REVIEW OF LITERATURE
REVIEW OF LITERATURE

Antigens identical to the ABO (H) blood group antigens are present on the leucocytes and platelets along with red blood cells. These antigens can also be detected from various body fluids and tissues (Wintrobe, 1975).

According to Davidson (1971) these blood group antigens are present in glandular secretions of 80% of persons who are known as "Secretors". In secretors the blood group substances are present in tissues in alcohol soluble as well as water soluble forms. These two forms are serologically same, but only biochemically different. In remaining 20% of non secretors, blood group specific substances are present only in alcohol soluble forms.

Along with secretions and excretions the blood group specific substances are also found in some normal tissues. These includes endothelial cells lining of blood vessels and lymph vessels, squamous epithelium of oropharynx, portio vaginalalis of cervix, columnar epithelium of gastrointestinal tract and bronchi, stratified squamous epithelium of skin, transitional cell epithelium of urinary bladder and epithelial cells of exocrine glands of pancreas etc (Szulman, 1961). These serve as built-in-positive control. The angifens are absent in the sebaceous gland and basal
layer of squamous epithelium, connective tissues, hepatocytes, Islets of Langerhan's in pancreas (Szulman, 1961) these serve as a built-in-negative control.

The blood group antigen are either produced or stored by the normal cell. They serve as a "tracer" substance for the study of changes in malignant transformation. According to Davidsøhn (1971) various organs which serve as built-in-positive control have been studied for the loss of antigens during malignant changes. Isolated cancer cells and culture of cancer cells also show the loss of antigen, which is interpreted as evidence of physiological and morphological dedifferentiation in cancer. Cancer cells these give a negative reaction are assumed to have lost the ability to produce antigens or to store them. This loss is not an all or none phenomenon. It can be explained as progressive loss of antigen in the course of cancerous transformation and this loss of antigen is parallel to the degree of anaplasia and pre-requisite for formation of distant metastasis (Dabelstein et al 1971; Davidsohn et al 1969; and Stall et al. ).

Tumour development is a play with many different dramatic personas. The main character is still the cell. It is a protean figure that can appear in many different forms and play different roles, most of which can classified as
stages in microinvolutionary process, known as tumour progression. The initiation of tumour development is most likely to involve changes at the genetic level. On its road to progression the neoplastic cell encounters many predators, including various effectors of immune system. Interalia it will depend on host genetics, on age and all of the many physiological modulators of immune system (Klein, 1980).

The state of knowledge of immunologic aspects of cancer till 1935 was not encouraging, mainly because the lack of specificity of the early tests made them useless and only too frequently misleading. "Very little worthwhile material remains after one bills down to essentials the vast literature that has accumulated on the subject of serologic diagnosis of cancer during the last 35-40 years" (Davidsohn, 1936). This was referred to the first 40 years of this century. Since 1935 solid progress has been made in immunology of cancer (Davidsohn, 1972).

**Genetics, Immunochemistry and Distribution of Blood Group specific antigens :**

**GENETICS AND IMMUNOCHEMISTRY :**

Out of total of 60 human blood groups antigens, only a few have been studied beyond their serological
behaviour and mode of genesis. Immunological character
have been analysed in ABC, Lewis, MNSs blood group
systems to date.

First human blood group system - A, B, O system
was discovered at the beginning of this century (Landsteiner
et al 1901). The ABC antigen are inherited as simple Mande-
lian characters. In this system genes controls the arrange-
ment of sugar residues - that determines blood group speci-
ficity. The blood group of an individual depends on the
presence of two out of three alleliagens A, B, and O. The
antibodies reacting with AB antigen are regularly found
in the serum when the corresponding antigen is absent from
the red cells. These antigens occur not only as surface
components of red cells, but also are found in water soluble
forms in various tissue fluids and secretions. The A, B and
H antigens present on the surface of red cell are predomi-
nantly glycolipids, their soluble counterparts in secretions
are glycoproteins. The capacity to secrete these substance
is under the control of a pair of alleliagens Se-se, Se is
dominant over se and only homozygous se se individuals are
therefore "nonsecretors" of A, B, H substance (Wintrobe, 1975).
**BIOCHEMICAL ASPECTS OF ABO ISOANTIGENS**

**Water soluble substances**

Biochemically the alcohol soluble and water soluble ABO isoantigens are glycoproteins and glycolipids respectively and the group specificity is associated with carbohydrate moiety that constitutes about 85% of the molecule, and an aminoacid moiety that make up the remaining 15%. The peptide resudies are always composed of the same 15 aminoacids and four of these threonine, alanine, serine, proline make up 2/3rd of all the aminoacids present. These peptides has structural functions only and form a film, spiny back bone to which a large number of relatively short oligosaccharides chains, constituting the blood group substances are attached at intervals. The carbohydrate moiety of all the ABO substances is qualitatively similar in composition. Each contain a methyl pentose, L-fucose, a-hexose, D-galactose, two aminosugars, N-acetyl-D-glucosamine and N-acetyl-D-galactosamine, and a nine carbon sugar, N-acetyl neuraminic acid. One of these sugars appears to be immunodominant in each of the blood group substances studied. In a substance, some of the chains have a terminal N-acetyl-D-galactosamine residue, joined in alpha-glycosidic linkage to the next sugar, and this terminal non-reducing sugar unit is an important part of the structure of the A determinant. The B specificity is similarly associated with a
terminal non reducing D-galactose residues joint in a
alpha-linkage to the next sugar. Both the N-acetyl-D- galactosaminyl and D-galactosyl residues are in the
pyranose forms. In H substance an alpha-fucosyl residue
is an important part (Wintrobe, 1975).

The appearance of ABO antigens begin with a
precursor mucopolysaccharide substance which is further
modulated into H substance and the H substance into AB
antigens under genetic control. The genes responsible
for this conversion regulate enzyme production for cata-
lyzing the transfer of sugar. The L-N acetyl-D-galactosa-
minyl and D-galactosyl transferases are the enzymes which
are necessary for the conversion of H substance with A
and B substances respectively (Watkins, 1966).

Cellular ABO substances:

These are difficult to isolate most of the
cellular extracts of the surface of RBC are glycolipids,
although some glycoproteins residues have been found as
well. The glycolipids are the compounds containing carbo-
hydrate moiety, jointed through sphingosine, to fatty acids.
The active glycolipids contain in addition to fatty acid,
sphingosine and glucose, the same five sugars that are
present in glycoproteins - Galactose, glucosamine, galac-
tosamines fucose and sialic acid, thereby accounting for
the similarities in the specificity of antigenic determinants on the red cells and in secretions (Wintrobe 1975).

**DISTRIBUTION:**

It has been investigated by Hartman (1941) using haemagglutination inhibition, complement fixation and similar other tests. The strongest blood group activity was associated with mucus secretions. Coomb's et al (1956) demonstrated blood group substances on free or degenerated cells. True histological localization was done by immunofluorescence (Coon's and Kaplan, 1950).

The ABH substances are broadly distributed into cell wall and as a water soluble entities in secretions.

**Water soluble form of A.B.H. antigens:**

Mucus born ABH and Le\(^a\) antigens represent the mucopolysaccharides and have been found in mucus secretions, columnar and goblet cell membrane and mucus acini. Thus sites like salivary gland, mural glands of trachea and oesophagus, stomach, tongue and small intestine, gall bladder, appendix, uterine cervix, ovarian mucus cystadenomas are found to be rich in blood group substances (Szulman, 1960).
In embryonic and foetal life, Szulman (1964, 1965) found blood group substances in the secretions of stomach and salivary gland at the age of 5-60 mm embryo and foetuses to the end of the first trimester of intrauterine life. These substances persists throughout the life. Kent (1964) suggested absence of 'A' in favour of 'H' substances, indicates non-conversation of all or small amounts of substances and represent a stage in a biosynthesis of A antigen. A₂ group person characteristically carry large proportions of "unfinished" H- chains (Watkins and Morgan, 1956-57).

The secretor and non-secretor phenomenon can be most simple interpreted by viewing the non secretor recessive gene (se se homozygous condition) as inhibiting the production of some of the water soluble forms of the blood group substances. Since the gene was never found responsible for dissociation of the H and A,B antigens, its inhibitory action is thought to be brought at the point of formation of the basic H substances or its precursor (Szulman, 1962).

Glynn et al (1957), Szulman (1960-1962) and Kent (1964) described the formation of ABH substances in the deeper glands of stomach, small and large intestine irrespective of secretor status. In non-secretors Leₐ substance in large amounts is confined to the superficial surface of mucosa, but slight in secretors (Glynn et al, 1957).
Eklund (1963) using double layer immunofluorescence described "secretor" pattern of ABH activity in secretor and non-secretors both.

Pancreatic (exocrine acini), the sweat gland secretions which are not under the control of secretor gene (se se or Se se), and breast, uterine, prostatic secretion which are under the control of secretor gene are also shown that breast secretes little substance except in pregnancy and lactation.

Table: Distribution of water soluble antigens (Szulman, 1960)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Organ</th>
<th>Secretor</th>
<th>Non-secretor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>MUCOUS BORN ANTIGENS</strong></td>
<td></td>
</tr>
<tr>
<td>1-</td>
<td>Salivary gland</td>
<td>Mucous acini</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>Stomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i)</td>
<td>Corpus</td>
<td>Surface epithelium, feveolae</td>
<td>Deep parts of feveolae</td>
</tr>
<tr>
<td>ii)</td>
<td>Pylorus</td>
<td>Surface epithelium, gland</td>
<td>Deep parts of glands</td>
</tr>
<tr>
<td>3-</td>
<td>Small bowel</td>
<td>Goblet cells of villi and crypts, Brunner's glands of duodenum.</td>
<td>Goblet cells in deeper parts of crypts, Brunner's gland.</td>
</tr>
<tr>
<td>4-</td>
<td>Colon</td>
<td>Goblet cells throughout the thickness of mucosa. Number of active goblet cells decreases rapidly post hepatic flexure.</td>
<td>-</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Organ</td>
<td>Secretor</td>
<td>Non-secretor</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------</td>
<td>-----------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>5</td>
<td>Appendix</td>
<td>Goblet cells throughout the thickness of mucosa</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Gall bladder</td>
<td>All mucous secreting cells of lining epithelium</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Respiratory passage</td>
<td>Goblet cells of trachea and bronchi. Mucous glands.</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Uterus, cervix</td>
<td>Mucous secreting epithelium glands.</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Ovarian cyst (Pseudomucinous)</td>
<td>Cells of lining epithelium secreting pseudomucin</td>
<td>-</td>
</tr>
</tbody>
</table>

**Antigens in Other Secretions and Excretions**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Secretion Type</th>
<th>Details</th>
<th>Non-secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Breast</td>
<td>Occasional acini or groups of epithelial acinar cells</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Endometrium</td>
<td>Glands in late proliferative phase</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Kidney</td>
<td>Collecting tubules, calyces</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Pancreas</td>
<td>Exocrine acini</td>
<td>Exocrine acini</td>
</tr>
<tr>
<td>5</td>
<td>Prostate</td>
<td>Single and small groups of acinar cells</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Sweat glands</td>
<td>Cells of secretory coils</td>
<td>Cells of secretory coils</td>
</tr>
</tbody>
</table>

**Cellular ABO Antigens:**

**Cell wall of endothelium:**

Szulman (1964, 1965) demonstrated ABH substances throughout the cell wall of endothelium of cardiovascular system.
Cell wall of epithelium:

Cell wall of stratified epithelium (in skin), non cornifying stratified squamous epithelium and transitional epithelium contain blood group substances (Szulman, 1962). Transitional epithelium of lower urinary tract possesses ABH antigens in cell wall and is demonstrated by MCAR (Coomb's et al, 1956), and by immunofluorescent method (Glynn and Holborow 1959, Szulman 1964). In the development stages in embryos they are present in most of the epithelial cells including mesonephric and Mullarian ducts in smallest embryo (5-36mm, 6-8 weeks ovulation age) Szulman 1964, 1965 and finally they disappear from organs sequentially until a vestigial stage of extra uterine life is reached coinciding with the morphological and functional advancement towards final adult model of organ.

Various other cells:

Ova and sperms are, in situ, free from ABH substances. A secretor acquire ABH antigens from seminal plasma and non-secretors one negative.

Perietal cells of stomach has abundant granular A,B substance, but no 'H' antigen, even in group 'O' persons. In tissue culture 'H' substance has been demonstrated in
Hela cells and AB antigens in short term culture of human foetal cells by Hogman (1960). Hogman in 1960 demonstrated that foetal cells containing antigens are capable of fixing the homologous antibody, which trend to disappear after 8-24 hours and can be recoated.

**GENESIS OF ISOANTIGENS IN TISSUES**

The problem of origin of the ABC isoantigen 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 autochthonous character of cell wall antigen, however, is their appearances in the embryo long antedating that of the water soluble forms and their presence in non-secretor location devoid of the water soluble substances (Szulman, 1964).
VARIOUS METHODS OF DEMONSTRATION OF ABO ISOANTIGENS:

The presence of ABO isoantigens in cells and tissue other than erythrocytes was first demonstrated through the use of an agglutination inhibition test (Kritschewsku et al, 1927; Landsteiner, 1926). In oral mucosa their presence was shown by absorption of iso-agglutinins in water soluble extracts of oral epithelium (Yosida, 1928).

Immunofluorescent (IF) staining technique (Coons and Kaplan, 1950), mixed cell agglutination reaction (MCAR) Glynn et al, 1957) and immunoperoxidase (IP) staining technique (Avrameas, 1969) were later used for their demonstration in tissue cells.

MIXED CELL AGGLUTINATION REACTION (MCAR) OR
SPECIFIC RED CELL ADHERENCE (SRCA):

Mixed cell agglutination reaction was first described by Topley and Wilson (1935) as quoted by Milgrom et al (1964). Later it was employed in serological testing (Finland and Curnen, 1938) Weiner and Harman, 1939), cell suspension (Coombs, 1961), in tissue cultures for the recognition of the species of origin of cell (Coombs et al, 1961). In the ABO grouping of human cells in culture (Hogman, 1960; Chessin et al, 1965) and in studying tissue antigen (Coombs et al, 1956; Cowan, 1962; Milgrom et al 1964).
Tonder et al (1966) used MCAR in frozen sections in order to preserve alcohol soluble antigens. Later Davidsohn and Ni (1970) reported that the test could be done on frozen sections of fresh and formalin fixed tissues, on section of recent and old paraffin blocks and on old and new H & E stained and decolorized slides for reaction. Moreover age of the sections and the paraffin blocks do not affect the sensitivity and specificity of the test. The principle of mixed cell agglutination reaction with tissue sections is very much similar to the principle of indirect immunofluorescence technique. In both the methods the antibodies are bound to the tissue sections and are localised by the corresponding antigammaglobulin. In immunofluorescence (IF) technique, the antigammaglobulin is 'labelled' with fluorescent dye, while in mixed cell agglutination technique it is labelled by RBC. Davidsohn et al (1971) stressed that positive reaction is not the amorphous clumping of agglutination but adherence and for this reason they used the term specific red cell adherence (SRCA) in lieu of mixed cell agglutination reaction.

MCAR on formalin fixed paraffin embedded tissue has been reported to be more sensitive than IF technique (Davidsohn et al, 1969) where as Debelsteen and Rygaard (1972) suggest that IF technique seems to be as sensitive as the MCAR but is superior to the later in allowing more
accurate localization of the antigens. The results, obtained by Holborow et al (1960) with MCAR, are in close association with those seen in frozen sections with IF technique. Negative IF test, but positive SRCA has been reported by Coombs (1956); Hogman (1960); Holborow et al (1960); Chessin (1965). Holborow et al (1960) explained the discrepancies by quantitative factors and higher sensitivity of MCAR.

It has been shown that the ABO isoantigens in tissue are not influenced by formaline fixation and paraffin embedding procedures, therefore immunofluorescent staining immunoperoxidase staining and specific red cell agglutination reaction can be successfully used in sections prepared from formalin fixed and paraffin embedded tissues (Kovarik et al, 1968; Dabelsteen and Rygaard, 1972). Dorsett and Ioachim (1978) have suggested that Bouin's fluid in the better fixative for immunofluorescent staining studies as in it the antigens and the antibodies are better preserved. Quantitatively ABO (H) isoantigens differ widely in their concentration in different tissues and quantitative analysis as such is not very much helpful in early diagnosis of malignant lesions (Dabelsteen, 1972).
The structural pattern of a malignant tumour cell is sufficiently distinct from the normal cell to be identified in most instances. Subjective differences in their interpretation are always possible, especially in severe dysplasia and intra epithelial neoplasia. The limiting factor in the cure of carcinoma is early diagnosis. Serological methods for diagnosis of malignant tumours (Davidsohn, 1936) are of historical importance and non-give promising results.

In the recent past much emphasis has been given on the immunological aspect of neoplasia. The antigenic constitution of the tumour 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 (Coombs, 1961).

The depletion of specific antigen within tissues has been considered an important factor of neoplastic behaviour, both in experimentally induced tumours in animals and spontaneous tumours in human. A loss of antigenicity of intercellular substance and basement membrane was observed in benign, premalignant and malignant tumours of epidermal origin and it has a parallel course with the grade of cellular anaplasia (Varelzidis et al, 1980).
With higher sensitive techniques A.B.O. isoantigens can serve as traces antigen for the study of changes in malignant transformation.

The effect of cancerous transformation on ABO isoantigen has been studied. The initial studies indicated that ABO isoantigens were not affected by malignant process. Further studies indicated that malignant transformation is essentially associated with antigenic loss (Kovarik et al, 1968). ABO isoantigenic status has been studied in tumours of different tissues separately.

Antigenic loss of varying degree has been found in almost all of them and in many of them it was parallel with degree of anaplasia and dedifferentiation.

Loss of isoantigen does not occur only in malignancy. It has also been demonstrated in wound healing, atypia to premalignant lesions (Dabelsteen et al, 1975), in adenomas of parathyroid glands (Woltering et al, 1979), in colon having adenomatous polyps and/or long standing chronic mucosal inflammation (Cooper et al, 1979; Sheehan, 1979), in breast having benign proliferative duct lesions associated with fibrocystic diseases (Strauchen, 1980), in urinary bladder mucosa having carcinoma in situ (Weinstein et al, 1978) and in tissue after several passages. In cultures, Hogman (1960) and Chessin (1965) reported that addition of carbohydrate essential for synthesis of A, B & O to the culture restored ability of the cells to produce antigen.

The vast literature on ABO isoantigenic status of various tissues in normal, neoplastic and non-neoplastic conditions indicate that loss of ABO isoantigen may serve as an early marker for neoplastic transformation (Davidsohn, 1972; Feizi and Picard, 1978). The uniform expression of ABO isoantigen by epithelial lining cells and general absence in connective tissue suggests that ABO isoantigen expression may be related to epithelial dedifferentiation. Absence of
ABO isoantigen in least differentiated basal layer of stratified squamous epithelium and presence in more differentiated superficial layers support the concept of ABO isoantigen expression as a marker of differentiated epithelial cell function (Davidsohn et al, 1969). So the loss of normal surface antigen from anaplastic cell may play a significant role in abnormalities of cell recognition such as escape from immune surveillance and loss of contact inhibition (Strauchen et al, 1980).

The loss of isoantigen is not an all or none phenomenon both positively reacting and negatively reacting cells in MCAR are frequently found in carcinomas. This is probably an evidence of progressive loss in the course of malignant transformation (Davidsohn and Ni, 1970). In locations like gastrointestinal tract, ovary and epidermis etc. Carcinoma is seen as a role as a fully developed lesion.

RELATION OF ANTIGENIC LOSS WITH METASTASIS:

It is reasonable to assume that radical change occurs in a 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 demonstrable criteria to distinguish the cancer cell that may succeed in
overcoming body's defence and form distant metastasis is not yet known. Loss of tissue ABC isoantigen precedes the formation of distant metastasis in squamous cell carcinoma in uterine cervix, and squamous cell carcinoma, oat cell carcinoma, adenocarcinoma and anaplastic carcinoma of bronchus (Davidsohn and Fi, 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 (Varelzidis et al, 1980).

ISOANTIGEN AND ORAL CAVITY NEOPLASIA:

The presence of blood group substances A and B in oral epithelium has been described by several authors (Yosida 1928; Holborow Brown, Glynn, Coombs, 1960; Szulman, 1960; Brandtzaeg, 1965).

The blood group substances in this squamous epithelium are found exclusively in the spinous cell layer, whereas the basal cell layer and the keratinized cell layers are negative (Szulman, 1969; Brandtzaeg, 1965; Davelsteen, 1972).

Among different individuals there exists a variation in expression of A antigen of buccal epithelial cells.
(Dabelsteen, 1972). Furthermore, Brandtzaeg (1965) has described that the epithelium in the deeper parts of the gingival crevice reacts weakly as compared to the surface epithelium of the oral mucosa. However, systematic investigation comparing the amount of blood group substances in different areas of the human oral mucosa are not available.

A variation in reactivity in different areas of normal epithelium would be important, as a loss or decrease in reactivity of antigens has been reported to occur in neoplasms originating from epithelium in which such substances are normally present (Davidsohn 1972; Dabelsteen & Pindborg, 1973) and it has been suggested that the loss of blood group antigens may be used in the diagnosis of early malignant diseases (Davidsohn, Kovarik & Ni, 1969; Dabelsteen and Fulling, 1971).

Expression of blood group antigen on oral epithelial cells taking part in wound healing is markedly decreased. This decrease is very similar to what is seen in oral carcinomas. Whether this change is caused by a masking of antigen by over production of cell coat or by an all over chemical change of the carbohydrate in the coat of the moving cells is not known (Dabelsteen et al, 1974).
It is however, more probable that the antigen changes found in the dysplastic lesions are associated with other factors, such as cell movement and growth rate, rather than malignancy per se. That blood group antigen expression and neoplastic transformation are not necessarily interdependent is demonstrated by the presence of blood group antigens in a few oral carcinomas and by the loss of antigens on epithelial cells taking part in wound healing (Dabelsteen et al, 1975).

These tissue isoantigens ABO (H) can be demonstrated readily by using antisera against A & B antigens by SRCA (specific red cell adherence) test (Kovarik et al 1966 and Tonder et al 1964). This method has been regarded as most sensitive and simple and the results are reproducible.

The use of SRCA in oral lesions may be considered as an early diagnostic tool in diagnosis of oral malignancies and to have a better prognosis.

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**FIG-1** THREE LAYERED SANDWICH REACTION, PRINCIPLE OF S.R.C.A. TEST.