REVIEW OF LITERATURE
Tumor development is a play with many different
dramatic persons. The main character is still the
cell. It is a protein figure that can appear in many
different forms and play different roles, most of
which can be classified as stages in neovascularization
process known as tumor progression. The initiation of
tumor development is most likely to involve changes
at the genetic level. In its road to progression
the neoplastic cell encounters many predators,
including various effectors of immune system.
Internally it will depend on host genetics, on age
and all of the many physiological mediators of immune
system (Mein, 1996).

The structural pattern of malignant tumor cell
is sufficiently distinct from the normal cell to be
identified in most instances (Spaeth, 1954).
Subjective differences in their interpretations are
always possible, especially in severe dysplasia and
intraepithelial neoplasia. The limiting factor in
the case of carcinoma is early diagnosis, pathological
methods for diagnosis of malignant tumors (Hartman, 1936)
are of historical importance and some give
promising results.
In the recent past much emphasis has been given on the immunological aspect of neoplasia. The antigenic constitution of the tumor may be different from that of the host. The changes in antigenic constitution may involve the acquisition of new antigenic substance or deletion or loss of antigenic component (Combe, 1961).

The depletion of specific antigens within tissues has been considered an important factor of neoplastic behaviour, both in experimentally induced tumors in animals and spontaneous tumors in humans. A loss of antigenicity of intercellular substance and basement membrane was observed in benign, premalignant and malignant tumors of epidermal origin and it had a parallel course with the grade of cellular malignancy (Vareliés et al., 1960). With higher sensitive techniques A B C I extracts can serve as tumor antigens for the study of changes in malignant transformation.

In the same way as it is impossible to study pathological changes in tissues without a knowledge of normal histology, so it is also necessary to have a full knowledge of the antigenic architecture of normal cells.
The ABO(0) antigens, in addition to their well recognized presence on RBC and secretion of certain individuals, are also expressed in variety of body tissues.

**Distribution of ABO Antigens in Various Tissues**

It has long been confirmed that ABO antigens are present in cells other than erythrocytes and in secretions other than saliva. They are present in platelets (Severin et al., 1954; Sorensen and Sander, 1954), white blood cells (Sorensen, 1950; Sorensen, 1954), in mucous secretions (Martnez, 1948) in epidermal and epithelial cells (Gomaa et al., 1946), human oral epithelium (Dahlin and Fujiwara, 1974) and in spermatozoa (Edwards et al., 1964; Rotherham, 1965). Sorensen (1950), Sorensen (1954) studied the histological distribution of ABO antigens in humans in intra and extraembryonic life by immunofluorescent (IF) technique in great details. According to him it can be summarized in following six convenient headings (1950).

1. The lining of the mouth of all children through out the body and those supplying salivary tissues contain ABO Antigens.
(ii) The stratified epithelium of skin, oral cavity, esophagus, uterine cervix, vagina, Hassall bodies in thymus and transitional epithelium of lower urinary and upper respiratory passages show that antigenes are confined to basal layer in stratified squamous epithelium with a predilection of granular layer in skin and are present in all layers of transitional epithelium.

(iii) The simple epithelium show various degree of completeness of outlining the cell wall wide independent of the secretory status. The parietal cells of endocrine glands and nervous system show absence of antigen.

(iv) The mucous secretion containing

In secretory - Salivary glands, lining epithelium of the glands of stomach, goblet cells of small and large intestine up to the level of transverse colon, mucous glands and goblet cells of upper respiratory passageway bladder, uterine cervix and pseudomucinous ovarian cyst contain large amount of ABO antigenes,

In nonsecretory - ABO antigenes are usually not present except in certain special locations like deep parts of gastric foveolae and the
pyloric glands, and varying small number of
paillet cells in the crypts of small and large
intestine.

(v) Among other organs of excretions and excretions.
The exocrine component of the pancreas, parotid
gland, kidney, sweat glands, endometrium, fallopian
tube, breast and male genital organs like epididymis,
seminal vesicles and prostate secretor ABO antigens
in secrete. No antigens is found in non secretare.

(vi) In miscellaneous group. The testes show presence
of ABO antigens in spermatocytes and cells in
prospermatozoal level. Ova show no antigens and
pailletal cells of gastric glands are uniformly
negative.

The term alcohol soluble and water soluble are
for the two varieties of ABO (antigens). The ethanol
resistant water soluble antigens are readily
demonstrable in formalin fixed paraffin embedded
tissues (Hovarth et al., 1966).

VARIUS METHODS OF DEMONSTRATION OF ABO ANTIGENS

The presence of ABO antigens in cells and
tissues other than erythrocytes was first demonstrated
through the use of an agglutination inhibition test
(Kritschewski et al., 1937; Landsteiner, 1936). In
oral vaccine their presence was shown by absorption of 
hemagglutinins in water soluble extracts of oral 
epithelium (Yasuda, 1928).

Immunochemical (IV) staining technique 
(Cowan and Kaplan, 1956), mixed cell agglutination 
reaction (MAC) (Glyn et al, 1957) and 
immunoperoxidase (IP) staining technique (Avennum, 1960) were 
later used for their demonstration in tissue cells.

Mixed cell agglutination reaction was first 
described by Topley and Wilson (1935) as quoted by 
Hilgrem et al (1964), later it was employed in 
zoological testing (Finland and Curwen, 1928; 
Wainar and Hansen, 1935), cell suspension (Cowan, 
1961), in tissue cultures for the recognition of 
the species of origin of cell (Cowan et al, 1961), 
in the ABO grouping of human cells in culture 
(Hagman, 1941; Chasnin et al, 1965) and in studying 
tissue antigens (Cowan, 1956; Cowan, 1963; Hilgrem 
et al, 1964); Tonder et al (1966) used MEM in tissue 
sections in order to preserve antibody soluble 
antigens. Later Davidsson and Kj (1970) reported 
that the test could be done on frozen sections of 
brain and formalin fixed tissue, on section of 
resect and old paraffin blocks and an old and new
HAS stained and decalcified slides for reaction. Moreover age of the sections and of the paraffin blocks do not affect the sensitivity and specificity of the test. Davidson and Stejskal (1971) stressed that positive reaction is not the pericellular clumping of agglutination but adherence and for this reason they used the same specific red cell agglutination (MAB) in films of mixed cell agglutination reaction.

MAB on formalin fixed paraffin embedded tissues has been reported to be more sensitive than IF technique (Davidson et al., 1969), whereas Nebelsteen and Apper (1972) suggest that IF technique seems to be as sensitive as the MAB but is superior to latter in allowing more accurate localization of the antigens.

It has been shown that the ABC immunogous in tissues are not influenced by formalin fixation and paraffin embedding procedures, therefore immediate-scent staining, immunoperoxidase staining and specific red cell agglutination reaction can be successfully used in sections prepared from formalin fixed paraffin embedded tissues (Mozurk et al., 1966) Nebelsteen and Apper (1972). Roest and Joestin (1973) have suggested that brain's fluid is the better fixative.
for immunofluorescent staining studies as in in situ the antigens and the antibodies are better preserved. Quantitatively ABO (H) antigens differ widely in their concentration in different tissues and quantitative analysis as such is not very much helpful in early diagnosis of malignant lesions (Dahlestone, 1978).

**BIOCHEMICAL ASPECTS OF ABO ANTIGENS**

Biochemically the alcohol soluble and water soluble ABO antigens are glycoproteins and glycolipids respectively and the group specificity is associated with the carbohydrate moiety. The occurrence of ABO antigens begin with a precursor mucopoly saccharide substance which is further modified into H substance and the H substance into AB antigens under genetic control. The genes responsible for this conversion regulate enzyme production for catalyzing the transfer of sugar. The L-N-acetyl-D-galactosaminyl and D-galactosyl transferases are the enzymes which are necessary for the conversion of H substance with A and B substances respectively (Mathies, 1968). ABO antigen loss may indicate defective biosynthesis (Pehsa, 1976).
The view according to which the isoantigen are derived from H, and may indeed be associated with one and the same neoplymphochoride gains a further circumstantial confirmation as while in group 0, tissue K constitutes the sole antigen of the ABO (K) system, it generally appears also in non-O tissues in amount varying from none to those appearing equivalent to or exceeding A or B (Sauter, 1964).

Alterations of glycercyl transferase enzymes occur frequently in carcinomatous tissue in relation to normal adjacent tissue. Schouten and Erbus (1976) have reported deficient enzymes in stomach and colon carcinomas. The accumulation of precursor substance, probably due to the block of synthesis of more complex determinants destined to heat, because of the possible activation of allelicomorph genes occurs in human cancer (Young and Nakoneczni, 1976).

AND ISOTYPIC AND CROSS-IMMUNOREACTIVE METHODS

Immunological studies show that carcinomembryonic antigens (CEA), the tumor incidence, are deficient or incomplete ABO blood group antigens and the determinants of blood group antigens and CEA share the same glycopeptide (Alastair et al., 1973; Nishizawa et al., 1976).
GENESIS OF EUCANTIGENS IN TISSUE

The problem of origin of the Euc antigens on the surface of the epithelial and endothelial cells is complicated by the fact that the absorption of antigen from the surrounding fluid onto the cell surface can be accomplished experimentally. It would seem doubtful whether the concentration of group substance in plasma and tissue fluid is sufficient to be a factor, although in salivary glands and in breast the secreted antigen may contribute to the outlining of the glandular epithelium. The most convincing circumstances arguing for the generally autostimulatory character of cell wall antigens, however, is their appearance in the hosts long antedating that of the water soluble forms and their presence in non-secretor locations devoid of the water soluble substances (Sakuma, 1964).

CONGENITAL TRANSFUSIONS AND EUCANTIGENS

The effect of congenital transfusion and Euc antigens is being studied for a long time. The initial studies indicated that ABO antigens were not affected by malignant process. Further studies indicate that malignant transformation is essentially associated with antigenic loss (Key, 1957; Navratil...
et al., 1968). And immunogenic status has been studied in tumors of different tissues separately.

Studies on gastrointestinal tract malignancies as a whole (Coon, 1968; Davidson et al., 1966; Cooper et al., 1970), oral malignancies (Dahlestein and Pindborg, 1973; Dahlestein et al., 1973; Gupta et al., 1981), stomach malignancies (Dank et al., 1976; Fein et al., 1977), colon malignancies (Schoenfeld, 1978; Cooper et al., 1970; Cooper et al., 1975), lung malignancies (Davidson and Li, 1969), nasopharyngeal malignancies (Hocking et al., 1974), laryngeal malignancies (Lee et al., 1977), ear, nose and throat malignancies (Beyani et al., 1972), breast malignancies (Torti, 1963; Gupta and Schewie, 1973; Attewell et al., 1980), skin tumors (Englund et al., 1978; Nicholas et al., 1980), malignant effusion (Smith et al., 1980), white blood cell cancers (Scikhnan and Ghazally, 1976), urinary bladder malignancies (Alley et al., 1978; Emoto et al., 1979; Lines et al., 1979; Emoto, 1979), prostate malignancies (Gupta et al., 1978), pancreas malignancies (Davidson et al., 1971), endometrial malignancies (Gupta, 1978), salivary tumor malignancies (Englund and Davidson, 1972), uterine cervix malignancies (Davidson et al., 1969; Davidson et al., 1972; Stadie and Wethington, 19...
1972; Lill et al., 1976; Bergfeldt and Pellaschew et al., 1976) and trophoblastic aneuplasms (Mittal et al., 1973) have been carried out. Antigenic loss of varying degree has been found in almost all of them and in many of them it was parallel with the degree of aneuplasia and dedifferentiation.

Loss of antigen does not occur only in malignancy. It has also been demonstrated in oral mucosa in wound healing, atypia in premalignant lesions (Bablisten and Pulling, 1971; Bablisten and Pellaschew, 1974; Bablisten et al., 1975), in adenomas of parathyroid glands (Welshing et al., 1979), in colon having adenomatous polyps and/or long standing chronic mucosal inflammations (Cooper et al., 1979; Sheahan, 1978) in breast having benign proliferative duct lesions associated with fibrocystic disease (Sheahan, 1980) in urinary bladder mucosa having carcinoma in situ (Weinstein et al., 1978) and in tissues after several passages. In cultures, Japanese (1980) and Cheahin (1985) reported that addition of carbohydrate essential for synthesis of N-3 & 0, to the culture restored ability of the cells to produce antigen.
The vast literature on ABO isoeantigens status of various tissues in normal, neoplastic and nonneoplastic conditions indicates that loss of ABO isoeantigen may serve as an early marker for neoplastic transformation (Davidson, 1972; Palki and Pictet, 1970). The uniform expression of ABO isoeantigen by epithelial lining type cells and general absence in nonneoplastic connective tissue suggests that ABO isoeantigen expression may be related to epithelial differentiation. Absence of ABO isoeantigen in least differentiated basal layer of stratified squamous epithelium and presence in more differentiated superficial layers support the concept of ABO isoeantigen expression as a marker of differentiated epithelial cell function (Davidson et al., 1969). As the loss of normal surface antigen from neoplastic cell may play a significant role in abnormalities of cell recognition such as escape from immune surveillance and loss of contact inhibition (Strunshan et al., 1965), the loss of isoeantigen is not an all or none phenomenon as both positively reacting and negatively reacting cells in NCA A R are frequently found in carcinomas. This is probably an evidence of progressive loss in the course of malignant transformation (Davidson and Ni, 1970).
In locations like gastrointestinal tract, ovary and epididymis etc. carcinoma is seen as a rule as a fully developed lesion, only rarely is transition from benign to malignant is encountered.

RELATION OF ANTIGENIC LOSS WITH METASTASIS

It is reasonable to assume that radical change occurs in cancer cell before it is released from the tissue and the site of its origin, to grow and multiply at a new location. Any morphologically demonstratable criteria to distinguish the cancer cell that any succeed in overcoming body's defense and form distant metastasis is not yet known. Loss of tissue ABC antigen proceeds the formation of distant metastasis in squamous cell carcinoma in uterine cervix, and squamous cell carcinoma, oat cell carcinoma, adenocarcinoma and anaplastic carcinoma of breast (Davidson and Ni, 1970) as the loss might be connected with impairment of normal control which limits the cell within the border of the organ of their origin with resultant dissemination of cancer cells and possibility of metastasis (Vassilis et al., 1983).

IDENTIFYING AND COUNTERING MEDIATION

The uterine cervix is the cancer site for the development of squamous cell carcinoma. It was shown...
For first in-depth study on the natural history of this squamous cell carcinoma provides an opportunity to follow the transition from benign lesions such as dysplasia to metastatic carcinoma through the stages of carcinoma in situ and invasive carcinoma, the study on the progressive changes in ASCI assessment status can easily be carried out on uterine cervix (Davidson, 1969).

A close relationship between the loss of cellular AB and C antigen and malignant transformation in uterine cervix has been shown (Davidson et al., 1969; Davidson et al., 1973; Smith et al., 1973; Sypecht et al., 1976). The degree of morphologically demonstrable cellular anaplasia and the degree of loss of antigens were parallel (Davidson et al., 1973).

In metaplastic and dysplastic epidermal lesions, antigens could be demonstrated consistently in amount and distribution compatible with that seen in normal epithelium. In metastatic carcinoma of the uterine cervix, antigens could not be demonstrated in primary as well as in metastatic lesions. In the intermediate group including carcinomas in situ and early invasive carcinoma, antigens were absent from the cells exhibiting cytological signs of malignancy.
Low density in the distribution of indicator red blood cells and putative parasit type of neovascular in NEAR were explained by the heterogeneity of cellular population of early carcinoma with resulting variation in the ability to produce or to share antigens. It has been suggested that loss of antigens in an early indicator of these cellular changes that are the prerequisite for ability to form metastasis (Davidson et al., 1975; Davidson et al., 1973).

The fate of purely benign and reversible lesions such as metaplasia and more serious lesions such as severe dysplasia and carcinoma-in-situ of uterine cervix has widely been studied. Dysplasia especially of severe degree is known to lead frequently to invasive carcinoma. Hill et al., (1975) studied the relationship of ABO antigens with dysplasia of uterine cervix and demonstrated that loss of ABO antigen did not correlate with the morphological grading of dysplasia as was not of significant value in diagnosing dysplastic lesions of cervix.

Mixed differences of opinion exist as to the pathological diagnosis of cervical biopsies. In such cases of disputable lesions, as regards their
benignness or malignant behaviour, a demonstrable loss of ABC antigen in tissues by SICA technique will greatly facilitate interpretations of cervical malignancies. On the other hand the presence of antigens will indicate benignness of the lesion. In SICA negative cases immunodiffusion technique involving use of labelled IIFC will further substantiate the findings of SICA and the diagnosis of cervical neoplasia.

Morphological examination of cervical dysplasia can not yet predict which lesion will progress to invasive carcinoma and which will regress. This applies also at present time to the immunological methods like SICA (Davidson et al, 1970).

Carcinoma of the uterine cervix is the commonest cancer in females in poor countries like Peru, China and India. The incidence of carcinoma cervix at J.N. Cancer Institute, Bombay was between 25.4 to 26.7% during 1971-76 and all cervical malignancies the incidence of invasive carcinoma cervix was found to be 94.3% (Upadhye et al, 1981). The use of SICA on cervical lesions may considerably increase the diagnosis of early stage cancer at which stage complete cure is possible.
Material and Method

For the study 96 samples of cervical lesions from the records of, and the fresh surgically removed specimens received in the Histopathology Section of Pathology Department of N.I.E. Medical College, Jammu, were selected to represent inflammatory non-neoplastic lesions, dysplasia of mild, moderate and severe degree and invasive carcinoma of well, moderate and poor differentiation. One representative block of each case was chosen out. Out of ninety six cases:

Forty six cases were of invasive carcinoma.
Twenty five cases were of dysplasia and
Twenty five cases were of inflammatory lesions.
Blood group of some of the specimens which were removed by major surgery requiring blood transfusion, were found out from the records of Blood Bank of N.I.E. Medical College Hospital, Jammu. In cases of fresh specimens blood sample of the patients were taken and the blood grouping was done by slide method. In rest of the cases, blood groups were determined by specific red cell agglutination (SCA) reaction by treating one of the two sections of the same case by anti-A serum and NAC of group A and another by anti-B
serum and RBC of group B. Positive reaction in either indicates the blood group A or B. Positive reaction in both indicates that the blood group is AB and if none show positive reaction, the blood group is 'O'.

In all the cases, SRCA test was done and in cases immunofluorescence (IF) technique was also applied.

Brief clinical findings, blood group, histological findings and the results of SRCA and IF were recorded on a planned proforma

**SPECIFIC RED CELL AGGLUTINATION REACTION (SRCA):**

**PRINCIPLE:**

The test is based on the three layered sandwich reaction, described by Davidson (1979) in which homologous bivalent or polyvalent antiserum act as the connecting link between the antigen A, B or H present on the tissue as well as on indicator RBC (Fig. No. 1).

In the three layered sandwich:

- the bottom layer = is of tissue,
- the middle layer = is of homologous blood grouping antiserum and
- the top layer = is of homologous indicator and blood cells.
Diagrammatic representation of specific red cell agglutination reaction.

Blood group isoaantigen.
Epithelial cell.

Anti-bora containing homologous antibodies.

Blood group antigen.
Red blood cell.

Three layered sandwich technique (Figure No.1)
MATERIAL:

The following material was used:

TISSUE:

Five micron thick sections from each block were mounted on separate microscope slides smeared with egg albumin. De-paraffinisation was done by passing the mounted sections through xylene, 90%, 50% alcohol and water for a short duration of time.

ANTISERA:

Commercially prepared anti A, anti B and anti AB sera with a titre of 512 and anti H sera with a titre of 256 were used. Anti-A, anti-B and anti-H sera were purchased from Associated Laboratories, Bombay and anti AB serum from Ocean Dispensary, Surat.

Indicator red blood cells:

Blood samples belonging to group A, B, AB and O were taken. RBC were washed in three changes of physiological saline and 5% suspension of RBC of each groups were prepared in the same saline.

Physiological saline:

0.9% sodium chloride solution in distilled water was prepared in the Chemical Laboratory of Pathology Department of K.Z.M.R Medical College, Jhansi.
PROCEDURE:

The test was performed in batches of 3-10 cases.

Each slide was treated in the following manner:

1. The slide, mounted with tissue section, was placed on a moist filter paper and antisera was poured on the section and was covered with a petri dish for 10 min. at room temperature.

2. The uncombined antisera remaining on the surface of the section was washed off in three changes of physiological saline each lasting for 10 minutes.

3. The excess saline was drained off and the individual slide was returned to the moist filter paper and covered with a suspension of indicator RED for 10 min. at room temperature. Slide was covered with petri dish in order to avoid drying.

4. Another petri dish was filled with minimal amount of physiological saline and the slide was turned upside down with a brisk movement and as such placed immediately on the two supporting wooden sticks in the saline filled petri dish so that it just touched the saline.

5. After a few min, the slide was shifted over a clear area and after allowing 20-30 min. for indicator RED that did not react specifically with the antisera,
to become detached and sink to the bottom of the petridish slide was finally moved aside on a clear area.

6. The slide still remaining in the petridish was then examined with low power of microscope through the thickness of the slide with tissue section remaining on the lower surface using 1X and 10X eye pieces.

CONTROL:

To ensure that the ANCA reaction were specific, the following controls were applied:

A. Tissue controls:
   1. Intrinsic positive control
      1. Endothelial lining of blood vessels.
      2. ASC present in the section.
      3. Epithelial cells of nasal tissue adjacent to lesion.

   II. Intrinsic negative control - connective tissue.

B. Patient controls:
   1. Heterologous antisera and homologous ASC were used e.g., In group A section anti-B serum and group B ASC were used.
   2. Heterologous antisera and homologous ASC were used e.g., In group A section anti-A serum and group B ASC were used.
3. Blood grouping by MNH reaction also served as a control.

**INTERPRETATION:**

'•' Negative - no adhesion.

'X' Weak positive - the result patchy with some areas show clear adhesions while other areas show no adhesion. Also included in this group are sections that show adhesion only in lower or top third of epithelium.

'••' Weak positive - all the cells do not show adhesion but weakly positive. Adhesion is diffuse not patchy.

'•••' Moderately positive almost all cells show adhesion.

'••••' Strongly positive - over crowding of adhered red blood cells.

**METHODS:**

Immunofluorescence (IF) staining technique is based upon a double layer fluorescence staining method used on sections cut from formalin fixed paraffin embedded tissues as described by Giese and Kaplan (1950) (Fig. 4a, 3).

In the double layer fluorescence staining the first layer is of homogenus blood grouping.
Diagrammatic Representation of Immunofluorescence Staining

Blood Group Isoantigen Epithelial Cell

Anti Serum Containing Homologous Antibodies

Anti-Human IgG Conjugated with Fluorescein Isothiocyanate

Double Layer Staining Technique
 antisera and
the second layer is of antihuman IgG (Coat),
conjugated with fluorescein
isothiocyanate (FITC).

MATERIAL:

Tissue:
Same as in SRCA reaction.
Antisera:
Anti-A, anti-B and anti-AB antisera as used in
SRCA reaction.
Conjugate:
Commercially prepared Goat antihuman IgG conjugated
with fluorescein isothiocyanate (FITC) was purchased
from DECRAH CORPORATION, Mumbai.
Phosphate Buffer saline (PBS) of pH 7.1:
Was prepared in the Chemical Laboratory of the
Pathology Department of M.A. Medical College,
Jhansi, using the following recipe:

- NaCl: 0.9 g
- Na₂HPO₄ (monohydrate): 1.87 g
- KCl: 0.13 g
- Distilled water: 1 liter

Reagents:
Glycerol and PBS in equal parts,

PROCEDURE:

1. Slides were incubated with appropriate antisera in
a moist chamber at room temperature for 20 minutes,
3. Slides were washed in three changes of PBS, each lasting for 5 minutes.

4. Slides were incubated for further 20 minutes with FITC (in 1:4 dilution in PBS).

5. Slides were washed again in three changes of PBS, each lasting for 5 minutes.

6. Slides were mounted in glycerol mountant and studied by fluorescence microscope.

A "Leitz" fluorescence microscope fitted for incidental illumination with Filter pack 2 (quack-change exciter mirror filter turret) in the microscope tube was used. The light source was 200W ultra high pressure mercury lamp. Immersion type objectives (10X and 25X) and low power oculars (4X) with built-in filter for protecting the eyes were used. Primary filter was FITC interference blue filter.

CONTROL:

A. Tissue control: same as in SCCA section.

B. Reagent control: Negative antisera was used which served as negative control.

INTERPRETATION:

+- Positive - showing apple green fluorescence.

-+ Negative - showing no fluorescence.