariectomized and carcinogen threaded animals (the third group) showed a maximum tumor incidence among all the groups i.e. 94.44%. A total number of seventeen animals were bearing tumorous cervices out of the treated 18. Five of them showed advanced carcinomatous changes, the neoplastic cells spreading to almost all the regions of the cervix. Eight preparations had early squamous cell carcinomas with the stromal infiltration of neoplastic cells at few places. Though the incidence was maximal it should be noted that only five animals were in advanced carcinomatous conditions whereas the number was ten in the carcinogen-treated control

group. Further in the third group three preparations showed adenocarcinomas and one with sarcoma. The fourth group of animals involved BHA feeding along with the carcinogen thread insertion and ovariectomy. A total number of 13 out of 19 preparations showed tumors amounting an incidence of 68.42%. It is more than double the amount when compared to the second group treated with carcinogen and BHA but without ovariectomy. Out of the 13 tumorous preparations in the fourth group five were with early carcinomas, seven with advanced carcinomas and one with adenocarcinoma. Four animals had marked dysplasia in the same group. The animals of the control groups (i.e. BHA only and BHA + wax) did not show any tumorous changes. But because of the irritation of wax threads possibly, two animals showed mild dysplasia and one with moderate dysplasia in the wax threaded BHA group.

Experiment No. 2 :

The second experiment was designed to find out the influence of retinoic acid on the cervical carcinogenesis. Two doses i.e. 200 mg/kg and 400 mg/kg of body weight were tried. The second group of animals fed with the minimal dose of RA (retinoic acid) with the carcinogen thread showed 42.85% of tumor incidence. This incidence is 50% lesser when compared to the 90% incidence in the

group without RA. Out of 21 treated animals in the second group 9 were bearing tumors. Five tumors were early carcinomas and three were advanced carcinomas. All of them were non-keratinizing types. One preparation had severe adenocarcinomatous lesion. More preparations showed dysplastic changes in the second group. Six had marked dysplasia with the typical atypia, all over the epithelial region with occasional buds towards the stromal region . Four preparations had moderate dysplasia and two were with mild dysplasia.

All the six tumor bearing animals had squamous cell carcinomas. Three of them were in the early stage and the rest three in the advanced carcinomatous conditions. Five preparations showed marked dysplastic changes, with two among them showing severe infections. Four animals had moderate dysplasia while another group of five had mild dysplasia.

A total number of 16 mice were fed with the maximal dose of RA 400 mg/kg of body weight. Out of them six were already threaded with beeswax threads. Out of the ten animals fed with RA only without any thread, nine had normal epithelia and only one showed mild dysplastic changes. The six RA-fed and wax threaded control animals did not show either tumor or marked dysplasia. However,

three preparations showed moderate dysplastic conditions with one having severe infections. Two were showing normal stratified squamous epithelia and one with mild dysplastic changes.

Experiment No. 3 :

MPG, the hepatotrophic detoxifier did not show any significant effect on the cervical carcinogenesis. However, there was 5% decrease in the tumor incidence in MPG treated group. Out of the 20 carcinogen threaded and MPG-treated animals, 17 were bearing tumors. Nine early squamous cell carcinomas with minimal invasion of neoplastic cells and five advanced differentiated squamous carcinomas were noted. Apart from these carcinomatous changes, two preparations showed adenosarcomas and one with sarcomatous lesions. Marked dysplastic changes were seen in two animals and moderate dysplasia was showed by one other animal in the same group. No animal had either mild dysplastic or normal epithelium.

Fifteen animals serving as controls received MPG administration alone. Five of them had been inserted with beeswax threads to see the difference if any among the controls. Eight among the ten MPG-treated animals had normal epithelium without any significant pathological

changes. The rest two had infectious cervices with mild dysplastic changes. Among the wax threaded and MPG treated animals two had moderate dysplastic changes. One preparation had mild dysplasia and the rest without any pathological changes in the squamous epithelium.

TABLE VIII

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES : EXPERIMENT NO. 1.

Groups	Mice at risk	Normal	Dysplasia			No.of tumor bearing mice			Total. No. of tumor bearing mice	%age of tumor incidence
			+	++	+++	Carcinoma Early	Carcinoma Advanced	6ther types (ACM & SCM)		
I.Carcinogen only	20	0	0	0	2*	5	10***	3	18/20	90%
II.Carcinogen + BHA	19	0	1	5*	7***	4	2	0	6/19[a]	31.58%
III. Carcinogen + Ovariectomy	18	0	0	0	1	8	5*	4	17/18[b]	94.44%
IV.Carcinogen + BHA + Ovariectomy	19	0	1	1	4	5*	7	1	13/19[c]	68.42%
V.BHA only	10	8**	2	0	0	0	0	0	0/10	0%
VI.BHA + Wax	5	2	2	1	0	0	0	0	0/5	0%

Note: The number of '*' denotes the number of preparations which showed infection.

SCM = Sarcoma; ACM = Adenocarcinoma; + = Mild; ++ = Moderate; +++ = Marked.

a = Significantly different from 'carcinogen only' group ($p < 0.01$)
b = Non significant different from carcinogen only group ($p<0.01$);
c = Non significant different from 'carcinogen only' and 'carcinogen + Ovariectomy' groups ($p < 0.05$).

TABLE IX

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES : EXPERIMENT NO. 2

Groups	Mice at risk	Normal	Dysplasia			No. of tumor bearing mice			Total No. of tumor bearing mice	%age of tumor incidence
			+	++	+++	Carcinoma Early	Carcinoma Advanced	Other types (ACM & SEM)		
I. Carcinogen only	20	0	0	0	2*	5	10***	3	18/20	90%
II. Carcinogen +Retinoic acid I	21	0	2	4	6	5	3	1	9/21@	42.85%
III. Carcinogen +Retinoic Acid II	20	0	5	4	5**	3	3	0	6/20¢	30%
IV. Retinoic acid II only	10	9	1	0	0	0	0	0	0/10	0%
V. Retinoic Acid+Wax	6	2	1	3*	0	0	0	0	0/6	0%

Note: The number of '*' denotes the number of preparation which showed infection.

ACM= Adenocarcinoma; SCM = Sarcoma;. + = Mild; ++ = Moderate; +++ = Marked.

@ = Significantly different from 'carcinogen only' group ($p<0.05$).
¢ = significantly different from 'carcinogen only' group ($p<0.01$).

TABLE X

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES : EXPERIMENT NO. 3

Groups	Mice at risk	Normal	Dysplasia			No.of tumor bearing mice			Total No. of tumor bearing mice	%age of tumor incidence
			+	++	+++	Carcinoma Early	Carcinoma Advanced	Other types (ACM & SCM)		
I.Carcinogen only	20	0	0	0	2*	5	10***	3	18/20	90%
II.Carcinogen + MPG	20	0	0	1	2	9	5	3	17/20@	85%
III.MPG only	10	8	2**	0	0	0	0	0	0/10	0%
IV.MPG + Wax	5	2	1	2	0	0	0	0	0/10	0%

Note: The number of '*' denotes the number of preaparations which showed infection.

ACM = Adenocarcinoma, SEM = Sarcoma; + = Mild; ++ = Moderate; +++ = Marked.

@ = Non/Significantly different from carcinogen only group ($p < 0.05$).

The potential mechanisms of modifications of chemical carcinogenesis are (i) the reversal of early carcinogenic processes; (ii) alteration in the metabolism of carcinogens by decreased activation and increased detoxification; (iii) scavenging the active molecular species of carcinogens to prevent their reaching critical target sites in the cell and (iv) competitive inhibition. BHA was tried as a possible chemical modifier based on the concept that the antioxidant may exert a scavenging effect on the reactive species of carcinogens thus protecting the constituents from attack. In the present set of experiments, BHA is found to inhibit the cervical tumor incidence to a level of about 60%. There are many earlier reports of such inhibitory effects of BHA on other cancer incidences, particularly with situations where the route of administration resulted in direct contact of carcinogen with the target tissues i.e. forestomach, lung, skin, breast etc (Wattenberg, 1972). Some studies of the mechanism of inhibition of chemical carcinogenesis by BHA have been performed. Most of them involve the carcinogens like B(a)P or DMBA and there is no report with MCA.

Generally polycyclic aromatic hydrocarbons are metabolized by the microsomal mixed function oxidase system which acts on a wide variety of xenobiotic compounds. Reactive metabolites as well as detoxification products are produced. The effects of administration of BHA on microsomal metabolism of BP in female A/HeJ mice has been studied with experimental conditions similar to those in which BHA inhibits neoplasia due to this carcinogen. Incubation of B(a)P and DNA with liver microsomes from the BHA-fed mice results in approximately half the binding of BP metabolites to DNA as compared to that found with microsomes from control mice (Spier and Wattenberg, 1975). Investigations were made by Wattenberg's group (Luk and Lam et al. 1977) to determine if the BP metabolites that were employed when the carcinogen was incubated with liver microsomes prepared from BHA fed mice, differ from those formed in controls. It was found that the liver microsomes isolated from mice within four hours after administration of BHA showed a depression of BP metabolism by more than 16%. A profound decrease in the concentration of metabolites in the polar region of the chromatogram was noted indicating a reduction in the formation of diolepoxides by BHA. Also, it was found that the formation of B(a)P 4,5-oxide was reduced with microsomes from BHA-fed mice (Wattenberg, 1977). The major metabolite in microsomal

incubations from BHA fed and control mice was 3-HDBP. This metabolite constituted a significantly higher percentage of the total metabolites formed when B(a)P was incubated with microsomes from BHA fed mice as compared to the percentage in the control.

Thus BHA alters microsomal metabolism by diminishing activation reactions leading to the formation of ultimate carcinogenic metabolites and also enhances formation of metabolites of detoxification.

The increase of carcinogen induced tumor incidence in the ovariectomized and BHA treated group is very difficult to explain. The absence of ovarian hormones may have affected the BHA's inhibitory action. But there is no report of ovarian hormone's influence on BHA's activity. Otherwise the ovariectomy itself might have an accelerating influence on the process of carcinogenesis. In support of this the carcinogen plus ovariectomy group shows a maximal incidence of 94.44%. There are some experimental reports on the influence of castration on the development of tumors. Koudstall et al. (1966) found ovariectomy to be a promoting factor in the cervical carcinogenesis by MCA Ppaintings. Krieg and Reagan (1961) observed that cervical carcinogenesis proceeded more rapidly after castration, and the latency period of the

development of cervical carcinoma was shortened. In addition to these reports Meisels (1964) reported that the average latency period of cervical cancer development was markedly lengthened in animals receiving estrogen systemically. So it is possible that ovariectomy and the absence of ovarian hormones is the main cause for the increase of tumor incidence in the third and fourth groups of the present investigation. It is possible to speculate that ovarian hormones exhibit an inhibiting effect on the cervical carcinogenesis.

Retinoic acid was the second chemical modifier tested in the present investigation. Only 42.85% of tumor incidence was noted in the second group administered with a retinoic acid dose of 200 µg/kg body weight. The incidence further reduced to 30% when the dose was increased to 400 mg/kg in the third group. Thus retinoic acid treatment gave significant inhibitory effect on the cervical carcinogenesis. Many such reports of inhibitory effect of retinoids on carcinogenesis are available (Varma et al., 1979; Harisiadis, 1978). The rationale behind the retinoids in cancer prevention is as follows. It has been pointed out that epithelia innately possess protective cellular mechanisms to repair or diminish the damage caused by chemical carcinogens (Cairns, 1975).

The biochemical and cellular nature of these protective mechanisms is not well understood. The intrinsic control of the form and territory of epithelial cells is thus one of body's primary mechanisms of defence against the development of epithelial cancer. Extrinsic enhancement of this defence mechanism by pharmacological agents represents a practical strategy for the prevention of cancer in man. Here retinoids are capable of having an important role.

It was found that in the absence of retinoids in the diet the normal mature differentiated cells in many epithelia are not formed. Instead they are replaced by keratinizing squamous cells which undermine the territory normally occupied by the specialized differentiated cells. Administration of retinoids to these keratinized metaplastic epithelia reverses the abnormal differentiation and causes reappearance of the normal mature cells; the retinoids thus act in a manner similar to a differentiation hormone controlling the types of cells which occupy specific epithelial territories.

There are also reports of retinoids controlling cell differentiation caused by chemical carcinogens in target epithelia. Exposure of prostatic or tracheal organ cultures to carcinogenic polycyclic aromatic hydrocarbons such as methylcholanthrene or benzopyrene causes cellular

lesions that are characterized by the appearance of hyperplastic or atypical cells. If retinoids are applied to these lesions after they are formed the abnormal cells tend to disappear and are replaced by relatively normal epithelial cells (Lane and Miller, 1976).

Many investigations point out that retinoids control the DNA synthetic activity. Many years ago Wolbach and Howe (1925) noted that there was an increase in mitotic activity in certain epithelia of vitamin A deficient animals and they suggested that the dedifferentiated metaplastic epithelial lesions of vitamin A deficiency had features in common with those of neoplastic lesions. It is presently known that there is very little DNA synthesis under some normal conditions in some epithelia such as those of trachea or bladder that depend on retinoids for maintenance of normal differentiation. Under normal conditions the basal cells of these epithelia are minimally labelled by thymidine, cell cycle times have been estimated to be as long as two weeks for tracheal epithelium and up to six weeks for bladder epithelium. However, these epithelia are not deficient in their capacity to synthesize DNA and undergo cell division as they are capable to rapid regeneration and repair after necrotizing damage by chemical agents (Clayson, 1970). After carcinogen administration enhanced DNA synthesis

occurs in these epithelia (Clayson, 1970; Schreiber et al., 1975). And quantitative studies have shown an immence increase in DNA synthetic activity and mitotic index in tracheal epithelium during vitamin A deficiency (Harris et al., 1973). It would thus appear that one physiological role of retinoids in an epithelium such as that of trachea is to suppress DNA synthesis and basal cell replication as well as to maintain proper differentiation. It is not presently known whether retinoids have any role in controlling DNA synthetic activity in cervical epithelia but the suppression of cervical tumor incidence in the present investigation may be because of the same mechanism. Also the recent finding of specific cellular receptor protein that binds both natural and synthetic retinoids aids further evidence to suggest that the effects of retinoids on the suppression of preneoplasia as the result of enhancement of intrinsic physiological mechanism (Ong and Chytil, 1975; Sani and Hill, 1974; Sani and Hill, 1976).

MPG was the third chemical modifier tested in the present investigation. There is no published report on the interference of MPG with chemical carcinogenesis. However, it is well known that MPG is a good radioprotector and a hepatotrophic detoxifier (Sugahara and Srivastava, 1976;

Kawasaki, 1977;1978). MPG is also reported to enhance the excretion of toxic compounds of mercury, copper and iron. A protective action of MPG against the toxic effects of antineoplastic agents was also noticed by Cacciari and his associates (Cacciari et al., 1972). The present investigation was to find out the influence of MPG on the cervical carcinogenesis. No significant change was noticed by its administration with the carcinogen. Out of the 20 carcinogen-threaded plus MPG-treated animals, seventeen were bearing tumors. This tumor incidence is 5% lesser when compared with that of the group with carcinogen threads only. However, the decrease in tumor incidence by the treatment of MPG is not statistically significant. So MPG did not have any significant effect on the cervical carcinogenesis by the carcinogen 3-methylcholanthrene. However, the recent unpublished results in our laboratory (Perra, 1980) show that MPG offers protection against the DMBA-induced biochemical changes at the initiational phase of the liver carcinogenesis. The DMBA induced changes in the levels of glycolytic enzymes like hexokinase, glucokinase, pyruvatekinase, phosphofructokinase and lactatedehydrogenase were either totally nullified or at least reduced. MPG was also found to have offered protection against the DMBA induced changes in the DNA content levels.

However, the above said protection of MPG against the DMBA induced changes in the liver is only at the initiational phase of the carcinogenesis. Its relationship with the tumor incidence at later stages is not reported with experimental evidences.

So MPG might have altered some of the biochemical changes indduced by the carcinogen. But these changes could not have built up any prominent antineoplastic effect at the later stage of tumor appearance.

CHAPTER - IV

INFLUENCE OF HORMONAL MODIFIERS ON THE CERVICAL CANCEROGENESIS

INTRODUCTION AND REVIEW OF LITERATURE

In mammals, hormones play a vital role in development, differentiation, homeostasis, survival and reproduction. Both steroidal as well as peptidal hormones play very important role in mammalian reproduction by involving in the structural, functional and behavioural aspects. The hormone-cell interactions may be important for maintenance of cells' wellbeing or conversely they may result in an altered state, making the cell more susceptible to neoplastic transformation by other agents.

Berenblum (1974) treats hormones primarily as "modifying or permissive influences" for they may act at the level of the action of the carcinogen on the host. A further distinction is needed as hormones may alter the target prior to neoplastic transformation or may enhance (or inhibit) the growth of the neoplasm after transformation. Examples of these effects are readily realized in the role of estrogens in the induction and growth of mammary tumors induced by the administration of the polycyclic aromatic hydrocarbons. Since many hormones exert trophic effects on target tissues their presence may be essential to respond to neoplastic transformation. Such a case may be exemplified by the difference in

incidence of breast cancer in men and women the former being about 1% of the latter (Silverberg, 1977).

Also the high incidence of cancer in accessory sex organs which are markedly influenced by endogenous and exogenous hormones gave way for the extensive investigation of relationships of hormones to the initiation and growth of cancer of the breast, Uterus, prostate, ovaries etc. In the last three decades an explosion of information on endocrine-cancer relationships took place and it was established that the hormones which influence growth occupy a place near the center of the cancer problem.

According to Huggins (1956) an effort to control cancer by hormonal means can be definitely made and this rests on two principles (a) Cancer is not necessarily autonomous. Cancer cells do not differ from normal cells as black differs from white. When the cells of origin of a cancer are dependant on hormones, for metabolic activity at a high rate, the cancers derived thereform can be similarly dependant and both cancer and normal cells undergo atrophy when hormonal support is withdrawn and this can be accomplished by a number of means. Such tumors are by definition called hormone-dependent tumors; (b) The second principle is that cancer can be sustained by hormonal function that is not exaggerated in

rate or abnormal in kind about which is operating at normal or even subnormal levels. It is now appreciated that trace amounts of hormones can lead to such exuberant neoplastic growth that cause death of the host.

Regarding the interaction of hormones with the processes of carcinogenesis the conclusions by Clifton (1961) may be mentioned here. According to him "(a) specific hormones at normal levels may be necessary for the development of carcinomas secondary to an oncogenic virus, chemical carcinogen, or radiation and once established some of these tumors may continue to require normal hormone levels for growth or successful transplantation; (b) specific hormones in elevated levels, may increase the susceptibility to an oncogenic virus, may reduce the threshold carcinogenic dose of chemicals or radiation, may increase the tumor frequency and may reduce tumor latency".

Recently endocrine cancer relationship have been reviewed (Jull, 1977; Noble, 1976; Noble, 1977; Clifton, 1975; Hilf, 1979). Mostly experimental studies on the hormonal modulation of chemical carcinogenesis have been done with mammary gland, ovary, prostate gland, liver etc. Though voluminous clinical data suggest the importance of hormones in cervical cancer, experimental studies are comparatively scarce.

Clemmeson (1951) emphasized the importance of the difference in the age distribution between breast and cervical cancers. The incidence of breast cancer always rise with age whereas cervical cancer decreases after menopause. So he suggested a possible implication of hormonal imbalance in the development of both tumors. Another feature of importance concerns the marital status of the host. Cervical cancer was rare among virgins and the reverse was true with breast cancer. The recent observation about the association between early coitus and increased risk for cervical cancer (Rotkins, 1973), as seen from the theoritical point of view should be placed in the same perspective with the rarity of cervical cancer among nuns (Gangnon, 1950; Taylor et al., 1959). These observations have been discussed in terms of viral infections. However, the distribution of antibody to the candidate Herpes-type 2 virus was not necessarily in accord with the occurrence of cervical cancer (Rawls et al., 1970; Pridam, 1971; Sabin, 1974). The results obtained were far from being proof for casuality (Wilbanks, 1973). Indeed Lombard and Potter (1950) suggested that the significance of early marriage and cervical cancer could be investigated in terms of excessive hormone manipulation and lack of

development. Wilbanks (1973) stated that active squamous metaplasia during adolescence may be critical in the genesis of cervical cancer. Several epidemiological observations about the marital instability of a patient could also be related to an endocrinologic disorder (Stern et al., 1967). Cowdrey (1968) suggested that the high incidence of cervical cancer in Bantu women is an end result of depressed estrogen detoxifying function of liver. The Bantu women with a characteristically high incidence of hepatic cirrhosis and primary hepatic cancer might have been exposed to excessive concentration of estrogen.

Mitsuo Kodama et al. (1978) found that cervical cancer patients were associated with deficient excretions of those steroids that were destined to increase during adolescence and slowly decline thereafter in a normal woman. This discrimination represented a mixture of major androgen and minor corticosteroid derivates of adrenal gland origin. But the question is that the observed charges in the urinary excretion of a patient only represent findings of nonspecific illness as in breast cancer, hepatitis, or in hepatic cirrhosis. But the situation is different in cervical cancer than the latter said diseases, that the steroid deviation in patients with cervical cancer was not affected by the

progress of the disease as in the latter examples. Also, Mitsuokodama et al. (1978) speculated that the deficiency of steroid excretions specific to cervical cancer patients may represent the arrested maturation of adrenal gland which in turn may promote hyper-estrogenic production at the expense of another factor namely early coitus.

Thus the importance of hormones in the development of cervical cancer was well established clinically. The present investigation deals with the influence of estrogen progesterone, prolactin and testosterone on the experimental cervical carcinogenesis.

Estrogen and Progesterone :

The ovarian hormones estrogen and progesterone exert some influence on a great many tissues of vertebrate organisms. However, a principle action usually taken as a measure of biologic acitivity is their remarkable trophic effect in tissues of the reproductive tract which require continuous/cycle exposure to the hormones for optimal growth and function. Perhaps the most general effect of estrogens is to promote tissue growth. This is most pronounced in the accessory sex tissues, but it occurs in other tissues as well. By stimulating cell divisions in the deeper layers of the

skin, estrogens cause a more rapid replacement of the outer cornified layers. There is some evidence that the estrogens may be potentially dangerous in as such as they encourage the formation of cancer in certain individuals. This may correlate with the concept that continued high rate of cell division is one factor predisposing a tissue to become cancerous. Apart from the influence on growth and development the ovarian secretions are also required for the transformation of the target tissues like breast and uterus from normal to neoplastic by other etiologic agents such as ionizing radiations, viruses, and chemical carcinogens. Estrogens have historically been implicated as a primary agent in breast cancer. And at present, removing, administering or antagonizing estrogens provide an approach for inducing objective responses in patients with breast cancer.

There are also reports of antineoplastic properties of estrogens. Haddow et al. (1944) reported on the efficiency of treating breast cancer with synthetic estrogens. Recently Manoharan and Rao (1980) reported that exogenous administration of 17β estradiol inhibited the oocytic depletion induced by 7,12 dimethylbenz(a) anthracene in mice. Estrogens have also been reported to increase the incidence, decrease the incidence or have no effect on the incidence of mammary tumors in rats induced

by polycyclic hydrocarbons (Geyer et al., 1953; Scholler and Caraes, 1958; Shay et al., 1956).

Progesterone and progestational compounds in contrast to the estrogens have received little attention in relation to tumor growth. Further, studies on the effect of progesterone on tumor growth have produced contradictory results. Heiman (1943) reported that progesterone inhibited the growth of mammary fibroadenoma in intact female rats whereas Miller and Noble (1954) did not obtain any effect when a smaller dose of progesterone was used. Progesterone increased the incidence of mammary tumors in animals fed a diet containing 2-acetylaminofluorine (Cantarow et al., 1950) or methylcholanthrene (Huggins et al., 1959).

Estrogens and progesterones act independently or in combination with other hormones to produce the great variety of effects. It is well established that estrogens can inhibit progesterone-induced endometrial proliferation (Courrier, 1950).

As regards the influence of ovarian hormones in the genesis of cervical cancer there are many experimental reports. Allen and Gardner (1941) succeeded in inducing cervical cancer in mice by means of estrogen. An adeno-

carcinoma of the uterus appeared in a rat experiencing constant estrous which suggested the carcinogenic role of endogenously produced estrogen (Pfeiffer, 1949). Uterine and extra-uterine leiomyomas have been induced in the guinea pig and hamster by administration of estrogen (Lipschutz and Iglerias, 1938).

Forseberg (1968) found that neonatal estradiol treatment resulted in a strong suppression of the mitotic rate in uterine cervix and vaginal fornix. Also because of the treatment both the regions developed atypical columnar epithelium instead of the normal stratified epithelium. In his later studies of carcinogenesis with 3-methylcholanthrene, Forseberg reported that the persistent production of ovarian estrogen in the neonatally estradiol treated animals retarded uterine cervical cancer growth (Forsberg, 1972).

According to Klavins (1962) weekly injections of 50 µg of estradiol benzoate subcutaneously did not affect the incidence of methylcholanthrene induced carcinoma of the cervix in mice. But the tumors were more highly differentiated and the incidence of dysplasia decreased by the treatment of estradiol with MCA.

Meisels (1964) investigated the effect of gonadal hormones on mouse cervix simultaneously treated with 9,10-dimethyl- 1,2-benzantharacene which was implanted after impregnation in the thread. He found that the average latent period was markedly lengthened in animals receiving estrogen systemically.

Kaminetzky (1966) observed that estrogen augmented the stromal penetration of dysplastic cells by the treatment of 3-MCA. Progesterone appeared to give protection against this action of estrogen.

Koprowska (1964) showed that stilbestrol and estradiol valerate probably promote the development of chemically provoked cervico-vaginal carcinomas in intact mice.

Murphy (1961) found that castration after limited exposure of the endocervical epithelium of strain A virgin females to MCA markedly decreased the incidence of invasive cervical carcinoma (26.7% in intact animals, 2% in castrates). So he postulated that normal levels of estrogen significantly promote chemically induced cervical carcinogenesis in the mouse.

Contrary to the Murphy's report, Laffargue et al.(1963) could not prove the promoting effect of estrogens. They

concluded that estrogens inhibit theinfiltrating capacity of the tumors. Supporting this, Kodstall et al. (1966) reported that ovariectomy appeared to be a tumor promoting factor, in the uterine cervix of mouse. It is notworthy that they characterized the carcinogen-induced lesions by enzyme histochemical methods. It was found that generally in cervical tumors the activity of 5-nucleotidase, aminopeptidase, reduced nicotinamide adenine dinucleotide (NADH) and NADPH tetrazolium reductase, lactic acid dehydrogenase and glucose-6-phosphate dehydrogenase increased while the activity of alkaline phosphatase, β-hydroxy butyric acid dehydrogenase and the nonspecific esterases decreased.

Glucksmann and Cherry (1962) reported that ovariectomy significantly increased the incidence of muco-epidermoid carcinomas of the cervix. Treatment of castrate mice with progesterone or with lutocycline increased the extent of the adenosquamous carcinomatous component of these tumors while stilbestrol treatment resulted in the induction of squamous cell carcinomas only. The percentage of papillomas of all epithelial tumors in the cervix of intact mice was only seven percent but rose to 31 percent in castrates with or without additional hormonal treatment.

There are many reports of possible association between the use of steroidal contraceptives and cervical cancer incidence. Stern et al. (1977) reported that in a prospective study of women with dysplasia of the cervix., there was an increase in the severity of dysplasia and of conversion to cancer in situ in users of the contraceptive pill compared with the users of other contraceptive methods. Similar results have been mentioned in many reports (Peritz et al., 1977; Melamed et al., 1969; Stern, 1973). However, contrary to this Ory et al. (1976) could not find any significant relation between the use of contraceptive pill and cervical cancer. Many recent epidemiological studies also report similar findings (Shulman, 1973; Berger, 1974; Fuertes-de-la Hoba et al., 1973).

So the controversy of the influence of estrogen and progesterone on the development of cervical carcinoma still remains. In the present investigation the influence of exogenously administered estrogen and progesterone on the cervical cancer indidence in mice has been studied.

Prolactin :

Of the numerous activities of prolactin in vertebrates its ability to stimulate mammary gland growth and development is most pertinent. So the literature

dealing with prolactin in cancer involves mainly breast cancer. The initial report of Loeb and Moskopkirtz (1939) using pituitary isografts confirmed that the enhanced secretion of prolactin from these transplanted hypophyses increased the occurrence of mammary cancer (Brot, 1970). Similar results were obtained after prolonged estrogen treatment which stimulates prolactin secretion (Meites, 1972). It has been proposed that estrogens interfere with the action of prolactin at the level of the neoplastic cell although how this might occur is not clear (Mietes, 1972). Asparagus racemosus which is known to have mammotrophic and lactogenic effect (by way of eliciting prolactin secretion) has been shown to inhibit mammary carcinogenesis in rats exposed to DMBA (Rao, 1981). Also experiments with R3230AC mammary tumor have shown that prolactin inhibits tumor growth (Hilf, 1972). Recently, the influence of prolactin on murine mammary tumorgenesis has been reviewed by Welsch and Nagasawa (1977).

No detailed study is available on the influence of prolactin in cervical cancer. However, recently Forseberg (1974) reported that prolactin stimulated DNA synthesis in cervical carcinomas. According to him the pronounced epidermization in estrogenized animals did not satisfactorily

explain the initial difference in cancer incidence in mice given estradiol or olive oil neonatally. Ovariectomy had no effect on this difference. In fact the persistent estrogen production in nonspayed estrogenized animals seemed to retard later cancer growth. Some other hormone, possibly prolactin, might have involved in this process. Interest was devoted to prolactin since it was supposed that the persistent output of estrogen from the ovaries of estrogenized mice increase the serum prolactin level (Forsberg, 1974). It is possible that a changed prolactin level is part of the estrogen induced disturbance in neonatal hypothalamic differentiation. Forseberg, (1974) suggested that prolactin significantly stimulates ^{3}H-thymidine incorporation in cervical tumors from animals given olive oil neonatally. So he speculated a probable effect in tumors from estrogenized animals. Also he demonstrated the stimulation of ^{3}H-thymidine incorporation in monolayer cultures from normal neonatal cervical epithelium. According to Freeman (1979) the electrical stimulation of the uterine cervix in rats resulted in prolactin surges. So prolactin is suspected to play an important role in cervical carcinogenesis.

Also it was found that 20% cases of secondary amenorrhoea hyperprolactinaemia prevents ovulation by

impairing normal follicular development. Dorington and Gore Langton (1981) suggested that the contraceptive action of prolactin was due to its ability to interfere with the action of follicle stimulating hormone (FSH) on the synthesis of estrogen. Since estrogen always plays a significant role in the uterine cervix, prolactin may be of a greater importance in the efforts to modulate cervical carcinogenesis. So in the present study, the influenee of exogenously administered prolactin on the development of experimental cervical cancer is investigated.

Testosterone :

Androgens such as testosterone propionate are employed in the treatment of cancer of breast. They are originally used on the basis that they antagonize estrogens. Andogens were first used in the treatment of clinical mammary cancer with metastases by Ulrich (1939), Loesser (1941). The effect of testogterone and related steroids on different types of tumors in animals has received a moderate amount of attention. Heiman and Krechbel (1936) recognized that the growth of mammary fibroadenomas was associated in part with factors relating to sex and pregnancy and began to study the effect of the known steroids on tumor growth. Since then, Heiman (1940) and others have shown that castration or testosterone

treatment decreases the number of takes of transplants of tumors and that testosterone decreases tumor growth in unoperated rats.

Dihydrotestosterone was studied more extensively than othe other compounds and was observed to inhibit the stimulation of tumor growth produced by 17β-estradiol or progesterone reducing tumor weight to the level slightly below that obtained in ovariectomized or untreated rats (Huggins et al., 1957). However, in tumors with a low degree of hormonal dependence dihydrotestosterone failed to inhibit tumor growth. Dihydrotestosterone delayed but did not prevent the formation of mammary cancer in rats fed 7,12-dimethyl-benz(a)anthracene. It produced regression of tumors induced by methylcholanthrene in 15 out of 17 rats, and increased tumor size in three animals (Huggins, 1959). There are some experimental reports on the influence of androgen in the development to cervical cancer. Kimura and Nandi (1967) observed that the cervico-vaginal tumor incidence in androgenized mice was lesser than the normal ones. This result was similar to the earlier neonatal oestrogenization experiments. However, Cherry and Glucksmann (1948) reported that testosterone and oestrogen affect carcinogenesis in different ways. In intact rats oestrogens inhibit the induction of sarcomas while testo-

sterone merely prolongs the induction period. The induction of epithelial tumors is promoted by testosterone but not oestrogens given continuously. In castrate rats testosterone promotes and accelerates the appearance of sarcomas and of epithelial tumors, while estrogens given continuously fail to promote carcinogenesis. The effect of estrogens on carcinogenesis is quite independent of its stimulating action on stroma and epithelium of the cervico-vaginal tract. Intermittant administration of low doses fail to restore normalcy in the castrate animals but greatly promote carcinogenesis. So the male and female gonadal hormones have the same inductive actions in the prenatal period, but divergent actions in the adult state. Meisels (1966) observed that the average latent period of cervical cancer development was markedly lengthened in animals receiving weekly injections of 28 µg of testosterone. But there was no report on the changes in tumor incidences.

According to Victor (1960) the various means by which androgenic steroids may act on tumors are outlined as follows. They may (i) affect tumor growth because of specific effects related to androgenicity; (2) inhibit pituitary function; (3) affect tumor growth because of specific anabolic effects e.g. nitrogen retention, calcium

retention which may influence tumor metabolism; (4) affect other endocrine activities of steroids (Progestational, antiestrogenic); (5) act through metabolic products; (6) produce specific effects on tumor unrelated to endocrine activity. Exogeneously administered testosterone in the present investigation is suspected to interfere with the process of cervical carcinogenesis and alter the tumor incidence.

MATERIALS AND METHODS

Randombred Swiss albino mice from Maulana Azad Medical College, New Delhi were maintained in an air-conditioned room. They were fed with rat food (Hindustan Lever Ltd, India) and tap water ad libitum.

The carcinogen 3-methylcholanthrene and the hormones 17β estradiol, Progesterone, Prolactin and Testosterone propionate were purchased from Sigma Chemical Company, U.S.A. The pure beeswax was procured from Mysore. The wax and carcinogen thread preparations and their intra-cervical insertions were done as explained in the I Chapter.

Four major experiments were setup to find out the influence of the above-mentioned four hormones namely 17β estradiol, progesterone, prolactin and testogterone propionate on the cervical carcinogenesis. The first experiment was designed to study the influence of 17β estradiol. Twenty one animals were inserted with carcinogen threads and 10 animals with just wax-coated threads. All of them were given each 0.5 µg of 17β estradiol dissolved in 0.5 ml of olive oil intramuscularly twice a week throughout the experimental period. A group of ten animals were given the same dose of 17β estradiol alone to serve as estrogen controls.

The second experiment was designed to study the influence of progesterone on the cervical carcinogenesis. Progesterone was dissolved in olive oil in the ratio of 0.1 mg in 0.5 ml. A total number of fortyfour animals were given the intramuscular injections of 0.1 mg progesterone each twice a week throughout the experimental period. Out of them 19 animals were already inserted with MCA coated threads and other twelve with wax-coated threads. The rest thirteen animals served as progesterone controls.

The influence of prolactin on the cervical carcinogenesis was studied in the third experiment. The hormone prolactin was dissolved in physiological saline in the ratio of 200 µg in one ml. Twenty two mice inserted with MCA-coated threads intracervically, were given each with 200 µg of prolactin, subcutaneously, twice a week throughout the experimental period. A group of 11 animals were given the same dose of prolactin to serve as prolactin controls. Another group of ten mice which had intracervical insertion of carcinogen coated threads, received 200 µg of prolactin in one ml of saline twice a week.

The last experiment was set up to study the influence of testosterone propionate in cervical carcinogenesis. Twenty three mice were inserted with carcinogen coated

threads and another 11 with wax threads. All of them received each 15 µg of testosterone propionate dissolved in olive oil, intramuscularly twice a week throughout the experimental period. A group of twelve animals were also given the same dose of the hormone to serve as testosterone controls.

After the experimental period of three months all the mice were autopsied. The upper part of the vagina, the uterine cervix and the posterior part of the uterine horns were removed en bloc and fixed in bouins solution. All preparations were embedded in paraplast paraffin wax purchased from Sherwood Medical Industries, St. Louis, Missouri, U.S.A. Sections cut transversely at 7 µ in various regions of the cervices were stained with Harris haemotoxylin and eosin.

RESULTS

Experiment No. 1: (Influence of 17β estradiol)

There was no mortality among the experimental animals before the autopsy period. The administration of 17β-estradiol in the MCA threaded animals had decreased the tumor incidence significantly to 44.44%. This incidence was 50% lesser than that of the carcinogen control group which had the tumor incidence of 90% (Table XI). It is to be noted that among the 21 animals of the carcinogen-plus-17β-estradiol group nine were bearing tumors. Out of the nine tumors eight were advanced carcinomas and only one was an early carcinoma. No other types of tumors like sarcoma or adenomas were found. Almost all the eight advanced carcinomas were of non-keratinizing type and the rest two were of keratinizing type. Six preparations of the same group were found to be in the marked dysplastic stage with the pleomorphic, keratinizing and nonkeratinizing cells above the basal limited membrane. Five preparations had moderate dysplasia and another one with mild dysplasia. No preparations showed normal squamous epithelium.

Twelve animals were treated with 17β-estradiol only to serve as hormone controls. None of them showed dysplastic

changes. However, nine preparations showed dysplastic changes one with marked dysplasia, four with moderate dysplasia and the rest four with mild dysplasia. Three preparations had normal squamous cervical epithelia. So it is evident that 17β estradiol could induce dysplastic changes in the cervices.

Ten wax-threaded animals were administered with 17β estradiol. Out of the ten treated mice five preparations showed moderate dysplastic changes one had marked dysplasia and three other preparations showed mild dysplasia. Only one mouse was found to have the normal squamous epithelium. So in the wax threaded cervices 17β-estradiol could increase the pathological changes upto the dysplastic stages but not to the tumorous states.

Experiment No. 2: (Influence of progesterone)

The second experiment was set upto find out the influence of progesterone on the cervical carcinogenesis. Three animals died with malignant tumors in the carcinogen plus progesterone group. Out of these three preparations, two showed well differentiated non-keratinizing squamous cell carcinomas in the advanced stage and the rest one preparation had severe adenocarcinomatous lesions. Totally out of the 19 preparations from progesterone administered carcinogen

threaded mice, 18 showed tumorous changes. Four of them had early squamous cell carcinomas and the other 12 showed advanced carcinomatous changes. The infiltrations of the neoplastic cells into the stromal region was very vigrous in almost all the advanced carcinomas. Islands of neoplastic cells throughout the stromal region could be seen in all those preparations. Two other preparations showed adenocarcinomatous lesions. The one preparation which did not show any tumorous change was in the moderate dysplasia stage. Patches of pleomorphic and keratinizing cells could be seen all through the squamous epithelium. Compared with carcinogen control, the administration of progesterone had a significantly increased the tumor incidence and other pathological changes.

Out of these 13 hormone treated animals five showed normal stratified squamous epithelia, six had mild dysplastic changes and the rest one with marked dysplasia. However, the preparations from wax threaded progesterone treated animals showed many pathological changes. One preparation had a well differentiated non-keratinizing early squamous cell carcinoma and another preparation with severe marked dysplastic changes with many hyperchromatic and pleomorphic cells throughout the squamous epithelium. Two preparations had moderate dysplasia and six had mild dysplasia in the

same group. Normal epithelium could be seen in only two preparations.

So the present iobservations show that exogenous administration of progesterone had a pronouncing influence on the cervical carcinogenesis.

Experiment No. 3: (Influence of prolactin)

The influence of prolactin on the cervical carcinogenesis was investigated in the third experiment. Out of the 22 MCA threaded prolactin administered mice 21 were bearing tumors. Twelve preparations showed advanced carcinomatous lesions of which four were from the mice died with the tumorous lesions before the experimental period. Five were keratinizing squamous cell carcinomas and the rest eight were non keratinizing squamous cell carcinomas. All the carcinomas were of well differentiated types. Three preparations showed adenosquamous carcinomas with the involvement of cervical glands. One preparation taken from another dead animal before the experimental period showed severe sacromatous lesions. Five preparations showed early squamous cell carcinomas. The one preparation without the tumorous lesion in the group showed moderate dysplastic changes. Thus the carcinogen-plus-prolactin group had a significantly increased tumor incidence and

other pathological changes when compared with the carcinogen only controls.

The 11 animals treated with only prolactin did not show significant pathological changes. Eight preparations showed normal stratified squamous epithelium and the rest had mild dysplastic changes. Also the wax threaded prolactin administered mice did not show any significant changes. Out of the ten preparations in the group four showed moderate dysplastic changes one had mild dysplasia and the rest five showed normal stratified squamous epithelia.

So the present observation shows that the administration of exogenous prolactin has a significant promoting influence on the cervical carcinogenesis.

Experiment No. 4: (Influence of Testosterone)

In the fourth experiment testosterone propionate was taken as a modifier in the cervical carcinogenesis. Three animals died due to malignant tumorous lesions in the carcinogen plus testosterone group. This group showed severe pathological changes. Out of the 23 treated animals 21 had tumors. Three preparations showed severe adenocarcinomatous lesions and another one had malignant sacromas. Among the 17 squamous cell carcinomas 9 were of advanced well differentiated carcinomatous types

and the rest 8 were of early carcinomatous types. Most of them were non-kertinizing squamous cell carcinomas. Two other preparations showed marked dysplastic changes. In total carcinogen plus testosterone group had a tumor incidence of 91.3% which is only slightly more than that of the carcinogen control group i.e. 90%. Nevertheless the mortality with cancerous lesions before the experimental period and the increased number of tumors per tumor bearing animal (1.73) in the carcinogen plus testosterone group show that the administration of exogenous testosterone had a slight promoting influence on the experimental cervical carcinogenesis.

Twelve mice were administered with testosterone only to serve as hormone controls. No significant pathological changes were observed. Out of them four preparations had mild dysplastic changes and the rest eight showed normal stratified squamous epithelium. The 11 wax threaded testosterone administered mice also did not show any prominent pathological changes. Six preparations in the same group had moderate dysplasia, three had mild dysplasia and the rest two showed normal squamous epithelia.

TABLE XI

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES (Influence of 17β estradiol)

Groups	Mice at risk	Normal	Dysplasia +	Dysplasia ++	Dysplasia +++	No. of tumor bearing mice: Carcinoma Early	No. of tumor bearing mice: Carcinoma Advanced	No. of tumor bearing mice: Other types (ACM & SCM)	%age of tumor incidence	Total No. of tumors	No. of tumors per tumor bearing animal
Carcinogen only	20	0	0	0	2*	5	10***	3	90%	22	1.22
Carcinogen + 17β estradiol	21	0	1	5	6**	1	8	0	44.4%	31	1.48
17β estradiol only	12	3**	4*	4	1	0	0	0	0%	0	0
Wax + 17B estradiol	10	1	3	5***	1	0	0	0	0%	0	0

Note : The number '*' denotes the number of preparations which show infection.

ACM = Adenocarcinoma; SCM= Sarcoma; + = Mild; ++ = Moderate; +++ = Marked.

TABLE XII

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES (Influence of Progesterone)

Groups	Mice at risk	Normal	Dysplasia			No.of tumor bearing mice			% age of tumor incidence	Total No. of tumors	No. of tumors per tumor bearing mouse
			+	++	+++	Carcinoma Early	Adavanced	Other types (ACM & SCM)			
Carcinogen only	20	0	0	0	2*	5%	10**	3	90%	22	1.22
Carcinogen + Progesterone	19	0	0	1	0	4*	12@@	2@	94.73%	32	1.68
Progesterone only	13	5**	6	1	0	0	0	0	0%	0	0
Wax + Progesterone	12	2	6**	2	1	1	0	0	8.3%	1	1

Note: The number of '*' denotes the number of preparations which showed infection.

The number of '@' denotes the mortality number of mice with tumors before the autospy period.

ACM = Adenocarcinoma; SCM = Sarcoma; + = Mild; ++ = Moderate; +++ = Marked.

TABLE XIII

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES (Influence of Prolactin)

Groups	Mice at risk	Normal	Dysplasia			No.of tumor bearing mice			%age of tumor incidence	Total No.of tumors	No. of tumors per tumor bearing mouse
			+	++	+++	Carcinoma Early	Carcinoma Advanced	Other types (ACM & SCM)			
Carcinogen only	20	0	0	0	2*	5	10***	3	90%	22	1.22
Carcinogen + Prolactin	22	0	0	1	0	5**	12@@@@	4@	95.46%	39	1.96
Prolactin only	11	8**	3	0	0	0	0	0	0%	0	0
Wax + Prolactin	10	5**	1	4	0	0	0	0	0%	0	0

Note : The number of '*' denotes the number of preparations which showed infection.

The number of '@' denotes the mortality number of mice with tumors before the autopsy period.

ACM = Adenocarcinoma; SCM = Sarcoma; + = Mild; ++ = Moderate; +++ = Marked.

TABLE XIV

THE TUMOR INCIDENCE AND OTHER PATHOLOGICAL CHANGES (Influence of Testosterone -propionate)

Groups	Mice at risk	Normal	Dysplasia			No.of tumor bearing mice			%age of tumor incidence	Total No.of tumors	No. of tumors of per tumor bearing mice
			+	++	+++	Carcinoma Early	Carcinoma Advanced	Other types (ACM & SCM)			
Carcinogen only	20	0	0	0	2*	5	10***	3	90%	22	1.22
Carcinogen+ Testosterone	23	0	0	1	2*	8	9@	4@@	91.3%	40	1.63
Testosterone only	12	8	4**	0	0	0	0	0	0%	0	0
Wax + Testosterone	11	2	3*	6	0	0	0	0	0%	0	0

Note: The number of '*' denotes the number of preparations which showed infection.

The number of '@' denotes the mortality number mice with tumor before the autospy period.

ACM = Adenocarcinoma; SCM = Sarcoma; + = Mild; ++ = Moderate; + = Marked.

DISCUSSION

Estrogen :

Though the influence of estrogen on the development of experimentally induced cervical carcinomas has been studied by some authors there are always contradictory reports and the controversy of estrogen's influence on the cervical cancer still remains. So the present investigation was undertaken to study the influence of 17β estradiol on the MCA induced cervical carcinogenesis. The tumor incidence in the carcinogen plus estradiol group was 44.44% whereas 90% incidence of tumors was noted in the carcinogen control group. This clearly shows that exogenous administration of 17β estradiol had an inhibiting influence on the development of cervical tumors. This is in agreement with the observation of Forsberg (1972) that persistent production of ovarian estrogen in the neonatally estradiol treated animals retarded cervical cancer growth. However, Murphy (1961) found that castration after limited exposure of endocervical epithelium of strain A mice to MCA, markedly decreased the tumor incidence and so he reported that normal levels of estrogen significantly promote cervical carcinogenesis. But as mentioned earlier (see Chapter III) the results of carcinogen plus ovariectomy group in one of the present investigations showed that castration had a

promoting influence. This is in agreement with Kodstall (1966) that ovariectomy appeared to be a promoting factor in the uterine cervix of mouse. Kodstall's conclusion was based on the observations of various histochemical changes during cervical carcinogenesis. Glucksmann and Cherry (1962) also reported that ovariectomy significantly increased the incidence of mucoepidermoid carcinomas. It is to be noted that in Murphy's experiment (Murphy, 1965) the cervices were not exposed to the carcinogen for the whole experimental period. So his results can not be compared with the present investigation, in the strict sense. Further according to Murphy (1965) an interference with the tumor promotion was the reason for the marked reduction in the tumor incidence by castration. With the present results it can not be stated whether estrogen interfered with the initiational phase of the promotional phase of carcinogenesis. However, in the present investigation a significant number of cervices from 17β estradiol group and the wax plus 17β esteradiel group showed mild and moderate dysplastic changes. Also in the carcinogen plus 17β estradiol group six preparations showed marked dysplasia and the other five had moderate dysplastic changes, though the ultimate tumor incidence was only 50% of the carcinogen control group.

Inhibition of DMBA induced carcinogenesis by estrogen particularly at the initiational level has been already reported by many authors. Manoharan and Rao (1980) reported that DMBA elicited the depletion of oocytes, but concomitant administration of estrogen with DMBA reduced this less of oocyte population significantly. Bates (1968) suggested that estrogen may interfere with the tumorigenic action of DMBA in female mice since animals in the estrous phase are less suspeptible than in diestrous phase, to skin tumorigenesis. Also 17β estradiol was known to inhibit their induction of the aryl hydrocarbon hydroxylase system in mouse tissues in vitro and in vivo (Nebert et al., 1970).

So it may not be an absurd idea that in the process of cervical carcinogenesis estrogen may have an inhibitory influence on the initiational phase and a promoting influence on the promotional phase. However, further investigations are needed to prove this possibility.

Progesterone :

In the present investigation, carcinogen plus progesterone group had a tumor incidence of 94.75%, whereas it was only 90% in the carcinogen control group. Also three animals died due to the vigorous lesions of cancer

before the experimental period in the same progesterone treated group. A significant number of wax threaded animals treated with progesterone had dysplastic changes and one single animal developed an early squamous cell carcinoma. So it is very clear that the exogenous progesterone administration had a promoting influence on the carcinogenic process. It seems even the dysplastic changes in the cervical epithelium by wax thread irritation may be induced to marked dysplasia or carcinoma.

At present,no report is available in agreement or in contradiction to this observation. However, Haminetzky (1966) reported that progesterone appeared to counteract the tendency of estrogen to induce stromal penetration with the treatment of MCA. He could not comment on the incidence of tumors because the study was limited to alterations in dysplastic changes only.

Similar reports of antagonistic effect of progesterone to estrogen were reported by many authors. Estrogen inhibited progesterone induced endometrial proliferation (Courrier, 1950). When estrogen induced tumors were studied, progesterone had an antagonistic effect (Noble and Coll, 1941; Mardones et al., 1954). Huggins (1950) reported that the stimulation effect of small doses of

estriol on tumor growth was increased by progesterone. On the other hand, the effect of larger doses of progesterone was inhibited by a high dose of estrone.

Inhibitory influence of estrogen at the initial level of cervical carcinogenesis was discussed earlier. Evidences for the antagonistic effect of progesterone to estrogen were already given. Adding these two a probable possibility emerges that in the present investigation the initiational level inhibition of endogenous estrogen might have been antagonised by the exogenously administered progesterone resulting in the increase in tumor incidence and other pathological changes. This possibility is in agreement with the report of Glucksmann and Cherry (1962) that treatment of castrate mice with progesterone or with lutocycline increased the incidence of adenosquamous carcinomas.

Prolactin :

The hormone prolactin was suspected to modulate the process of cervical carcinogenesis because of the observations by many authors (Forsberg, 1974; Chem, 1974) that it has close association with the actions of estrogen. In the present investigation 21 out of 22 mice developed the cervical tumors, the incidence being 95.44% whereas

the carcinogen control group had 90% tumor incidence. Also, it is to be noted that five animals died with severe cancerous lesions before the experimental period of three months. Exogenous administration of prolactin alone or with wax thread insertion did not elicit significant pathological changes (Table XIII). So it is very clear that the carcinogenic action of MCA in the cervix had been enhanced by prolactin.

Reports available on the influence of prolactin in carcinogenesis mostly dealt with mammary carcinogenesis (Moskop Kitty, 1939; Brot, 1970; Meites, 1972; Hilf, 1972). The influence of prolactin on cervical carcinogenesis was not given due attention for detailed experimental studies. However, Forseberg (1974) described the importance of prolactin in the cervical tumor development and demonstrated the incorporation of ^{3}H-thymidine in monolayer cultures from normal cervical epithelium and in cervical tumors.

The mechanism of the promoting influence of prolactin on the cervical carcinogenesis is difficult to explain. However, circumstantial evidence (Dorlington and Gorelangton, 1981) suggests that increased levels of prolactin has the ability to interfere with the actions

of follicle stimulating hormone and inhibit the estrogen synthesis in the ovary. Additionally it was concluded in the earlier experiments that estrogen might possibly have an inhibitory influence in cervical carcinogenesis. It is thus speculated that prolactin might have inhibited the estrogen synthesis on ovary and thereby increased the cervical tumor incidence.

Testosterone :

The male gonadal hormone had a slight promoting influence on the cervical carcinogenesis, in the present investigation. Out of 23 carcinogen threaded testosterone propionate administered mice, 21 had the cervical tumors. Before the experimental autopsy period three animals died due to cancerous lesions indicating the severity. However, the preparations from wax threaded testosterone treated animals and the hormone treated animals did not show any significant pathological changes. Overall the tumor incidence in the carcinogen plus testosterone group was 91.3%, whereas it was 90% in the carcinogen control group. Considering the mortality and the average number of tumors per tumor bearing animals (Table XIV) it is clear that the hormone testosterone propionate did have a promoting influence on the carcinogenic action of MCA in the cervices of mice.

Meisels (1964) reported that average latent period of cervical tumor development was markedly lengthened in animals receiving weekly injections of 28 µgms of testosterone. But the alteration in the tumor incidence was not mentioned. The interaction of testosterone with the breast tumor was mostly reported in the literature. Dihydrotestosterone was studied more extensively than the other compounds and was observed to inhibit the stimulation of mammary tumor growth (Huggins, 1957).

The promoting influence of testosterone on the cervical carcinogenesis in the present investigation is not much significant. Further studies have to be made to prove this possibility. However, the progestational activity of testosterone and other androgens have been amply demonstrated (Drill, 1960). Since the promoting influence of progesterone on the cervical carcinogenesis has been already discussed, the testosterone propionate might have had the same influence. Also the antiestrogenic property of the androgens like testosterone had been well documented in the literature (Drill, 1960). Further it was concluded by the earlier experiments that estrogen showed an inhibitory influence on cervical carcinogenesis in mice. So it may be speculated

that the slight alteration in the tumor incidences and other pathological changes by the administration of exogenous testosterone is due to the expression of its antiestrogenic and progestational properties.

EPILOGUE.

GENERAL SUMMARY AND CONCLUSIONS

High cervical cancer incidence has been recorded in several human populations of the world. Also it is well known that the uterine cervical cancer is one of the major oncologic problems in India. The influence of several extrinsic and intrinsic factors on the incidence and frequency of cervical cancer has been discussed in the literature. However, most of them pertain to clinical observations involving the study of exfoliated cells, from the human cervices. No report is available on a standardized animal model system with detailed histopathological observations at different stages of cervical cancer development.

Also a study of the modilatory influence of some exogenous and endogenous factors may provide a way for further developments towards the control of this cancer in human beings.

So the present investigation was undertaken to make a detailed histopathological study of the experimental cervical carcinogenesis.

The uterine cervical cancer development by the intracervical insertion of 3-methylcholanthrene impregnated thread in 8 weeks old Swiss albino mice was studied sequentially at different interval times. The salient findings are as follows:

(i) Mild, moderate, and marked dysplastic changes could be observed upto 30 days intervals and thereafter mostly marked dysplasia was found. The mild dysplasia was present from 10 days to 50 days interval while moderate dysplasia could be observed only in three intervals namely 20 days, 30 days and 40 days after the carcinogen thread insertion. Marked dysplasia appeared at the 20 days interval and it's presence continued till the last 90 days intervals.

(ii) The proportion of animals showing marked dysplastic changes decreased gradually from 30 days to 90 days interval.

(iii) The tumor appearance started from 30 days after the carcinogen thread insertion and a steady increase of the percentage of tumor incidence was noted with the increase in time.

(iv) The appearance of the tumors was maximal at 70 days and 90 days intervals, the percentage of incidence being 87.5% and 90% respectively.

(v) Unlike the human situationn, the presence of carcinoma in situ or epidermoid carcinoma is very rare in the mouse cervical carcinogenesis.

(vi) The bifurcated cervical canal occupied the maximal number of tumors in general. It is to be noted that the squamocolumnar junction which is suspected to play a role in the human cervical cancer development is present in this region. So it may be speculated that even under experimental conditions the squamocolumnar junction plays an important role in the origin and development of cervical cancer.

The aging process altered the cervical cancer development in mice appreciably.

(i) When all the tumors (tumors appearing both in epithelial and extraepithelial areas of cervix) were taken into account the tumor incidence oscillated from 90% (in 8 weeks age group) to 100% (in 80 weeks age group).

(ii) Further when the tumors recorded only in the cervical epithelial area (excluding extraepithelial and glandular epithelial regions) were taken into account the incidence varied from 75% (in 8 weeks age group) to 90% (in 80 weeks age group).

(iii) The average number of tumors per tumor bearing animal increased from 1.22 (in 8 weeks age group) to 2 (in 65 weeks age group) with the increase in age. However, the oldest animal group at the time of treatment showed a drop in the trend i.e. the number of tumors per tumor bearing animal in the 80 weeks age group was 1.45.

(iv) The mortality number due to tumorous lesions also increased with the increase in age at the time of insertion of the carcinogen thread.

Butylated hydroxyanisole, retinoic acid and 2-mercapto-propionylglycine were the three chemical modifiers tested for their influence on the experimental cervical carcinogenesis. The salient findings are as follows :

i. The incidence of cervical cancer decreased from 90% in the carcinogen control group to 31.58% in the carcinogen plus BHA treated group.

ii. Ovariectomized mice showed an enhanced tumor incidence of 94.44%. However, when BHA was fed with the diet, the incidence in these ovariectomized animals decreased to 68.42%.

iii. Administration of retinoic acid and at 200 µg/kg dose level decreased the cervical cancer incidence to 42.85%.

iv. The tumor incidence decreased further to 30% when 400 mg/kg retinoic acid was administered at weekly intervals.

v. MPG could not alter the cervical tumor incidence significantly, when it was administered at the given dose level 25 mg/kg twice weekly. However, it is suggested that appreciable inhibitory action of this compound could be accomplished if it is administered continuously rather than at weekly intervals.

Four exogenous hormones were tested for their modulatory influences in the cervical carcinogenesis in the present investigation. They were estrogen, progesterone, prolactin and testosterone. Their modulatory influences are as follows:

(i) Administration of exogenous 17β estradiol decreased the tumor incidence from 90% (in the carcinogen control group) to 44.44%.

(ii) The average number of tumors per tumor bearing animal increased from 1.22 in the carcinogen control group to 1.48 in the carcinogen plus 17β estradiol group.

(iii) A moderate increase in the cervical tumor incidence (94.73%) was noted by the progesterone administration in the carcinogen threaded animals.

(iv) However, a good number of progesterone treated animals died of cervical tumor lesions quite before the termination of the experiment thereby indicating reduction in the latency period.

(v) An enhancement of cervical tumor incidence to 95.46% by the prolactin administration to the carcinogen threaded animals was noted.

(vi) Also the mortality number with tumorous lesions and the increased average number of tumors per tumor bearing animals (1.96) indicated that the exogenous prolactin enhanced the cervical carcinogenesis in mice.

(vii) Exogenous administration of testosterone propionate did not alter the cervical tumor incidence significantly

(iii) However, a significant mortality number with tumorous lesions and the increased average number of tumors per tumor bearing animal (1.73) was observed in the carcinogen plus testosterone group. So the exogenous testosterone did have a slight promotory influence on the cervical carcinogenesis.

Thus, the present study reveals that the incidence of cervical cancer by a chemical carcinogen can be altered by using chemical and hormonal modifiers. Further discovery of the various natural and synthetic modifiers and studies on the mechanisms of these modulations may provide a way for the alteration and ultimately inhibition of the widely prevalent uterine cervical cancer in humans.

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