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Changes in Reactivity of Blood Group Substances a and B on Cell Surfaces of Neoplastic Tissue of the Oral Epithelium

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CHANGES IN REACTIVITY OF BLOOD GROUP SUBSTANCES A AND B ON CELL SURFACES OF NEOPLASTIC TISSUE OF THE ORAL EPITHELIUM

by

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Dedicated to

My Wife

for the lasting patience and many sacrifices made in order to further my education.

LIFE

Richard C. Prendergast was born in Kankakee, Illinois on November 26, 1936. At the age of two months he was moved to Chicago where his childhood was spent.

His secondary schooling was taken at Mount Carmel high school in Chicago. He enrolled at the University of Notre Dame in September, 1954, and graduated in June 1958. He attended one year of night school at Loyola University from September 1958 to August 1959. In September of that year he entered the Chicago College of Dental Surgery, Loyola University and graduated in June 1963. The next two years were spent in the United States Navy. In July 1965 he entered into a two year program in Periodontics at the Chicago College of Dental Surgery, Loyola University, leading to a Master of Science Degree in Oral Biology.

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INTRODUCTION

It has been known for many years that blood group substances exist on surfaces other than red blood cells. The importance of this knowledge has not been fully appreciated. It is only recently that this information has been utilized to any degree.

It was soon learned that several different antigenic materials are present on cell surfaces throughout the body. The question arose as to whether there is an alteration or loss of these antigens during neoplastic changes. Subsequent research indicated that a loss of antigen does occur. Exactly how this comes about is not understood, nor is it known whether some or all of the antigenic groups on the same cell are equally affected.

It was the specific intent of this paper to show any detectable changes in the reaction of "A" and "B" blood group substances in malignant tissues of the oral cavity. It was also desired to confirm or refute previous work done in localizing these antigenic substances in normal oral tissues. However, it was not intended to categorically explain any of these changes.

REVIEW OF LITERATURE

AGGLUTINATION REACTION; NORMAL TISSUE:

Blood group substances were first shown to exist on the surface of other tissue cells as early as 1926. Landsteiner (1926) using an agglutination inhibition reaction showed that a substance identical or similar to the isoagglutinable factors A and B of human red cells, was present on human spermatocytes. In developing this technique he reacted type A red blood cells and spermatocytes from type A individuals separately with anti A sera. These two groups of cells were then mixed and incubated for several hours. Agglutination did not occur.

The mixed agglutination reaction differs from this technique in that anti sera is first reacted to the red blood cells. This complex is then mixed with the test cells. Agglutination will occur if identical antigenic material is present on both cell groups.

In the succeeding years, attempts to identify the presence of these and other antigenic materials on cell surfaces proved difficult. Yosida (1928) using a mixed agglutination reaction in attempting to show the presence of blood group substances on the cell surface of surgically excised tissue, was unsuccessful. He therefore, concluded no such material existed. Co Tui, Campbell Rathburd, and Fand (1954) using a tissue explant technique

in which excised tissue cells were cultured through succeeding generations in test tubes, were also unable to show the presence of blood group substances on cell surfaces.

Then in 1957 Nelken, Gurevitch and Neuman, improving on the technique of Co Tui et al (1954) were able to show the presence of A and B blood group substances on the cells of human epidermis. Simultaneously, Coombs, Bedford and Rouillard (1956) and Kay (1957) were able to show the presence of A and B substance on human epidermis and urinary tract tissue respectively. Furthermore, Kay was able to show a quantitative difference in the A and B antigen on the cell surface of normal and malignant tissues. He also noted with regard to normal tissue, that agglutination does not appear to be an "all or none" phenomena: that with weakly positive reactions there was usually some completely agglutinated cells, as well as many cells with a reduced complement of adherent red blood cells and a correspondingly large area of free surface. Kay further believed that while there is a loss of antigen with neoplastic change, it is highly improbable that there is a related loss of the specific gene necessary for the formation of the A and B antigenic material.

FLUORESCENT ANTIBODY TECHNIQUE:

It is interesting to note that prior to this time, most studies on blood group substances utilized an agglutination inhibition or mixed agglutination technique. However, with the

development by Coons, Creech, Jones and Berliner (1942) of fluorescein isocyanate utilized in detecting the presence of antigenic material on pneumococcal organisms, the potential of this technique for the search of blood group substances on cell surfaces was quickly realized. But because of the difficulty involved in its preparation, it was not often used. Though further refinements in handling and preparing the material were introduced by Coons and Kaplan (1950) it still remained generally unacceptable.

Then Riggs, Seiwald, Burckhalter, Downs and Metcalf (1958) developed fluorescein isothiocyanate as a labeling agent. They found this material much easier to prepare and work with. This fact was apparent from the number of men who subsequently incorporated this material in their work.

BLOOD GROUPS:

Glynn and Holborow (1959) working with fluorescein isothiocyanate found that the only groups so far isolated in a sufficient state of purity to attempt chemical characterization were A, B, Le^a, and H substance because they exist in large enough quantities in ovarian cysts to be utilized as antigens in developing the corresponding antisera. They also thought that fresh specimens would be best for use in the preparation of the antigenic material as post-mortum changes might obscure the detection of these substances.

SECRETOR STATUS:

It was further noted by these authors that the order of the tissues with regard to their content of blood group substances was the same for secretors and non-secretors, but the average was ten to twelve times greater for secretors.

Szulman (1960) using acetone fixed specimens with a quick freeze technique was able to work out in detail the distribution of blood group substances in man. His work concurred with previous work on the effects of secretor status on the distribution of blood group substances. He also found alcohol soluble antigen in small amounts practically in all tissues of individuals of appropriate blood type regardless of secretor status. The effect of secretor status was further brought out by Swinburne, Frank and Coombs (1961) when reporting on the presence of blood group substances A on buccal epithelial cells. They noted that the secretor factor only affected the quantity of antigen present and not the location with regard to these tissues.

WATER SOLUBLE AND ALCOHOL SOLUBLE GROUPS:

Swinburne et al (1961) further noted that when buccal cells from group A secretors were treated with 70% alcohol alone the reaction with human type A antisera, if anything, was stronger.

Then Brandtzaeg (1965) localized substance A and B in alcohol fixed human gingiva utilizing a direct immunofluorescent technique. He found that frozen sections were unsatisfactory for

the application of the fluorescent antibody technique.

In preparing his tissues, Brandtzaeg fixed half his specimens in pre-cooled 95% ethanol. He washed the other half in buffered isotonic saline and then fixed this tissue in 95% ethanol also.

The results of the two methods of tissue preparation showed little difference. In both A and B tissue, the pattern of fluorescence was the same although in general the A tissue reacted more strongly.

He also noted, in general, a negative reaction in the first five basal layers. This finding concurred with those of Szulman (1960).

RELATION OF H SUBSTANCE AND THE ABO SYSTEM:

Kent (1964) in working with the H antigen was able to show that both H and A or H and B antigen could be present simultaneously on respective tissues. If either A or B antigen was in great quantity, the H antigen was correspondingly weak. Watkins (1966) in working with the blood groups A, B, Le^a and H and the secretor phenomena found that the ABO and H systems worked independently. His findings on the effects of secretor status concurred with those of Glynn and Holborow (1959), Szulman (1960), and Swinburne (1961) when he noted that only the presence of A or B substance in secretions was affected.

He further noted that chemically, antigen specificities were related to non-reducing sugar groups added to a pre-existing

glycoprotein complex. He thought that different genes controlled the formation of specific glycosyl transferase enzymes that add these sugar groups to the carbohydrate chain. And that if a specific genetic substance such as the "A" antigen was isolated and treated in such a way as to remove these non-reducing sugar groups, eventually a substance identical to "H" substance would be derived.

NEOPLASTIC TISSUE:

While the mapping of the blood group substances on normal cells continued, some investigators already foresaw the possibility that a change in the reactivity of the cell surface in a malignant cell might be detectable.

Weiler (1952) described an organ specific antigen for all healthy tissues of rat liver but absent in hepatoma. He also observed a correlation in the degree of tumor and the loss of antigenicity of the tissue. Kay (1957) working with A and B tissue found that with cancerous specimens there was always a reduced or completely negative reaction in testing for the corresponding blood group.

Utilizing the single layer stain technique with fluorochromes frequently made precise interpretation of the results difficult. This was especially true when dealing with very weak antigenic material. A solution to this problem was arrived at by Nairn, Richmond, McEntegart, and Fothergill (1960) when they compared

the reaction of tissue specific antigen in normal and malignant tissues. In this study they developed the sandwich layer technique which incorporated an intermediate stain designed to intensify the specific fluorescent reaction. With it they noted the following: 1) a positive reaction in cytoplasmic granules, although it was chiefly confined to the cytoplasmic membrane and, 2) benign tumors stained normally while malignant tissue was found to stain in a reduced fashion.

It wasn't until 1961 that Coombs while studying normal and malignant tissue expanded somewhat the existing knowledge of cell surface antigens. He noted that isoantigens, species specific, organ specific and heterophile antigens can all be found on cell surfaces. His findings were in accord with the current findings of Green (1954) and Weiler (1959) who thought that with malignant change there was a corresponding loss of tissue specific antigen.

The fact there is a difference in the surface of normal cells and homologous tumor cells was further shown by Ambrose, James and Lowick (1956) when the electrical charge carried by normal and homologous mouse kidney tumor cells was compared. Their findings indicated that tumor cells had an average charge density of twice the normal kidney cell. These findings were in accord with those of Abercombie and Heaysman (1953) who showed that sarcoma cells of fibroblasts did not exhibit inhibition with respect to each other or normal fibroblasts.

Ambrose and Abercombie (1955) had shown that this difference was dependent on a loss of adhesiveness of the surfaces of the tumor cells. This suggested to them that the electrical properties of the surfaces may have altered during malignant transformation. Their findings corroborated those of Dunham, Nichols and Brunschwig (1946) who, working with carcinoma of the stomach and colon showed a decrease in the calcium level in the respective cancer cells. This decrease was not apparent in benign tumors.

Coman and Anderson (1955) noted that the behavior of a cancer cell is determined by peculiarities of the external surface. They also noted that cancer cells are unable to bind calcium to any degree and as a consequence they bind to each other very poorly. The cells tend to separate, migrate, and invade adjacent tissue. Using the electron microscope they were able to show definite changes in the ultra structure of the malignant cell surface. In the normal epidermal cells of the rabbit, fine particles 30 to 60 A degrees in diameter predominate; with homologous cancer cells the particle size varies from 30 to 3000 A with great irregularity.

THEORIES OF CARCINOGENESIS:

Muhlbock and Boot (1959) working on the mechanism of hormonal carcinogenesis made the following observations: Hormones capable of inducing neoplasia in animals fall into two groups, the

trophic hormones of the hypophysis and the steroids. These two groups have specific targets which they normally affect. Other hormones as thyroxin and insulin are not known to be carcinogenic. They further noted that experiments with growth stimulating hormone caused multiple tumors to grow in many areas. This agent was thought to act more as an accelerator than a causative factor.

While the carcinogenic effects of hormones on their respective target sites were related to the quantity of the hormone, it was also necessary for it to work continuously on the target or no tumor would arise. Therefore, hormonal carcinogenesis differs from chemical carcinogenesis in that one large dose of the chemical carcinogen can and does induce certain types of cancer.

Green (1959) in discussing his original immunologic theory of cancer (Green, 1954) noted that carcinogenic polycyclic hydrocarbons were shown to have a specific inhibition on homologous transplanted tumors and therefore, probably directly affected the immune reaction induced by such tumors. He believed the hormone mimetic action of polycyclic carcinogens indicated a common focal point of effect on the cells for hormones and carcinogens.

He also thought that when a hormone dependent tumor became independent it could be presumed to have completely lost the protein complexes which conferred on it both tissue and hormonal specificity. He therefore concluded that tissues may no longer be under control of hormones because the identity antigen was missing and the potential for hookup with the hormone was corres-

pondingly absent.

Prehn (1964) in discussing mechanisms of carcinogenesis believed the "clonal selection theory" best explained those observations most often noted in different cancers. He believed the process often begins prior to the application of carcinogens with the occurrence of hereditarily stable variants in a cell population. Some of these variants will contain less than average amounts of "cancer control factor", some might have different antigens. He thought these variants did not have a competitive advantage over normal cells. However, with any form of hyperplasia there will be an increase in the potential for more deviations to occur.

The greater the degree of deviation the greater the chance for antigenic stimulation. However, the number of cells with one type of antigen is small and therefore, the chance for antigenic stimulation is correspondingly small. For this reason, variant clones are able to exist for long periods undisturbed.

Because of their lack of competitive advantage and small number, there is little chance for any deviant clone to give rise to successive variants which are usually necessary before a critically low level of "cancer control factor" is reached and progressive autonomous growth can begin. Occasionally this does occur, but not until after many successive variants have occurred. Therefore, tumors arising in this manner without the intervention of a carcinogen tend to occur only in old animals.

Cole and Norwell (1965) in discussing the sequence of events in radiation carcinogenesis, follow closely the "clonal selection theory". However, they believe their variant is a synthetically induced factor utilizing a radiation source, and that it can occur in one step, or more possibly in a series, one following the other. For the tumor to actually form, the variant must first enter into mitosis.

To date, research has shown that certain antigenic substances are regularly found on cell surfaces. In malignancy a detection of change in the quantity and/or distribution of this material has been noted. Whether this change occurs in all tissues, or if all antigen groups are affected is not known.

In view of information presently at hand, questions which have arisen as a result of it, it is obvious that expansion of this knowledge is necessary.

MATERIALS AND METHODS

Fifteen samples of clinically normal gingiva were obtained from the free nargin of the buccal molar region of males ranging in age from 23 to 30. Each section was approximately 2x4 mm extending to the periosteum. Eight of the specimens were from type A, six from type B, and one from type AB blood group phenotypes.

In addition, specimens were obtained from the soft tissue of the oral cavity of eight individuals with epidermoid carcinoma. Four of these specimens were from type A, three from type B and one from type AB blood phenotypes. Biopsies of clinically normal oral epithelium were also obtained from three of the above individuals from whom neoplastic tissue had already been obtained.

Fifteen specimens of the clinically normal, and eight of the cancerous type were fixed in 95% ethanol pre-cooled to 4 degrees centigrade. The sections were then processed and stained according to Ste. Marie (1962), utilizing the double layer stain technique.

This technique consisted of deparafinizing the sections in three consecutive one minute washes of xylol using fresh solvent each wash. This was followed by three washes in 95% ethanol for 15 seconds each. The specimens were then washed in three consecutive saline baths for one minute per wash. Staining was as

follows: application of the corresponding antisera for 30 minutes in a high humidity environment, followed by a twenty minute bath in 95% ethanol. The specimen was then washed in buffered saline for three minutes, dried and then reacted with the fluorochrome for thirty minutes. Fixation and washing of this layer was carried out as previously shown. Glycerol was used to mount the coverslips. The slides were then ready for immediate examination with the fluorescent scope.

Three sections of clinically normal tissue were fixed in buffered 10% formalin solution and also stained with the layering technique. All sections were cut at 6 microns with the A.O. Spencer 820 microtome.

The antisera used in the intermediate layer was human typing sera* without coloring agent. It was concentrated to one-half its original volume by evaporation at 4 degrees centigrade. Fluorescein isothiocyanate conjugated to goat antihuman globulin fraction was used as the second layer in the stain technique.

Blocking reactions were accomplished in the following manner: Type A tissue was reacted with anti B and then with goat antihuman globulin fraction. The resulting negative intercellular reaction was due to the inability of the intermediate layer to react with the A antigen and therefore the multiplier effect was absent.

*Purchased from Hyland Laboratories, Los Angeles, California.

A theoretical explanation of the multiplier effect is as follows: a weakly reacting antigen can have two or three reactive sites on each molecule of antigen. By reacting the corresponding antisera to this antigen a new complex is formed. It is possible that each of these antigen-antibody complexes can have two or three new reactive sites to which a molecule of an antibody from a second antisera can react to. (Figure 1, Table II). It is therefore theoretically possible to have increased the overall immunologic reaction threefold. If the second layer was conjugated to a fluorochrome, the specific fluorescent reaction would then be of a much greater intensity than if a single layer technique was utilized. By utilizing the incorrect antisera for the intermediate layer, it was possible to block the multiplier effect. (Figure II, Table II).

The conjugate was treated in two ways to reduce non-specific staining. The unreactive fluorescent material was reduced by continuous elution of the conjugate through a "sephedex" column with buffered saline. The column consisted of loosely packed plastic beads 50 microns in diameter. As the elutant passed downward the larger molecules of conjugated material passed more rapidly and were easily collected. The slower moving unconjugated material was discarded. The elutant was then concentrated by evaporation at 4 degrees centigrade. The non-specific protein material was reduced by repeated adsorptions with bovine liver powder using 100 mg. of powder to each cubic centimeter of anti-

sera. The mixture was centifuged and the supernate was collecte and used as the purified antisera.

Sections of the cancerous tissue were stained with hematoxy lin and eosin for identification and localization of the tumor cells.

A Reichert Zetopan research microscope with an HBO high pressure mercury vapor lamp was used to examine the slide sections. All examinations were carried out at 400 and 600 magnifications.

Photographs were taken immediately on preparation of the fluorescent material. Exposures were at 90 seconds using out door high speed ektachrome* film with an ASA of 160. H & E sections were photographed at 15 second exposures.

*Kodak, Rochester, New York.

FINDINGS

NORMAL TISSUE FIXED IN ETHANOL:

The normal tissue specimens when incubated with the corresponding antisera and conjugated fluorochrome all reacted positively in the intercellular spaces. However, positive intercellular reaction was confined chiefly to the stratum spinosum. The four or five cell layers adjacent to the basement membrane as well as the four or five cell layers adjacent to the stratum corneum were generally negative. (Figure 1). In two isolated areas a positive intercellular reaction was seen adjacent to the basal cell layer.

In one specimen an irregular pattern of intercellular fluorescence was seen. The positive reaction could not be detected through the eighth or ninth cell layer. (Figure 2).

The intensity of the intercellular reaction in the type A tissue was always greater than that seen in type B tissue. This was also true in the one normal specimen of AB tissue. When reacted with AB antisera the intercellular reaction was intense. With A antisera alone the degree of intensity was less. It was further reduced when only B antisera was used.

Blocking techniques carried out on normal tissue gave a negative intercellular reaction. (Figure 3). The level of background cytoplasmic fluorescence was approximately the same as that

seen in the straight reaction.

NEOPLASTIC TISSUE FIXED IN ETHANOL:

The sections derived from neoplastic tissue reacted in three ways: positively, negatively, and in combination. (Figures 4, 5, 6). The positive reactions were generally weaker than those seen in normal tissue. A slight "ghost-like" fluorescence was seen both in the negatively and positively reacting malignant cells. The positive intercellular reaction of type A tissue was generally more intense than that seen in type B tissue.

When the sections of type AB tissue were reacted with both types A and B antisera followed by the second layer, a fairly well defined positive reaction was apparent. (Figure 7). However, the intensity of this reaction decreased when the A and B antisera was applied separately. With anti A sera alone, a positive reaction of slightly diminished intensity was noted whereas with anti B sera there was a barely discernible. (Figure 8).

In some sections both a positive and negative intercellular reaction was seen (Figure 6); while in others there was a great contrast in the intensity of adjacent positively reacting intercellular areas. However, a combination of both negative and positive reactions was most frequently observed.

Hematoxylin and eosin stained sections were made of each specimen of neoplastic tissue to show tumor differentiation. There was no consistent correlation between the degree of differ-

entiation of the tumor cells and the intensity of the specific fluorescent reaction.

A comment should be made at this point in regard to the age of the normal tissue specimens. Since the fifteen specimens of normal tissue were obtained from individuals whose average age was twenty-three to thirty, and the average age of those from whom the neoplastic specimens were obtained was between 45 and 75 years. An attempt was made to eliminate the age differential, between the normal samples and the neoplastic specimens as a major factor in correctly interpreting the results. This was accomplished by treating normal specimens obtained from the oral cavity of three patients from whom neoplastic tissue had previously been obtained with the corresponding antisera. The normal tissue from these three individuals reacted very strongly positively. (Figure 9). While this number is too small to draw valid statistical conclusions from, it does give an indication of the neutral effect of age on the degree of intercellular fluorescence that is seen.

SEPHADEX FILTRATION AND LIVER ADSORPTION:

Sections of normal tissue when reacted with the corresponding antisera followed by the untreated fluorochrome gave a very strong intercellular reaction. (Figure 1). The background cytoplasmic and connective tissues fluorescence was also very high. When the blocking reaction was utilized, a similarly high degree of background fluorescence remained, although the intercellular areas

reacted negatively. (Figure 3).

A reduction in the nonspecific fluorescence was noted following treatment of the conjugate by gel filtration. (Figure 10). A corresponding reduction in the intensity of the specific reaction was also noted.

Following treatment of the fluorescent compound with "Sephadex" and liver adsorption, a further decrease in the specific and nonspecific reaction was seen. (Figure 11). In some sections the microscopic field was too dark to distinguish histologic features.

ACETONE FIXATION:

The sections of normal tissue that were fixed in buffered formalin and stained according to Ste. Marie with the appropriate antisera, all gave positive intercellular reactions. (Figure 12). However, they were more diffuse than those seen in tissues fixed in ethanol. Blocking reactions effectively eliminated all intercellular fluorescence. (Figure 13).

DISCUSSION

The surfaces of the cells in the body are known to contain at least three different antigenic substances. They are the blood group, specie specific, and organ specific materials. Their exact functions are not known. It is the intent of this paper to include in its discussion only the blood group substances.

NORMAL TISSUE; ETHANOL FIXATION:

The distribution of the intercellular reaction chiefly within the stratum spinosum in normal tissue, as found in this work, concurred with the findings of Szulman (1960) and Brandtzaeg (1965). The fact that some cells within the normally positive staining intercellular areas did not react, concurs with Kay (1957) and further points out the need for this information in correctly interpreting any work with neoplastic tissue. Whether the lack of this reaction is due to a change in the cell surface electrical charge, or an incomplete development of the antigen or lack of it, is unknown. The possibility that these areas might represent a "variant clone" with an altered surface antigen also exists (Prehn 1964).

The sections of normal tissue were prepared from fresh biopsy material in order to prevent any changes on the cell surface as suggested by Glynn and Holborow (1959). It was also

necessary to examine and photograph the prepared slides immediately as a decrease in the intensity of the specific reaction was noted after the second day.

Even though type A normal tissue reacted with greater intensity than B and this corresponded with Brandtzaeg (1965) and Kay (1960), it was not the intent of this study to attempt a quantitative comparison of the fluorescent reactions. For this reason, the secretor status of the donors was not established; as Glynn et al (1957), and Szulman (1960) have shown that only the water soluble antigen in secretions is affected, qualitatively, by the secretor status; and that with alcohol soluble factors there is only a quantitative effect.

It is interesting to note that the blood group substances have been categorized into water and alcohol soluble groups. Glynn et al (1959), Szulman (1960), and Davidsohn (1966) have shown that the alcohol soluble antigen is found on the surfaces of all epithelial and endothelial cells, whereas water soluble material is found chiefly in secretions and mucous secreting cells.

It was noted in this work that both ethanol fixed and formalin fixed normal tissue sections reacted positively, and raised the question of whether both forms of the antigen were present and, if so, in what proportion.

NEOPLASTIC TISSUE;ETHANOL FIXATION:

It is obvious that a change in the reactivity of the blood

group substances does occur. The question as to why it occurs deserves some attention. If a lack of maturation of the epithelial cell prevented the differentiation of this material, the negative intercellular reaction would be expected to progress away from the basal cell layer. This did not occur. Instead, mixed reactions were seen with negative intercellular areas interposed throughout areas of positive reactivity.

While the possibility of a mutation of the gene responsible for the formation of the blood group substance might exist, it has been held by many authors to be highly unlikely. For as Kay (1957) suggests an "all or none" reaction would probably be detectable.

The findings of this work as previously noted, suggested an "all or none" reaction on those cells which lacked any positive intercellular reaction at all, and an incomplete reaction on other cells where the fluorescent reaction was mixed. In view of the fact that all of the synthesized products of any cell are controlled by the genetic mechanism, it is my feeling that any change in the surface reactivity of select cells in a neoplasm, must be the result of a change in the genetic apparatus itself. The mode of this change or the reason why only certain parts of a cell surface is affected is not understood as yet.

Another possible explanation is that these blood group substances are absent from the cell surface because they have not been synthesized, although the cell maintains the ability to do

so. It is also possible that the necessary materials for synthesis are not present in the cell because they are unable to traverse the cell membrane; or once in the cytoplasm the enzymes necessary for utilization of this material are absent (Kalckar, H.M., 1965). The fact that Szulman (1962) has shown that "H" substance is present in malignant tissue where as A and B substance is undetectable, and that Watkins (1966) has shown that A and B substances are derivatives of "H" substance, could possibly be used as an argument in support of this hypothesis.

Davidsohn et al (1966) has suggested that the loss of the ability of the cell to secrete the antigen is an expression of functional dedifferentiation in neoplasia. This implies that these cells have first reached maturity. This is not the case, as frequently the area of negative cellular reactivity extends from the basal cell layer up to the stratum corneum.

As previously noted, there is more than one antigen present on cell surfaces. These groups are found regularly on normal cell surfaces. Nairn et al (1960) has shown that tissue specific antigen of skin reacts only sporadically, if at all, in carcinoma. He also noted that in normal skin this antigen was largely confined to the basal layers rather than the prickle cell layer. Szulman (1960) and Brandtzaeg (1965), in working with normal epithelium and blood group substances showed that these groups are confined primarily to the stratum spinosum. With malignancy they noted a corresponding decrease in this reaction. These findings

concurred exactly with those found in this work.

It would be interesting to note whether these different antigenic substances all complex onto one macro-molecule with each specificity being added on as the cell moves away from the basement membrane. Furthermore, the question of whether each specificity is equally affected, or if one is affected earlier or to a greater degree should be investigated.

SUMMARY AND CONCLUSIONS

Normal tissue specimens were obtained from both young and old individuals of type "A" and "B" blood group phenotype. They were fixed in 95% ethanol and buffered formalin. Neoplastic tissues were obtained from older individuals of corresponding blood types. These tissue sections were reacted with the corresponding antisera followed by a second layer of conjugated fluorochrome. Blocking reactions were carried out on all specimens.

The findings concurred with work done previously on normal oral epithelium. Results from neoplastic tissue indicated a change in the reaction seen normally. In general the findings may be stated as:

1) Initial findings tend to indicate that age is not a factor in the ability of cells to react with the corresponding antisera and fluorochrome. However, because of the narrow distribution involved, positive conclusions cannot be drawn.
2) Malignant tissues react negatively or with a reduced intensity as compared to normal tissues.
3) A mixed reaction of reduced intensity was most commonly seen in neoplastic tissue.
4) The level of background fluorescence was greatly reduced by the use of "Sephadex" filtration and

liver adsorptions.

5) Tissue sections fixed in ethanol and/or formalin fluoresced positively when reacted with the corresponding antisera.

6) When type AB normal or neoplastic tissue was incubated with only one antisera such as anti B, the reaction was much weaker than when both anti A and anti B sera were reacted together.

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APPENDIX

A) PHOTOMICROGRAPHS

Fig. 1 Normal tissue. Well defined intercellular reaction chiefly in prickle cell layer. High degree of background fluorescence.

Fig. 2 Normal tissue. Irregular intercellular reaction.

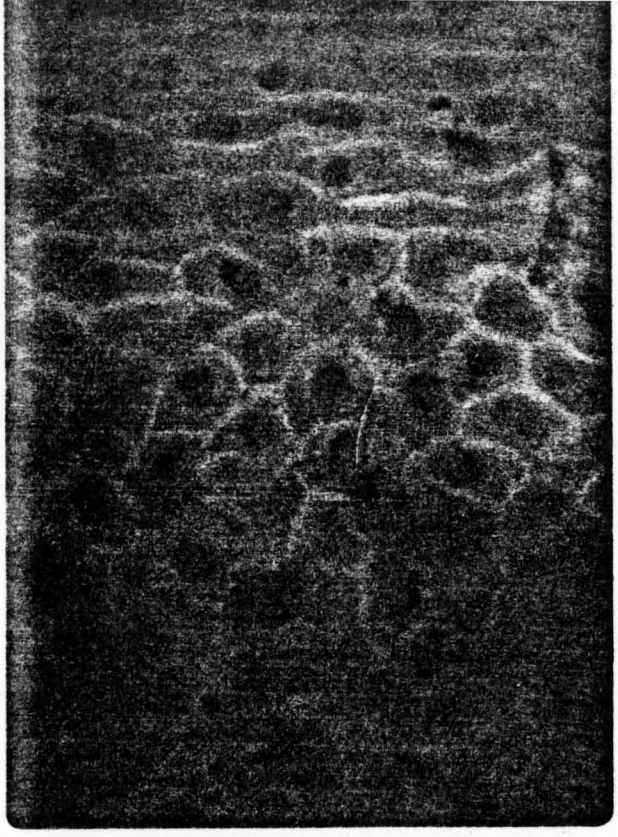


Fig. 3 Normal tissue, blocking reaction. Negative reacting intercellular areas.

Fig. 4 Neoplastic tissue. Positive, slightly diffuse intercellular reaction.

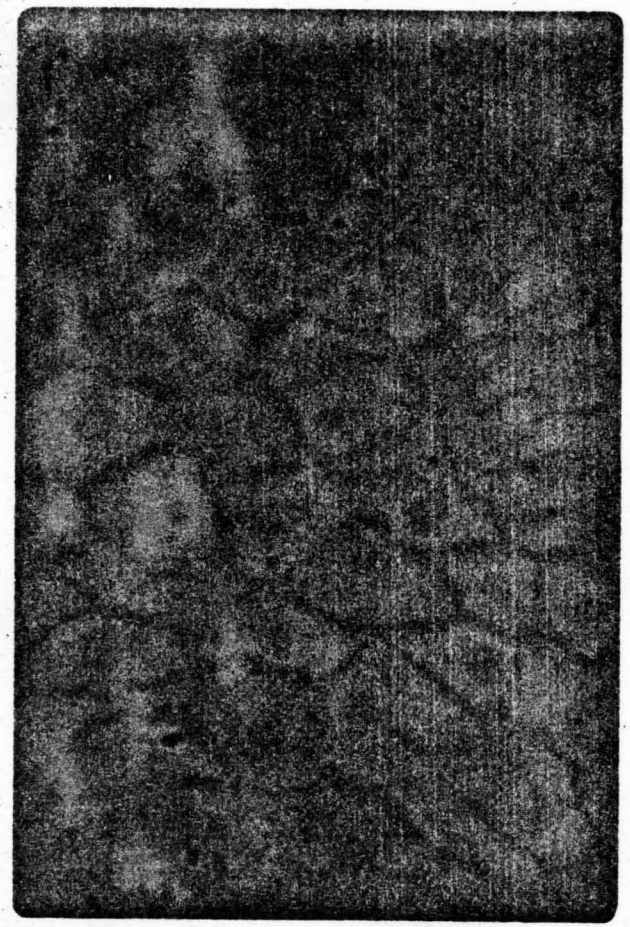


Fig. 5 Neoplastic tissue. Negative reaction.

Fig. 6 Neoplastic tissue. Clonal area of positive reaction with adjacent surrounding negative areas.

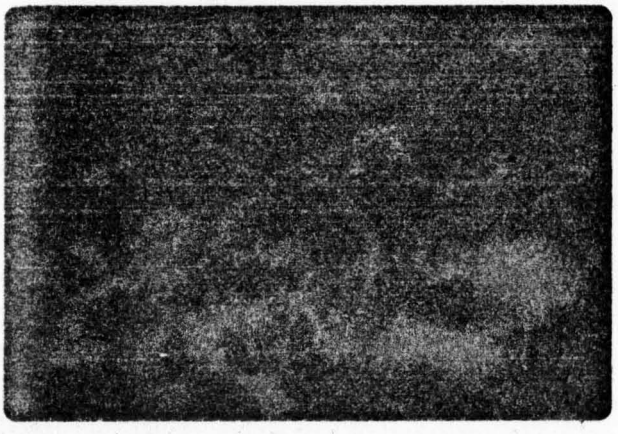
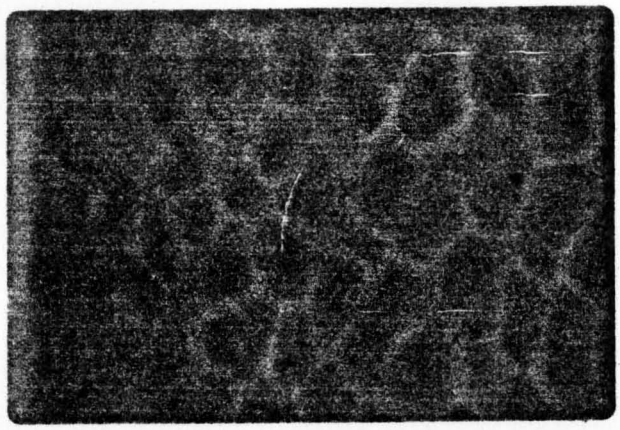


Fig. 7 Neoplastic tissue. Type AB reacted with "A" and "B" antisera together.

Fig. 8 Neoplastic tissue. Type AB reacted only with anti B sera.

Fig. 9 Normal tissue. Obtained from normal tissue from individual with neoplastic lesion.

Fig. 10 Section reacted with fluorochrome treated with "Sephadex" gel filtration. Note reduction in background fluorescence as compared to Figs. 1 and 2.



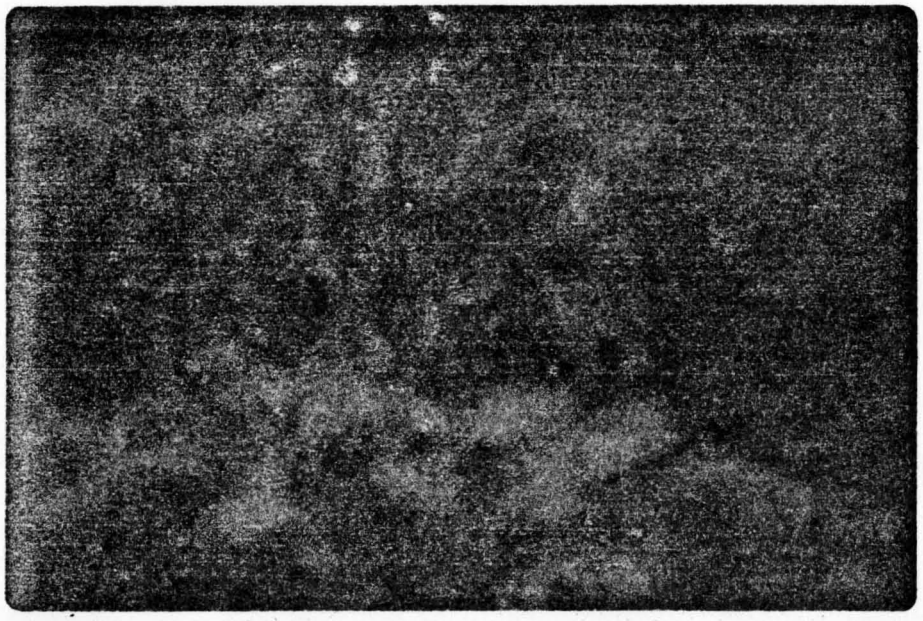


Fig. 11 Section reacted with fluorochrome treated with Sephadex and liver adsorption. Note further reduction in background fluorescence.

Fig. 12 Normal tissue fixed in formalin. Straight reaction.

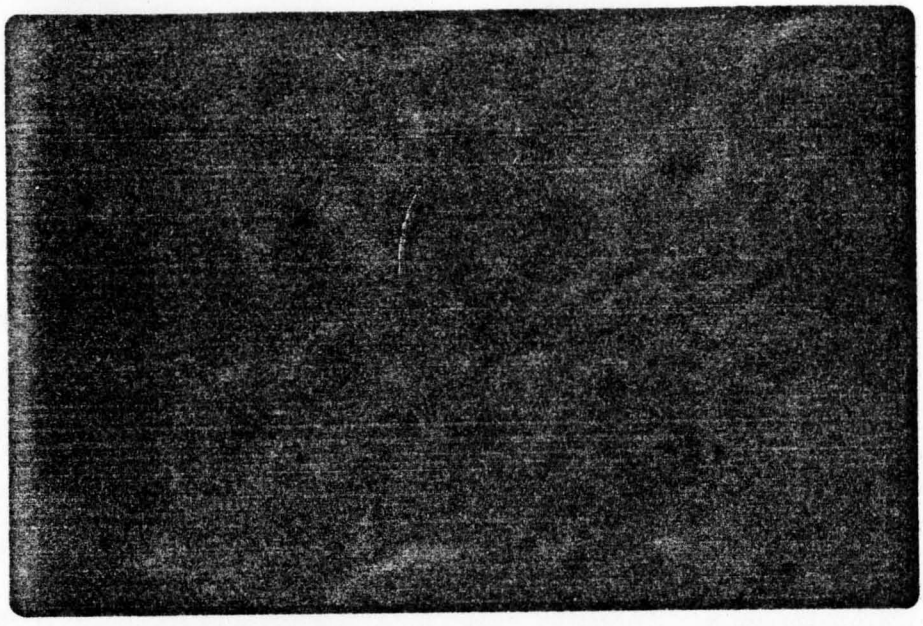


Fig. 13 Normal tissue fixed in formalin. Blocking reaction.

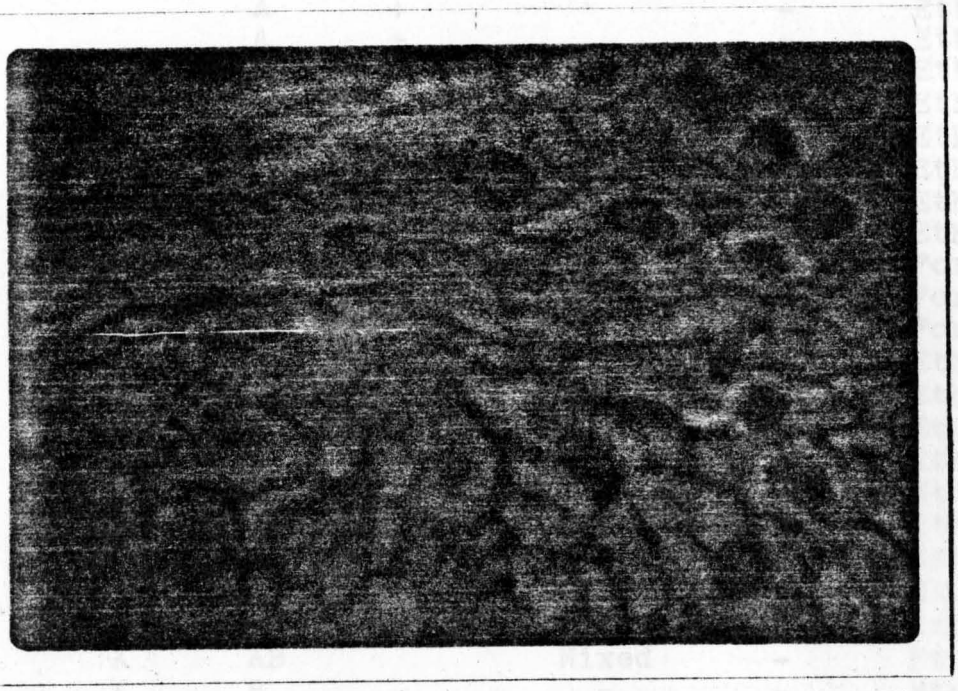


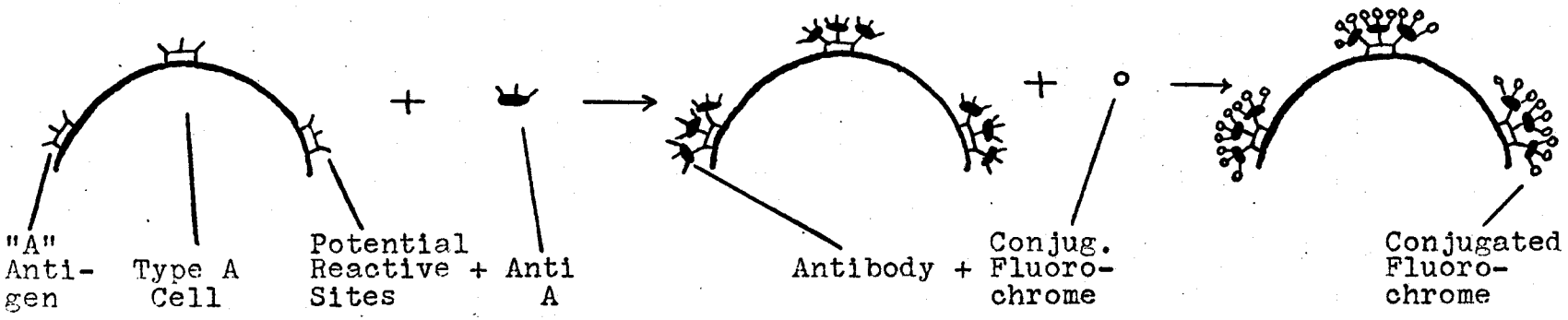
TABLE I

SEX	AGE UNDER 30	AGE OVER 30	BLOOD TYPE	NORMAL* TISSUE	NEOPLASTIC* TISSUE	BLOCKED TISSUE	FIXATIVE
M	X		A	+		-	Ethanol
M	X		A	+		-	Ethanol
M	X		A	+		-	Ethanol
M	X		A	+		-	Ethanol
M	X		A	+		-	Ethanol
M	X		A	+		-	Ethanol
M	X		B	+		-	Ethanol
M	X		B	+		-	Ethanol
M	X		B	+		-	Ethanol
M	X		B	+		-	Ethanol
M	X		B	+		-	Ethanol
M	X		AB	+		-	Ethanol
M	X		A	+		-	Formalin
M	X		A	+		-	Formalin
M	X		B	+		-	Formalin
M		X	A	+		-	Ethanol
M		X	B	+		-	Ethanol
M		X	B	+		-	Ethanol
M		X	A		Mixed	-	Ethanol
F		X	A		Mixed	-	Ethanol
M		X	B		Mixed	-	Ethanol
M		X	A		+	-	Ethanol
M		X	A		+	-	Ethanol
M		X	B		Mixed	-	Ethanol
M		X	AB		Mixed	-	Ethanol
M		X	B		-	-	Ethanol

*The reaction was determined as positive or negative or mixed. A quantitation of the degree of reactivity was not attempted.

FIGURE I

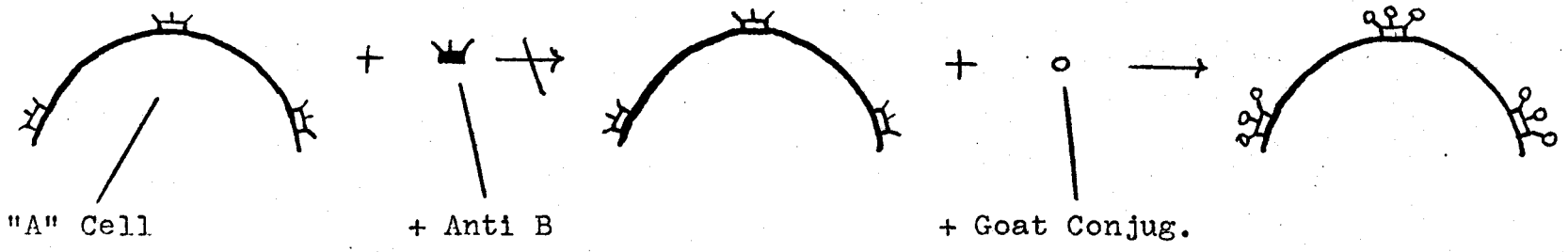
STRAIGHT REACTION



NB. Reactions Increased Threefold.

FIGURE II

BLOCKING REACTION



Multiplier Affect Blocked. Reaction Too Weak For Detection.

APPROVAL SHEET

The thesis submitted by Dr. Richard C. Prendergast has been read and approved by four members of the Department of Oral Biology.

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

May 25, 1967
DATE

Patrick D. Tate
SIGNATURE OF ADVISOR