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# A Comparison of the Ground-Substance of the Connective Tissue of the Alveolar Mucosa and the Ground-Substance of the Connective Tissue of the Eruptive Mucosa

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A COMPARISON OF THE GROUND-SUBSTANCE OF  
THE CONNECTIVE TISSUE OF THE ALVEOLAR  
MUCOSA AND THE GROUND-SUBSTANCE OF  
THE CONNECTIVE TISSUE OF THE  
ERUPTIVE MUCOSA

BY

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DEPARTMENT OF  
LOYOLA UNIVERSITY MEDICAL CENTER

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF  
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## LIFE

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## INTRODUCTION

THE TEETH ARE SURROUNDED BY BONE AND CONNECTIVE TISSUE DURING THE PERIOD OF EARLY GROWTH AND ERUPTION. AFTER THE EMERGENCE OF THE TOOTH FROM THE BONY CRYPT, DURING ERUPTION, THE REDUCED DENTAL EPITHELIUM IS SEPARATED FROM THE ORAL EPITHELIUM BY THE INTERVENING CONNECTIVE TISSUE. DURING ERUPTION THE TIP OF THE TOOTH APPROACHES THE ORAL MUCOSA AND THE REDUCED DENTAL EPITHELIUM FUSES WITH THE ORAL EPITHELIUM.

THE CHARACTERISTICS OF THE CONNECTIVE TISSUE INTERVENING THE REDUCED DENTAL EPITHELIUM AND THE ORAL EPITHELIUM ARE STUDIED IN AN ATTEMPT TO EXPLAIN THE MANNER IN WHICH THE RESISTANCE OFFERED BY THE CONNECTIVE TISSUE TO THE AXIAL MOVEMENT OF THE TOOTH IS REDUCED, PRIOR TO ITS EMERGENCE INTO THE ORAL CAVITY.

THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE HAS BEEN A SUBJECT OF INTENSIVE INVESTIGATION. IT HAS BEEN SHOWN THAT IN INFLAMED GINGIVA THE AMOUNT OF WATER SOLUBLE MUCOPROTEINS AND CARBOHYDRATES INCREASES AND THIS IS PRESUMED TO BE DUE TO DEPOLYMERIZATION OF THE GROUND SUBSTANCE OR TO ALTERED SYNTHESIS OF CONNECTIVE TISSUE GROUND SUBSTANCE. THE CONNECTIVE TISSUE OBTAINED FROM DIFFERENT SOURCES HAS BEEN STUDIED HISTOCHEMICALLY AND MORPHOLOGICALLY, AND IT HAS BEEN REPORTED THAT THE GROUND SUBSTANCE UNDERGOES DEPOLYMERIZATION AND THAT THERE IS AN EVIDENCE OF THE SOLUTION OF SOME COMPONENTS OF THE TISSUE.

THE COMPARATIVE QUANTITIES OF MUCOPOLYSACCHARIDES BINDING THE CONNECTIVE TISSUE IN THE ORAL MUCOSA MAY DIFFER SIGNI-

FICANTLY IN ATTACHED GINGIVA AS AGAINST THOSE OF THE CONNECTIVE TISSUE OF THE ORAL MUCOSA OVERLYING AN ERUPTING MOLAR.

THE PURPOSE OF THIS EXPERIMENT IS TO STUDY THE DEPOLYMERIZATION OF THE GROUND SUBSTANCE, WHICH MAY CONTRIBUTE TO THE ERUPTION OF A TOOTH THROUGH THE CONNECTIVE TISSUE BARRIER OF THE ORAL MUCOSA.

## REVIEW OF LITERATURE

CONNECTIVE TISSUE IS ONE OF THE SUPPORTING TISSUES OF THE BODY, IN WHICH THE INTERCELLULAR SUBSTANCE IS AT A MAXIMUM. IT IS WIDELY DISTRIBUTED IN THE BODY IN VARIOUS FORMS, LIKE AREOLAR, ADIPOSE, FIBROUS, ELASTIC, AND RETICULAR TISSUE, CARTILAGE AND BONE. THEY ARE ALL DERIVED FROM THE MESODERM OR THE MESENCHYME OF THE EMBRYO BUT THE DIFFERENCE LIES MAINLY IN THE NATURE OF THE INTERCELLULAR SUBSTANCE AND THE APPEARANCE OF THE DIFFERENT KINDS OF FIBRES IN IT. THE BASIC STRUCTURAL UNITS ARE CELLS, FIBERS, AND THE "GROUND SUBSTANCE".

### A. GROUND SUBSTANCE

ENGLE, ET.AL (1960) DESCRIBED THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE AS AN HETEROGENOUS COLLOIDAL MIXTURE OF MACROMOLECULES OF CARBOHYDRATE-PROTEIN COMPLEXES (MUCOPROTEINS AND GLYCOPROTEINS) AND MUCOPOLYSACCHARIDES, WITH A NEGATIVE-ELECTRICAL CHARGE. THE DEGREE OF THIS CHARGE IS DIRECTLY PROPORTIONAL TO THE DENSITY OF THE CONNECTIVE TISSUE. BY THE VIRTUE OF THIS NEGATIVE CHARGE, THE BIOLOGICAL COLLOID HAS GAINED UNIQUE PROPERTIES AFFECTING THE WATER AND SALT DISTRIBUTION AND THE ORGANIC AND INORGANIC CATION BINDINGS IN THE TISSUE. SLACK (1959) IN DISCUSSING THE COMPOSITION AND METABOLISM OF CONNECTIVE TISSUE EMPHASIZES THE INFLUENCE OF COLLOIDAL COMPONENTS ON THE DIFFUSION AND EXCHANGES OF ELECTROLYTES AND OTHER SOLUTES. THE COLLOIDAL



COMPONENTS OF THE GROUND SUBSTANCE ARE NOT AN INERT MASS THROUGH WHICH THE ELECTROLYTES AND METABOLITES CAN PASS FROM THE BLOOD CIRCULATION TO THE CELLS. THE DISTRIBUTION OF THE DIFFUSIBLE IONS IS DETERMINED PRIMARILY BY INDIFFUSIBLE CHARGED MACROMOLECULES OF ACID MUCOPOLYSACCHARIDES AND CARBOHYDRATE-PROTEIN COMPLEXES.

THE COMPOSITION OF THE GROUND SUBSTANCE AS STATED BY GERSH AND CATCHPOLE IS AS FOLLOWS:

TABLE 1

- |                          |                                  |
|--------------------------|----------------------------------|
| ACID MUCOPOLYSACCHARIDES | 1. HYALURONIC ACID               |
|                          | 2. CHONDROITIN SO <sub>4</sub> A |
|                          | 3. CHONDROITIN SO <sub>4</sub> B |
|                          | 4. CHONDROITIN SO <sub>4</sub> C |
|                          | 5. CHONDROITIN                   |
|                          | 6. KERATO SULPHATE               |
|                          | 7. HEPARITIN SULPHATE            |

NEUTRAL HETEROPOLYSACCHARIDES

PROTEINS

SOLUBLE COLLAGEN

PROBABLY OF LOCAL ORIGIN - WATER, ENZYMES, METABOLITES

PLASMA ORIGIN - IMMUNE BODIES

GLOBULIN

ALBUMIN

VITAMINS, HORMONES,

IONS

THE GROUND SUBSTANCE IS ORGANIZED AS A TWO-PHASE SYSTEM, COLLOID-RICH WATER POOR AND WATER-RICH COLLOID POOR. THE WATER-RICH PHASE EXISTS AS SUBMICROSCOPIC VACUOLES ENCLOSED AND SEPARATED FROM EACH OTHER BY DENSE PHASE (GERSH & CATCHPOLE, 1960). THESE VACUOLES ACCORDING TO BONDAREFF (1957) RANGE FROM  $500^{\circ}\text{A}$  TO  $2,000^{\circ}\text{A}$  IN DIMENSIONS. THE CHIEF CONSTITUENTS WITHIN THE VACUOLES IS WATER AND THE WALLS OF THE VACUOLES ARE BELIEVED TO BE RICH IN PROTEINS.

GERSH AND CATCHPOLE FURTHER STATE THAT EACH PHASE INCLUDES A VARIETY OF COMPONENTS DERIVED IN PART LOCALLY FROM CONNECTIVE TISSUE CELLS, IN PART FROM PLASMA. SOME OF THE COLLOID COMPONENTS OF THE GROUND SUBSTANCE HAVE THE PROPERTY OF FORMING AGGREGATES AND, WHEN THE PROPORTION OF THESE IS HIGH, THE GROUND SUBSTANCE IS LESS DENSE, OR PLASTIC. IN THIS STATE, THE TISSUE HOLDS MORE WATER AND MAY BECOME EVEN MORE EDEMATOUS. AS LONG AS THE TWO SYSTEMS ARE IN EQUILIBRIUM, EDEMA IS REVERSIBLE.

THE AMOUNT OF WATER IN EITHER OF THESE PHASES DEPENDS ON THE INTRINSIC HYDROPHILIC PROPERTIES OF THE SYSTEM, THE DENSITY OF THE COLLOID, OSMOTIC PRESSURE OF THE BLOOD PLASMA, LYMPHATIC DRAINAGE AND THE BLOOD PRESSURE (GERSH AND CATCHPOLE 1960). SLACK (1959) CONSIDERS THAT THE WATER IN CONNECTIVE TISSUE IS BOUND TO THE MUCOPOLYSACCHARIDE-MUCOPROTEIN-FIBROUS PROTEIN COMPLEXES. DURING THE WATER RICH PHASE THE WATER CONTENT OF THE GROUND SUBSTANCE AND THAT OF THE COLLAGEN FIBERS INCREASES; HYDRATION OF THE GROUND SUBSTANCE PRECEDES THE SWELLING OF THE

COLLAGEN FIBERS. THE DEGREE OF HYDRATION ALSO DEPENDS UPON THE AMOUNT OF HYALURONIC ACID PRESENT IN THE GROUND SUBSTANCE. HVIDBERG (1960) STATES THAT HYALURONIC ACID IS STRONGLY HYDROPHILIC. GERSH AND CATCHPOLE REPORT THE DEGREE OF HYDRATION BEING PROPORTIONAL TO THE DEGREE OF POLYMERISATION OF THE GROUND SUBSTANCE.

BENSELEY (1934) IN HER DESCRIPTION OF THE PROPERTIES OF THE GROUND SUBSTANCE STATES THAT 1) THE GROUND SUBSTANCE APPEARS AS A CONTINUOUS, FINELY GRANULAR, TRANSPARENT SUBSTANCE IN WHICH THE FIBERS AND CONNECTIVE TISSUE CELLS ARE EMBEDDED. 2) IT IS ELASTIC AND TENDS TO RETRACT WHEN CUT. 3) IT IS EXTRACTABLE BY 10% NaCl AND HALF SATURATED LIME WATER. CELLS ARE FIRST TO BE EXTRACTED, GROUND SUBSTANCE NEXT, AND FIBERS ARE THE LAST TO BE EXTRACTED. 4) GROUND SUBSTANCE AND CONNECTIVE TISSUE FIBERS ARE STAINED DIFFERENTIALLY BY TOLUIDINE BLUE. 5) IT HAS AFFINITY FOR COPPER SALTS SHOWING THAT CHONDROITIN SULPHURIC ACID IS PRESENT IN IT.

SHE FURTHER STATES THAT GROUND SUBSTANCE VARIES WITH PHYSIOLOGICAL AGE AND DEVELOPMENT OF THE CONNECTIVE TISSUE. IN EMBRYONIC AND UNDIFFERENTIATED TISSUES, SUCH AS RETICULAR TISSUE, IT IS YOUNG, VISCID AND A CONTINUOUS FORM. IN FULLY DEVELOPED SUBCUTANEOUS TISSUE, IT IS LESS ABUNDANT, INTERRUPTED BY TISSUE SPACES AND CHIEFLY ENCLOSES THE CONNECTIVE TISSUE FIBERS.

THE MAJOR COMPONENTS OF THE MUCOPOLYSACCHARIDE FRACTION OF THE GROUND SUBSTANCE ARE HEXOSAMINE AND HEXURONIC ACID. HEXOSAMINE IS A STABLE AMINO SUGAR WHICH IS AN AMINO DERIVATIVE OF

SUGAR WITH THE ALKYL RADICAL AT POSITION TWO OF THE MOLECULE; WHILE GLUCORONIC ACID IS A MONOBASIC ACID WITH AN ALDEHYDE REMAINING AT THE OPPOSITE END OF THE STRAIGHT CARBON CHAIN FROM THE CARBOXYL GROUP.

K. MEYER HAS SUGGESTED THE CLASSIFICATION OF MUCOPOLYSACCHARIDES AS FOLLOWS:

TABLE II

NEUTRAL: WITH NO ACID GROUPS AT ALL, EG. CHITIN (COMPOSED OF ACETYLGLUCOSAMINE ONLY)

ACID: 1) SIMPLE - IN WHICH THE ACID COMPONENT IS URONIC ACID, EG. HYALURONIC ACID COMPOSED OF ACETYLGLUCOSAMINE AND GLUCORONIC ACID.

2) COMPLEX - ACID COMPONENT IS GLUCORONIC ACID AND SULPHURIC OR PHOSPERIC ACID, EG. HEPARIN, COMPOSED OF ACETYLGLUCOSAMINE AND URONIC ACID AND SULPHURIC ACID.

CHONDROITIN SULPHURIC ACID COMPOSED OF ACETYLGALACTOSAMINE, GLUCORONIC AND SULPHURIC ACID.

STACY HAS GIVEN THE APPROXIMATE VALUES FOR THE COMPONENTS OF THE HYALURONIC ACID AS FOLLOWS:

NITROGEN 3 TO 3.5%

GLUCOSEAMINE 30 TO 40%

ACETYL 8 TO 12%

GLUCORONIC ACID 40 TO 50%

**B. LABILE NATURE OF THE GROUND SUBSTANCE**

THE GROUND SUBSTANCE AND THE BASEMENT MEMBRANE ARE CLOSELY RELATED. THEY ARE OPTICALLY HOMOGENOUS COMPONENTS OF INTERCELLULAR MATERIAL WHOSE PHYSICAL STATE MAY VARY FROM GEL LIKE CONSISTENCY TO FAIRLY FLUID CONSISTENCY ACCORDING TO GERSH AND CATCHPOLE (1949). GROUND SUBSTANCE IS CONCENTRATED TO FORM A DENSER REGION BETWEEN CONNECTIVE TISSUE AND EPITHELIUM. THIS ALTERED REGION OF THE GROUND SUBSTANCE IS CALLED THE BASEMENT MEMBRANE. THE GROUND SUBSTANCE EXISTS IN A POLYMERIZED STATE; THE DEGREE OF POLYMERIZATION BEING DIFFERENT IN THE SAME ORGAN AT THE SAME TIME. THE GROUND SUBSTANCE IS STRUCTURALLY ORGANIZED ON A SUBMICROSCOPIC OR MOLECULAR LEVEL. MOLECULES ARE BOUND TO EACH OTHER IN SUCH A WAY AS TO RESULT IN A MEDIUM WHOSE CONSISTENCY MAY VARY. THIS CHANGE IN PHYSICAL STATE IS BELIEVED TO BE CAUSED BY THE ACTIVITY OF CELLS OF THE CONNECTIVE TISSUE AND DUE TO SOME DEPOLYMERIZING ENZYMES.

GERSH (1941), IN DISCUSSING THE PLASTICITY OF CONNECTIVE TISSUE STATES THAT THE GROUND SUBSTANCE OF CONNECTIVE TISSUE IS NORMALLY OF GEL LIKE CONSISTENCY BUT UNDER CERTAIN CONDITIONS IT MAY BECOME MORE FLUID. IN HIS EXPERIMENTS WITH INTRAVITAL STAINING WITH THE DYE EVANS BLUE, HE STATES THAT NORMALLY LITTLE DYE ESCAPES FROM CAPILLARIES INTO THE INTERCELLULAR SPACES. HOWEVER, FOLLOWING INTRAVENOUS ADMINISTRATION OF PAPAIN IN RABBITS, EXCESSIVE AMOUNTS OF THE DYE LEAVE THE CAPILLARIES TO STAIN THE GROUND SUBSTANCES IN A MATTER OF SECONDS. IT APPEARS THAT THE CONSISTENCY OF THE GROUND SUBSTANCE, A FUNCTION OF THE STATE OF ITS ORGANIZATION,

WHICH DEPENDS PRIMARILY ON THE PROPERTIES OF GLYCOPROTEINS OF THE CONNECTIVE TISSUE AND THE DEGREE OF INTRAVITAL STAINING WITH EVANS BLUE, ARE ALL RELATED PHENOMENON. THE LOSS THROUGH CAPILLARIES OF EXCESSIVE AMOUNT OF EVANS BLUE HAS BEEN FOUND TO BE NEARLY SPECIFIC FOR REGIONS CONTAINING ALTERED OR WATER SOLUBLE GROUND SUBSTANCE.

IT HAS BEEN POSSIBLE TO DETECT ABNORMALLY HIGH LEVELS OF HYALURONIDASE AND COLLAGENASE ACTIVITY IN THE GROUND SUBSTANCE (GERSH & CATCHPOLE 1949). THESE DEPOLYMERIZING ENZYMES CAUSE THE ALTERED SOLUBILITY AND LOSS OF THE GROUND SUBSTANCE WHICH WEAKENS THE COLLAGENOUS FIBERS. THE BREAKING UP INTO SMALLER COMPONENTS WITH MORE DISAGGREGATED MOLECULES PRESENTS INCREASED REACTIVE GLYCOL GROUPS AND HENCE THE INCREASED STAINABILITY OF THE GROUND SUBSTANCE. HOWEVER, STAINABILITY IS REDUCED AS WATER SOLUBLE HYDROLYSIS PRODUCTS ARE LOST FROM THE GROUND SUBSTANCE.

IN THE EARLY STAGES OF DEVELOPMENT OF AN ORGAN, THE GROUND SUBSTANCE IS EXTRACTABLE AND, BECAUSE EXTRACTED IT STAINS VERY FAINTLY. IN SIMILAR SECTIONS OF ANIMALS 42 DAYS OLD, THE GROUND SUBSTANCE UNDERGOES PROGRESSIVE POLYMERIZATION AND, SUBSEQUENTLY GLUCOPROTEINS ARE NOT EXTRACTABLE, HENCE INCREASED STAINING PROPERTIES.

TO LOCATE THE WATER SOLUBLE AND ALCOHOL INSOLUBLE GLYCOPROTEINS OF THE GROUND SUBSTANCE, GERSH AND CATCHPOLE (1949) USED A METHOD TO DETECT THE EFFECTS OF A REAGENT ON GLYCOPRO-

**TEINS OF THE GROUND SUBSTANCE IN A SECTION.**

SMALL PIECES OF TISSUE FROM MOUSE SKIN WERE REMOVED AS EARLY AS POSSIBLE AFTER SPONTANEOUS DEATH OF AN ANIMAL AND FROZEN BY IMMERSION IN ISOPENTENE AT  $-160^{\circ}$  C. THEY WERE DRIED IN VACCUUM AT ABOUT  $-30^{\circ}$  C., INFILTRATED WITH PARAFFIN FOR ABOUT 15 MINUTES AND EMBEDDED. SECTIONS 6 MICRONS THICK WERE CUT AND MOUNTED ON ALBUMINIZED SLIDES. SUCH A SECTION WAS WARMED TO MELT AWAY THE PARAFFIN AND THEN FLATTENED WITH SLIGHT FINGER PRESSURE. IT WAS THEN STAINED BY ALCOHOLIC PERIODIC ACID LEUCOFUCHSIN METHOD.

ANOTHER SERIES OF SECTIONS 16 MICRONS THICK WERE DEPARAFFINIZED WITH PETROLATUM ETHER AND IMMersed IN ABSOLUTE ALCOHOL FOR AT LEAST 16 HOURS. ONE SET OF THESE SECTIONS WAS STAINED FOR POLYSACCHARIDES AND ANOTHER SET OF THE SECTIONS WAS TREATED WITH DROPS OF PHOSPHATE BUFFER AT PH7.0. THE PHOSPHATE BUFFER WAS PROMPTLY DECANTED AND FRESH BUFFER WAS ADDED, BUT AGAIN DECANTED AFTER TWO HOURS. SECTIONS WERE KEPT IN ALCOHOL FOR AT LEAST 16 HOURS AND STAINED BY PAS.

AN EARLY STAGE OF DEPOLYMERISATION OF GLYCOPROTEINS IS POSSIBLY REPRESENTED HISTOCHEMICALLY BY THE INCREASE IN THE COLOR VALUE OF THE GROUND SUBSTANCE. THIS MAY BE DUE TO GREATER ACCESSIBILITY AND AVAILABILITY OF REACTIVE GLYCOL GROUPS. DECREASE IN COLOR VALUE IS DUE TO LOSS OF THESE REACTIVE GROUPS THROUGH SOLUTION OF THE GLYCOPROTEINS CONTAINING THEM, POSSIBLY REPRESENTING A LATER STAGE OF THE DEPOLYMERIZATION OF THE GLYCO-

## PROTEINS.

GERSH AND CATCHPOLE (1949) OBSERVED BROWNIAN MOVEMENT BETWEEN COLLAGENOUS BUNDLES, WITHIN THE BUNDLES AND THEN BETWEEN THE FIBRILS WHEN CONNECTIVE TISSUE WAS EXPOSED TO ACTION OF CLOSTRIDIUM WELCHII TOXIN. CLOSTRIDIUM WELCHII TOXIN CONTAINS COLLAGENASE WHICH SEEMS TO REMOVE THE CEMENTING SUBSTANCE BETWEEN COLLAGENOUS FIBRILS. THEY STATE THAT THE REACTIVE MATERIAL OF THE GROUND SUBSTANCE MAY BE SELECTIVELY SEPARATED FROM FIBRILLAR STRUCTURES WHICH REMAIN MORPHOLOGICALLY INTACT BUT ARE FRAGILE. IT IS PROBABLE THAT REACTIVE MATERIAL IS A CARBOHYDRATE, CONTAINING PROTEIN, WHICH MAY BE HIGHLY POLYMERIZED AND DIFFICULT TO EXTRACT. THIS MATERIAL IS PRESENT IN EXTRAVASATED LYMPHOCYTES AND MACROPHAGES IN THE REGION OF INFLAMMATION. IT IS POSSIBLE THAT THE REMOVAL AND DISPOSAL OF GLYCOPROTEINS FROM GROUND SUBSTANCE BY THE PHAGOCYTTIC ACTIVITY REPRESENTS ONE OF THE NORMAL FUNCTIONS OF MACROPHAGES. THE GRANULES ARE ALSO SEEN IN FIBROBLASTS BUT ARE OBSCURED BY THE GROUND SUBSTANCE. SUCH GRANULES ARE BELIEVED TO BE THE SECRETION PRODUCTS OF FIBROBLASTS WHICH BECOME INCORPORATED IN THE GROUND SUBSTANCE (GERSH & CATCHPOLE 1949).

ENGLE (1951) STUDIED THE CONNECTIVE TISSUE OBTAINED FROM DIFFERENT SOURCES. ORAL MUCOUS MEMBRANE OVERLYING THE ERUPTING TEETH WAS OBTAINED FROM PATIENTS OF THE CHILDREN'S CLINIC. HE ALSO STUDIED THE JAWS FROM YOUNG RATS (NEWBORN TO 21 DAYS) AND FROM FETAL PIGS FOR HISTOCHEMICAL AND MORPHOLOGICAL STUDY. THE



TISSUES WERE TREATED WITH BUFFER SUBSTANCES AND ENZYMES PRIOR TO STAINING TO MAKE THE CHARACTERIZATION OF THE TISSUE COMPONENT MORE SPECIFIC BY COMPARING WITH THE TISSUE WHICH WAS STAINED WITHOUT ANY TREATMENT WITH BUFFER OR ENZYME.

BUFFERS	ENZYMES
ACETATE BUFFER PH3 TO 4.5	HYALURONIDASE PH6
PHOSPHATE BUFFER PH 5,6,7,	COLLAGENASE (CLOSTRIDIUM WELCHII FILTRATE)
BORATE BUFFER PH 9 TO 11	TRYPSIN PH7, PEPSIN PH 2

THE DECREASE IN THE STAINING PROPERTIES OF THE TISSUE AFTER TREATMENT WITH ENZYMES WAS REGARDED AS EVIDENCE OF THE SOLUTION OF SOME COMPONENTS OF THE TISSUE.

WHEN THE MUCOSA OVER THE DEVELOPING TEETH OF FETAL PIGS OR CALVES IS DISSECTED, A JELLY-LIKE MASS IS UNCOVERED WHICH FORMS A CONTINUUM FROM THE SUBMUCOSA TO THE TOOTH GERM. ENGLE STUDIED THE SOLUBILITY CHARACTERISTICS OF THE GROUND SUBSTANCE IN SUCH MUCOSA BY MAKING WATER EXTRACTS OF THIS JELLY-LIKE MASS. HE REPORTED A DECREASE IN THE VISCOSITY OF THIS EXTRACT UPON TREATMENT WITH TESTICULAR HYALURONIDASE.

THE ABILITY OF THE GROUND SUBSTANCE TO BIND DYE WAS TESTED BY INTRACARDIAL INJECTION OF THE DYE, EVANS BLUE, IN YOUNG RATS. IF THE ANIMALS WERE SACRIFICED WITHIN AN HOUR AFTER INJECTION, THE DYE WAS FOUND TO BE EITHER IN BLOOD VESSELS OR IN EXTRA-CELLULAR GROUND SUBSTANCE. AS THE TOOTH BECAME MORE DIFFEREN-

TIATED AND APPROACHED THE SURFACE OF THE ORAL CAVITY, THE CONCENTRATION OF THE DYE IN THE SUBMUCOSA INCREASED. THIS SUGGESTED THAT THERE WAS DEPOLYMERIZATION OF THE GROUND SUBSTANCE LIBERATING THE REACTIVE GROUPS FOR THE BINDING OF THE DYE.

### C. HISTOCHEMISTRY

**METACHROMASIA:** HISTOCHEMICALLY, METACHROMASIA CAN BE DEFINED AS THE STAINING OF THE TISSUE COMPONENT SO THAT THE ABSORPTION SPECTRUM OF THE RESULTING TISSUE DYE COMPLEX DIFFERS SUFFICIENTLY FROM THAT OF THE ORIGINAL DYE, AND FROM ITS ORDINARY TISSUE COMPLEXES, TO GIVE A MARKED CONTRAST IN COLOR.

#### MECHANISM OF METACHROMATIC REACTION:

MICHAELIS IN COLLABORATION WITH GRANICK (1945) HAS INTRODUCED THE CONCEPTION OF POLYMER FORMATION. THE MONOMERIC FORM OF THE DYE TOLUIDINE BLUE, IS BLUE OR VIOLET, DIMERIC OR TRIMERIC ARE PROGRESSIVELY VIOLET AND POLYMERIC FORMS ARE RED OR PINK. IT IS STATED THAT THE POLYMERIZATION OF THE SUBSTRATE WITH WHICH THE DYE COMBINES, INDUCES THE POLYMERIZATION OF THE DYE, AND HENCE THE CHANGE IN COLOR.

ACCORDING TO PEARSE (1960), THE DYE TOLUIDINE BLUE HAS AN ABSORPTION SPECTRUM WITH THREE BANDS, ALPHA, BETA AND GAMMA. MONOMERIC ALPHA FORM IS BLUE, DIMERIC BETA FORM IS VIOLET AND THE POLYMERIC GAMMA FORM IS RED. THE PRODUCTION OF GAMMA RED METACHROMASIA WITH TOLUIDINE BLUE IN TISSUE SECTIONS IS DUE TO PREDOMINANCE, IN OR ON THE SUBSTANCE STAINED METACHROMATICALLY OF THE GAMMA FORM OF THE DYE.

SYLVEN (1954, 1959) CONSIDERS METACHROMASIA AS A "SPECIAL TYPE OF ORDERLY DYE AGGREGATION CHARACTERIZED BY THE FORMATION OF NEW INTERMOLECULAR BOND BETWEEN ADJACENT TWO MOLECULES". THE SUBSTANCES (CHROMOTROPES) WHICH PRODUCE METACHROMASIA IN ANIMAL TISSUES ARE LARGE MACROMOLECULAR COLLOIDS, WITH SPACED ACIDIC GROUPS, I.E.  $-\text{OSO}_2\text{OH}$ ,  $-\text{COOH}$  AND PERHAPS  $-\text{OPO}(\text{OH})_2$  TO WHICH THE BASIC DYE IS FIXED. AN ORDERLY ALIGNMENT OF THE ATTACHED DYE MOLECULES IS NECESSARY FOR METACHROMASIA. THIS IS FAVORED IF THE DYE MOLECULES HAVE HYDROPHILIC AND HYDROPHOBIC PARTS. IT IS A TWO STAGE REACTION: DURING THE INITIAL STAGE THERE IS REACTION BETWEEN THE DYE AND THE SUBSTRATE MOLECULE; FOLLOWED BY THE INTERACTION OF DYE MOLECULES, IN THE SECOND STAGE. A MOLECULE OF WATER MAY BE INTERCALATED BETWEEN THE DYE MOLECULES, THUS GIVING A HYDROGEN BOND BETWEEN THEM.

SYLVEN AND PEARSE BELIEVE THAT METACHROMASIA SIGNIFIES ONLY THE PRESENCE OF FREE ELECTRONEGATIVE SURFACE CHARGES OF A CERTAIN MINIMUM DENSITY. THE DISTRIBUTION OF THIS CHARGE ON THE SURFACE OF THE SUBSTRATE DETERMINES THE ATTACHING ALIGNMENT OF THE DYE MOLECULES AND THE DEGREE OF METACHROMASIA. MINIMUM INTERCHARGE DISTANCE ESTIMATED TO BE  $50\text{\AA}$  IS ALSO NECESSARY FOR THE REACTION. HIGHER AGGREGATION OF THE ATTACHED DYE MOLECULES WOULD DECREASE THE INTERCHARGE DISTANCE, PERHAPS LESS THAN  $50\text{\AA}$  AND WOULD INCREASE THE DEGREE OF METACHROMASIA. HIGH CONCENTRATION OF THE DYE, AND INCREASED HYDRATION OF THE TISSUE WOULD ALSO FAVOR THE STAIN. THE QUALITY OF THE CHARGE, IN TERMS OF ACID RADICAL PRESENT, WOULD

ALSO ALTER THE DEGREE OF METACHROMASIA. FOR EXAMPLE, A SULPHATE RADICAL WOULD GIVE MORE INTENSE REACTION THAN A CARBOXYL GROUP. QUANTITATIVE DYE-SUBSTRATE RATIO IS ALSO IMPORTANT AS IT HAS BEEN OBSERVED THAT AN EXCESSIVE AMOUNT OF DYE CAN INDUCE ORTHOCHROMATIC APPEARANCE TO A METACHROMATIC SUBSTANCE. SYLVEN (GRIMAS 1962) DEMONSTRATED THAT HYALURONIC ACID NEEDS TO BE HIGHLY POLYMERIZED AND IN A MINIMUM CONCENTRATION OF 1% (W/VOL.).

#### HISTOCHEMISTRY OF MUCOPOLYSACCHARIDES AND MUCOPROTEINS:

THE HISTOCHEMISTRY OF THESE SUBSTANCES IS RELATED WITH PERIODIC ACID - SCHIFF (PAS) REACTION AND IT IS THEREFORE NECESSARY TO CONSIDER THE PRINCIPLES ON WHICH THIS REACTION IS BASED. ITS USE IN HISTOLOGY WAS FIRST INTRODUCED BY MCMAHON FOR THE DEMONSTRATION OF MUCIN BUT LATER LILLIE AND HOTCHKISS ELABORATED THE METHOD INTO A HISTOCHEMICAL ONE WHICH COULD BE USED FOR THE DETECTION OF VARIOUS POLYSACCHARIDES IN THE TISSUES.

PERIODIC ACID IS AN OXIDANT WHICH BREAKS THE C-C BONDS IN VARIOUS STRUCTURES, WHERE THEY ARE PRESENT AS 1:2 GLYCOL GROUPS (CHOH-CHOH), CONVERTING THEM INTO DIALDEHYDES. THE EQUIVALENT AMINO OR ALKYL AMINO DERIVATIVES OF 1:2 GLYCOL OR ITS OXIDATION PRODUCTS (CHOH-CO) ARE ALSO ATTACKED AND CONVERTED INTO DIALDEHYDES. THE PARTICULAR PROPERTY OF PERIODIC ACID THAT MAKES IT INMEASURABLY SUPERIOR TO OTHER REAGENTS COMMONLY USED IN HISTOCHEMISTRY FOR OXIDATION OF C-C BONDS IS THAT IT DOES NOT FURTHER OXIDIZE THE RESULTING ALDEHYDES AND THESE CAN, THEREFORE BE LOCALIZED BY COMBINATION WITH SCHIFF'S REAGENT TO GIVE A SUBSTI-

TUTED DYE WHICH IS RED IN COLOR (PEARSE 1960).

THE AMOUNT OF COLOR DEVELOPED BY THE REACTION IS DEPENDENT ON THE AMOUNT OF REACTIVE GLYCOL STRUCTURES PRESENT IN THE TISSUES. GLEGG ET AL (1952) CONSIDERED THAT THE REACTIVE GROUPS CONCERNED ARE THOSE OF HEXOSE SUGARS, GLUCOSE, GALACTOSE, MANOSE, AND OF THE METHYLPENTOSE SUGAR FUCOSE AND HEXURONIC ACIDS. THEY AGREED IN THE FACT THAT THE ACID MUCOPOLYSACCHARIDES ARE NOT PAS POSITIVE. BRADE, HOOGHWINKEL AND SMITS (1957) CARRIED IN VITRO TESTS TO DEMONSTRATE THE PAS REACTION OF ACID MUCOPOLYSACCHARIDES. THEY FOUND THAT CHONDROITIN SULPHURIC ACID AND HYALURONIC ACID, BOTH WERE PAS NEGATIVE. THEY NOTED THAT ALTHOUGH HEXURONIC ACIDS WERE OXIDIZED, NO ALDEHYDES COULD BE DEMONSTRATED. IT WAS THEREFORE CONCLUDED THAT A SUBSTANCE IN ORDER TO BE PAS POSITIVE, HAS TO BE BUILT UP ENTIRELY OR PARTLY OF CARBOHYDRATES OTHER THAN HEXOSAMINE AND HEXURONIC ACID.

ACCORDING TO HOTCHKISS A SUBSTANCE HAS TO FULFILL THE FOLLOWING FOUR CRITERIA IN ORDER TO BE PAS POSITIVE:

1. IT HAS TO CONTAIN 1-2 GLYCOL GROUPING OR THE EQUIVALENT AMINO OR ALKYLAMINO DERIVATIVE OR THE OXIDATION PRODUCT  $\text{CHOH-CO}$ .
2. IT SHOULD NOT DIFFUSE AWAY IN THE COURSE OF FIXATION.
3. THE OXIDATION PRODUCT OF THE SUBSTANCE SHOULD BE STABLE AND NOT DIFFUSABLE.
4. IT SHOULD BE PRESENT IN SUFFICIENT CONCENTRATION TO GIVE A DETECTABLE FINAL COLOR.

ACCORDING TO HOTCHKISS THE FOLLOWING COMPOUNDS ARE PAS POSITIVE: MUCOPOLYSACCHARIDES, GLYCOPROTEINS, PHOSPHORYLATED SUGARS, CEREBROSIDES AND INOSITOL CONTAINING LIPIDS.

#### ENZYMAL ANALYSIS:

THE TREATMENT OF THE TISSUES WITH VARIOUS ENZYMES WILL BRING ABOUT CHANGES IN THE STAINING REACTIONS MENTIONED ABOVE. THEY CAN EITHER ACT BY REMOVING THE REACTIVE GROUPS BY HYDROLYSIS, AS IN THE CASE OF NYALURONIDASE OR AMYLASE WITH THE CONSEQUENT REDUCTION IN METACHROMASIA AND PAS, OR UNCOVERING REACTIVE GROUPS BY BREAKING POLYSACCHARIDE PROTEIN COMPLEXES AS IN THE CASE OF PROTEOLYTIC ENZYMES IN WHICH THE MORE AVAILABLE GROUPS WILL GIVE MORE INTENSE RESULTS WITH PAS.

ON THE BASIS OF THESE OBSERVATIONS IT IS ASSUMED THAT AS THE ERUPTING TEETH MOVE THROUGH THE ORAL MUCOSA, THE CONNECTIVE TISSUE GROUND SUBSTANCE UNDERGOES CHANGES WHICH WOULD SEEM TO PERMIT THIS MOVEMENT TO TAKE PLACE. IT IS SUGGESTED THAT THIS PROCESS IS AN INSTANCE OF THE LABILE NATURE OF THE GROUND SUBSTANCE AS DESCRIBED BY GERSH AND CATCHPOLE.

ALTERATIONS OCCURRING IN THE PHYSICAL STATE OF THE GROUND SUBSTANCE SUCH AS THE DEPOLYMERIZATION OF THE GROUND SUBSTANCE IN THE ORAL MUCOSA, DURING ERUPTION OF A TOOTH SHOULD BE REFLECTED IN THE STAINING REACTION OF THE GROUND SUBSTANCE, USING PERIODIC ACID - LEUCOFUCHSIN METHOD.

THE STAINABILITY OF THE GROUND SUBSTANCE IN THE ORAL MUCOSA MAY VARY ACCORDING TO ITS PHYSIOLOGIC STATE. THE MUCOSA

OVER AN ERUPTING TOOTH HAS BEEN SHOWN TO UNDERGO A CHANGE FROM A GEL TO SOL STATE, LOSING SOME OF ITS POLYSACCHARIDES BY SOLUTION. THE ATTACHED MUCOSA ON THE BUCCAL OR LABIAL SURFACE OF THE MANDIBLE AND MAXILLA, SHOULD SHOW LESS SIGNIFICANT CHANGE IN THE PHYSICAL STATE OF THE GROUND SUBSTANCE EVEN THOUGH IT IS ANATOMICALLY APPROXIMATING THE MUCOSA OVERLYING THE ERUPTING TOOTH.

THE STAINING REACTIONS WITH PERIODIC ACID-SCHIFF REAGENT SHOULD DEMONSTRATE THE PRESENCE OF NEUTRAL POLYSACCHARIDES; TOLUIDINE BLUE SHOULD DEMONSTRATE ANY ACID MUCOPOLYSACCHARIDES. TREATMENT OF THE MUCOSA WITH AMYLASE SHOULD REMOVE GLYCOGEN. SUBSEQUENT STAINING WITH PAS WILL SERVE TO SEMIQUANTITATIVELY DETERMINE LOSS OF GLYCOGEN. THE USE OF TESTICULAR HYALURONIDASE WILL REMOVE ACID MUCOPOLYSACCHARIDES (CHONDROITIN A AND C AND HYALURONIC ACID). SUCH A LOSS MAY BE DEMONSTRATED BY STAINING WITH TOLUIDINE BLUE. THUS, A COMPARISON OF THE ADJACENT ATTACHED MUCOSA MAY SHOW DIFFERENT PHYSIOLOGIC STATES ATTRIBUTED TO CHANGE IN THE GROUND SUBSTANCE ATTRIBUTING TO THE ERUPTING TOOTH.

#### D. MECHANISM OF ERUPTION IN CONTINUOUSLY GROWING TEETH

THE ERUPTIVE MOVEMENTS OF A TOOTH ARE THE EFFECT OF DIFFERENTIAL GROWTH. DIFFERENTIAL GROWTH IS ONE OF THE MOST IMPORTANT FACTORS IN MORPHOGENESIS. IN THE JAWS, IT IS THE DIFFERENTIAL GROWTH BETWEEN TOOTH AND BONE THAT LEADS TO THE MOVEMENT OF A TOOTH.

THE MOVEMENTS OF THE TEETH DURING ERUPTION ARE VERY

INTRICATE AND ARE ACCOMPLISHED BY MINUTE COORDINATION OF GROWTH OF THE TOOTH, GROWTH OF THE ALVEOLAR BONE AND GROWTH OF THE JAWS (ORBAN).

ACCORDING TO SINGER (1942) THE TOOTH ELONGATION IS THE CAUSE OF ERUPTION. THE LONGITUDINAL GROWTH OF THE DENTAL PULP GENERATES THE MOST OBVIOUS ERUPTIVE FORCE. THE PULP, SEPARATED FROM THE PERIAPICAL TISSUE BY THE EPITHELIAL DIAPHRAGM AND PART OF THE HAMMOCK LIGAMENT, GROWS AS AN ORGAN IN ITSELF NOT BY INCORPORATION OF PERIAPICAL CONNECTIVE TISSUE BUT BY INTERSTITIAL GROWTH IN A CIRCUMSCRIPT AND WELL-DEFINED AREA FORMED BY THE INFOLDING OF THE HERTWIGS EPITHELIAL SHEATH. THIS LOCALIZED GROWTH OF THE PULP IN LENGTH IS OF EXACTLY THE SAME RATE AS THE GROWTH OF THE HERTWIGS EPITHELIAL SHEATH.

THE PROLIFERATION OF THE PULP AS AN ORGAN IN ITSELF IN COORDINATION WITH THE GROWTH OF HERTWIGS EPITHELIAL SHEATH PRECEDES THE FORMATION OF DENTIN AND IS THE IMPORTANT AND LEADING PROCESS IN TOOTH ELONGATION.

THE TOOTH IS FIXED TO AND SUSPENDED FROM THE BONE BY THE FIBERS OF THE PERIODONTAL LIGAMENT. THESE FIBERS SHOW AN INTERMEDIATE PLEXUS, WHICH CONSISTS OF PRECOLLAGENOUS FIBERS. AS THE TOOTH ERUPTS INTERMEDIATE PLEXUS ALLOWS THE REARRANGEMENT OF THE PERIODONTAL LIGAMENT. THIS CONTINUAL CHANGE IN THE INTERMEDIATE PLEXUS AIDS IN MAINTAINING THE FUNCTION OF THE ATTACHMENT OF THE ERUPTING TOOTH TO THE UNDERLYING BONE. (SINGER 1942)

SCHOUR (1934), IN HIS EXPERIMENTS, ON CHANGES IN INCISORS



OF THE WHITE RATS FOLLOWING HYPOPHYSECTOMY OBSERVED THE SLOWING DOWN OF ERUPTION AND THE FOLDING OF THE DENTIN AT THE BASAL END OF THE TOOTH. ACCORDING TO SIGHER, BOTH THESE FINDINGS CAN BE EXPLAINED BY THE SPECIFIC INHIBITION OF PULPAL GROWTH (MESODERMAL) WHEREAS EPITHELIAL GROWTH AND INDUCTION OF DENTIN FORMATION ARE FAR LESS AFFECTED.

IN RATS, PRIOR TO THE ACTUAL ERUPTION OF THE INCISORS THE BASAL END GROWS BACKWARD INTO THE JAW-BONE WHICH RESORBS BEFORE ITS ADVANCE. AT 8 DAYS OF AGE ACTUAL ERUPTION BEGINS IN A FORWARD DIRECTION (ADDISON, W.H.F. & J.L. APPELTON, 1915) AND THE INCISAL TIP APPEARS IN THE ORAL CAVITY AT ABOUT 8 TO 10TH DAY. THE RATE OF ERUPTION IS 2.1 MM. IN THE UPPER AND 2.8 MM. IN THE LOWER INCISORS.

THE ERUPTION OF NOT ONLY THE INCISORS BUT ALSO THAT OF THE MOLARS IS CONTINUOUS THROUGHOUT THE LIFE OF THE RAT BUT IS AT A VERY SLOW RATE. UNDER THE STIMULUS OF THE SLOW BUT CONTINUOUS ATTRITION OF THE MOLAR TEETH, ODONTOBLASTS, CEMENTOBLAST AND OSTEOBLASTS ARE DIFFERENTIATED FOR SECONDARY APPPOSITION OF DENTIN, CEMENTUM AND BONE RESPECTIVELY. THE GROWTH OF THESE TISSUES, AS WELL AS ATTRITION AND ERUPTION ARE CONTINUOUS PROCESSES (GRIFFITH AND FARRIS, 1942).

IT HAS BEEN STATED THAT THE CONNECTIVE TISSUE COVERING THE CROWN ATROPHIES WHEN THE TOOTH MOVES TOWARDS THE ORAL EPITHELIUM (WEINMANN, SVOBODA AND WOODS 1945). THEY ASSUMED THAT THE PRESENCE OF AN EPITHELIAL COVERING OF THE CROWN IS

NECESSARY TO BRING ABOUT THIS ATROPHY OF THE TISSUES BLOCKING THE PATH OF ERUPTION. THE LACK OF A LAYER OF EPITHELIUM ON THE OCCLUSAL PART OF A CROWN WOULD RESULT IN A LACK OF STIMULUS INDUCING THE ATROPHY OF THE CONNECTIVE TISSUE BETWEEN CROWN AND EPITHELIUM, THUS MAKING THE NORMAL ERUPTION OF A TOOTH IN MANY CASES IMPOSSIBLE.

IN THE PAST, THE TERM ERUPTION WAS GENERALLY APPLIED ONLY TO THE APPEARANCE OF TEETH IN THE ORAL CAVITY. IT IS KNOWN THAT THE MOVEMENTS OF THE TEETH DO NOT CEASE WHEN THE TEETH MEET THEIR ANTAGONISTS. MOVEMENTS OF ERUPTION BEGIN AT THE TIME OF ROOT FORMATION. THE EMERGENCE THROUGH THE GINGIVA IS AN INCIDENT IN THE PROCESS OF ERUPTION.

DURING THE PRE-ERUPTIVE PHASE OF THE DENTAL ORGAN, AFTER THE COMPLETE FORMATION OF THE CROWN, THE TOOTH GERM IS SURROUNDED BY THE LOOSE CONNECTIVE TISSUE OF THE DENTAL SAC AND BY THE BONE OF THE DENTAL CRYPT. WHEN THE ROOT FORMATION STARTS, THE CROWNS OF THE TEETH UNDERGO INTRICATE MOVEMENTS. DURING THESE MOVEMENTS, BONE GROWTH TAKES PLACE IN THESE AREAS OF THE DENTAL CRYPT FROM WHICH THE TOOTH MOVES; RESORPTION OCCURS IN THE AREAS TOWARDS WHICH THE TOOTH MOVES AND THE TOOTH THUS MOVES OUT OF THE BONY CRYPT. IT IS THEN SURROUNDED BY THE CONNECTIVE TISSUE OF THE ORAL MUCOSA. IN THIS EXPERIMENT, AN ATTEMPT IS MADE TO STUDY THE CHANGES IN THE CONNECTIVE TISSUE ABOVE AN ERUPTING TOOTH.

## MATERIAL AND METHODS

EIGHTEEN ALBINO RATS WERE USED IN THIS INVESTIGATION. THE ANIMALS WERE RECEIVED IN OUR LABORATORY AT THE AGE OF FOURTEEN DAYS. THEY WERE SACRIFICED AT THE AGE OF 15 DAYS BY ETHER.

SPECIMENS OF TISSUES WERE DISSECTED OUT FROM THE ATTACHED GINGIVA OF THE MAXILLARY AND MANDIBULAR MOLAR REGIONS AND FROM THE GINGIVAL TISSUE OVER THE UNERUPTED MOLARS.

ALVEOLAR MUCOSA CAN BE DEFINED AS THE MUCOUS MEMBRANE WHICH COVERS THE ALVEOLAR PROCESS TO THE MUCOGINGIVAL JUNCTION. IN THIS INVESTIGATION THE ALVEOLAR MUCOSA CAN BE CALLED THE MUCOUS MEMBRANE COVERING THE ALVEOLAR PROCESS TO A LINE WHERE IT TURNS AROUND THE OCCLUSAL SURFACE OF AN UNERUPTED TOOTH. THE PORTION OF THE GINGIVAL TISSUE OVERLYING AN UNERUPTED TOOTH CAN BE CALLED AS THE GINGIVAL PAD. ANATOMICALLY, BOTH ARE CONTINUOUS, ONE WITH ANOTHER, THEREFORE IN VERY CLOSE PROXIMITY.

THE TISSUE SPECIMENS WERE FIXED BY FREEZE-DRY METHOD. FIRST, THEY WERE SUBMERGED IN ISO-PENTANE PRE-CHILLED IN LIQUID NITROGEN TO  $-160^{\circ}\text{C}$ . THEY WERE THEN DEHYDRATED IN A VACUUM CHAMBER FOR A PERIOD OF 48 HOURS AT A TEMPERATURE OF  $-60^{\circ}\text{C}$ , TO ELIMINATE THE WATER CONTENT OF THE TISSUE BY SUBLIMATION.

THE EMBEDDING WAS DONE IN VACUO WITH PARAFFIN (TISSUE MAT FISHER CO.) AT A MELTING POINT OF  $56.5^{\circ}\text{C}$ . UNDER A PRESSURE

OF 15 LBS./SQ. INCH FOR A PERIOD OF 15 MINUTES.

SECTIONS WERE THEN CUT FROM 6 TO 10 MICRONS. THE THICKER SECTIONS WERE USED FOR ENZYME INCUBATION PROCEDURE.

THE MOUNTED SLIDES WERE DEPARAFFINIZED IN THE FOLLOWING MANNER. IMMERSION FOR FIVE MINUTES EACH IN TWO CHANGES OF XYLOL, THEN FOR FIVE MINUTES IN ABSOLUTE ALCOHOL, FOLLOWED BY THREE MINUTES IN 95% ALCOHOL AND SUBSEQUENTLY IN 75% ALCOHOL FOR THREE MINUTES. FINALLY, THE SECTIONS WERE WASHED IN DISTILLED WATER FOR FIVE MINUTES.

THE METHOD EMPLOYED FOR THE DEMONSTRATION OF ACID MUGO-POLYSACCHARIDES WAS TOLUIDINE BLUE STAIN METHOD. TOLUIDINE BLUE WAS USED AT A CONCENTRATION OF 1:1000 AND A PH OF 7.

SECTIONS WERE TREATED AS FOLLOWS:

- SOLUTION A    TOLUIDINE BLUE 1:1000  
 SOLUTION B    AMMONIUM MOLYBDATE 5% AQ. SOLN.  
 SOLUTION C    POTASSIUM FERRICYANIDE 1% AQ. SOLN.  
 SOLUTION D    1 VOL. OF SOLN. B PLUS 1 VOL. OF SOLN. C.

1. STAIN IN SOLUTION A FOR 2 MINUTES
2. RINSE IN DISTILLED WATER
3. STAIN IN SOLUTION D FOR 2 MINUTES

THIS PREVENTS LOSS OF METACHROMASIA, WHICH WOULD OTHERWISE OCCUR, WHEN TISSUE SECTIONS ARE DEHYDRATED IN ALCOHOL.

4. RINSE IN DISTILLED WATER.

5. DEHYDRATE IN 75% ALCOHOL, FOLLOWED BY 95% AND FINALLY IN ABSOLUTE ALCOHOL.
6. REMOVAL OF ALCOHOL BY TWO CHANGES OF XYLOL.

THE SECTIONS WERE THEN MOUNTED WITH PERMOUNT.

TESTICULAR HYALURONIDASE (GENERAL BIOCHEMICALS INC.)

INCUBATION WAS CARRIED OUT FOR THE REMOVAL OF HYALURONIC ACID, CHONDROITIN SULPHATE AND CHONDROITIN SULPHATE C. A SOLUTION OF A CONCENTRATION OF 1 MG. OF TESTICULAR HYALURONIDASE IN 1 CC. OF SORENSON'S PHOSPHATE BUFFER AT A PH OF 7 WAS MADE. FIVE DROPS OF THE SOLUTION WERE PLACED ON A SECTION ON A SLIDE. THE SLIDES WERE THEN PLACED IN WET CHAMBER, PETRI-DISH CONTAINING WET FILTER PAPER AND THEN INCUBATED AT 37° C. FOR TWO HOURS. SECTIONS FROM BOTH, THE ALVEOLAR MUCOSA AND THE ERUPTIVE MUCOSA, WERE TREATED FOR HYALURONIDASE INCUBATION. CONTROL SLIDES WERE TREATED WITH SORENSON'S BUFFER SOLUTION ALONE AND THEN INCUBATED AT THE SAME TEMPERATURE AND FOR THE SAME LENGTH OF TIME. IMMEDIATELY FOLLOWING INCUBATION THE SECTIONS WERE STAINED WITH TOLUIDINE BLUE.

THE PERIODIC-ACID SCHIFF METHOD (MCMANUS MODIFICATION OF GOLEMAN) WAS USED FOR THE DEMONSTRATION OF NEUTRAL POLYSACCHARIDES.

REMOVAL OF GLYCOGEN WAS CARRIED OUT BY INCUBATING THE SECTIONS WITH DIASTASE ENZYME. (NUTRITIONAL BIOCHEMICAL CORP.) A SOLUTION OF A CONCENTRATION OF 1 MG. OF ENZYME IN 1 CC. OF SORENSON'S BUFFER AT A PH OF 6 WAS MADE. BOTH THE SECTIONS,

THOSE OF THE ALVEOLAR MUCOSA AND ERUPTIVE MUCOSA, WERE TREATED WITH DIASTASE ENZYME SOLUTION. FIVE DROPS OF THE SOLUTION WERE USED TO COVER THE SECTION AND THE SLIDES WERE THEN PLACED IN A PETRI-DISH CONTAINING A WET CHAMBER FOR 15 MINUTES AT 37° C.

IMMEDIATELY FOLLOWING THIS TREATMENT WITH DIASTASE, THE SECTIONS WERE STAINED WITH PERIODIC-ACID SCHIFF METHOD.

TO STUDY THE GENERAL MORPHOLOGY OF THE TISSUES, SECTIONS WERE STAINED WITH HAEMATOXYLIN AND EOSIN METHOD.

## FINDINGS

### HAEMATOXYLIN AND EOSIN:

#### A. ALVEOLAR MUCOSA:

THE EPITHELIUM OF THE ALVEOLAR MUCOSA CONSISTED OF BASAL, SPINOUS, GRANULAR AND KERATIN LAYERS. BASAL CELL LAYER WAS FORMED BY A SINGLE LAYER OF CUBOIDAL CELLS ANCHORED TO THE BASEMENT MEMBRANE. INTERCELLULAR SPACES WERE OBSERVED ALONG THE EPITHELIAL LAYERS WITH THE EXCEPTION OF THE BASAL CELL LAYER. THE KERATINOUS LAYER WAS CHARACTERIZED BY ITS ACIDOPHILIC NATURE AND STAINED RED. THE EPITHELIAL RETE PEGS OR EPITHELIAL RIDGES WERE NUMEROUS AND SLENDER WITH THE PAPILLAE OF THE CONNECTIVE TISSUE INTERVENING. THE LAMINA PROPRIA CONSISTED OF DENSE CONNECTIVE TISSUE WITH COMPACT FIBER BUNDLES. THE LAMINA PROPRIA CONSISTED OF DENSE CONNECTIVE TISSUE WITH COMPACT FIBER BUNDLES. THE LAMINA PROPRIA WAS HIGHLY CELLULAR, WITH FIBROBLASTS PREDOMINATING. A FEW MAST CELLS WERE ALSO OBSERVED. THE BLOOD VESSELS SHOWED NARROW LUMENS.

THE GROUND SUBSTANCE HAD AN HOMOGENOUS APPEARANCE.

#### B. GINGIVAL PAD:

THE EPITHELIUM OF THE GINGIVAL PAD SHOWED DECREASED NUMBER OF CELL LAYERS, THAN THAT OF THE ALVEOLAR MUCOSA. THE DEGREE OF KERATINIZATION WAS ALSO LESS. THE EPITHELIAL RIDGES WERE NOT FREQUENT AND WERE LESS PROMINENT.

THE LAMINA PROPRIA CONSISTED OF LOOSE CONNECTIVE TISSUE. THE FIBERS WERE SEEN TO BE DISSOCIATED FROM WITHIN THE BUNDLES. THE AREA OF THE LAMINA PROPRIA, ABOVE THE ERUPTING TOOTH SHOWED VERY LOOSE AND WATERY APPEARANCE. MAST CELLS WERE PREDOMINANT. THE BLOOD VESSELS SHOWED DILATED APPEARANCE. ON THE WHOLE, THE LAMINA PROPRIA OF THE GINGIVAL PAD SHOWED A LOOSE AND WATERY APPEARANCE.

#### TOLUIDINE BLUE:

THE EPITHELIUM OF THE ALVEOLAR MUCOSA CONSISTED OF THE USUAL BASAL, SPINOUS, GRANULAR AND KERATIN LAYERS. THE THICKNESS OF THESE LAYERS, HOWEVER, WAS GREATER THAN THAT OF THE GINGIVAL PAD. THE KERATIN LAYER WAS ALSO THICKER AND THE NUMBER OF EPITHELIAL RETE PEGS WAS ALSO MORE NUMEROUS IN ALVEOLAR MUCOSA. IN BOTH THE ALVEOLAR MUCOSA AND GINGIVAL PAD THE NUCLEI OF THE BASAL CELL LAYER SHOWED A MORE INTENSE METACHROMATIC REACTION WHILE THE CYTOPLASM OF THE EPITHELIAL CELLS ALSO SHOWED A METACHROMATIC REACTION. IT WAS IRREGULAR, FAINT AND DECREASED TOWARDS THE SURFACE LAYERS. THE KERATIN LAYER GAVE A CONSTANT METACHROMATIC REACTION. THE INTERCELLULAR SUBSTANCE WAS OBSERVED TO STAIN WITH A LIGHT METACHROMASIA.

THE BASEMENT MEMBRANE AT THE JUNCTION OF THE EPITHELIUM AND CONNECTIVE TISSUE DID NOT STAIN AND APPEARED AS A CLEAR TRANSPARENT ZONE.

IN THE GROUND SUBSTANCE OF THE ALVEOLAR MUCOSA AN INTENSE METACHROMASIA WAS OBSERVED AS COMPARED TO THE GINGIVAL PAD. THE



METACHROMATIC AREAS IN THE ALVEOLAR MUCOSA WERE MORE INTENSE THAN THOSE IN THE GINGIVAL PAD. THE DISTRIBUTION OF THESE AREAS WAS ALSO DIFFERENT IN BOTH. THOSE IN THE GINGIVAL PAD WERE MORE WIDELY SPACED.

CELLULARITY IN THE LAMINA PROPRIA WAS NOTED AND COMPARED WITH THE GINGIVAL PAD. THE PREDOMINANT CELLS WERE THE FIBROBLASTS. THESE WERE LONG, SLENDER, CONNECTIVE TISSUE CELLS WITH FEW PALE METACHROMATIC GRANULES IN THE CYTOPLASM. THE NUCLEI AND THE CYTOPLASM OF THE FIBROBLASTS GAVE A METACHROMATIC REACTION WITH THE NUCLEI STAINING MORE INTENSELY.

A FEW MAST CELLS WERE ALSO OBSERVED IN THE LAMINA PROPRIA OF THE ALVEOLAR MUCOSA. HOWEVER, THE MAST CELLS WERE IN GREATER ABUNDANCE IN THE LAMINA PROPRIA OF THE GINGIVAL PAD. THESE CELLS WERE FILLED WITH NUMEROUS LARGE GRANULES WHICH WERE INTENSELY METACHROMATIC. THE HEAVY CONCENTRATION OF THESE GRANULES MADE IT DIFFICULT TO OBSERVE THE CELL DETAILS. SOME OF THE MAST CELLS WERE FOUND LIBERATING THEIR GRANULES INTO THE EXTRACELLULAR SPACE.

THE CONNECTIVE TISSUE FIBERS WERE OUTLINED BY PALE UNSTAINED REACTION.

#### TESTICULAR HYALURONIDASE:

A REDUCTION IN THE METACHROMASIA OF THE GROUND SUBSTANCE OF THE ALVEOLAR MUCOSA WAS OBSERVED, FOLLOWING INCUBATION WITH HYALURONIDASE FOR TWO HOURS. HOWEVER, THE REDUCTION IN THE METACHROMASIA OF THE GROUND SUBSTANCE OF THE GINGIVAL PAD WAS MORE AS COMPARED TO THAT OF THE ALVEOLAR MUCOSA. THE GROUND

SUBSTANCE SHOWED MUCINOUS APPEARANCE.

THE CONNECTIVE TISSUE FIBERS WERE UNSTAINED AND, IN SOME SECTIONS THEY WERE SEEN TO BE SEPARATING WITHIN THE BUNDLES.

THE STAINABILITY OF THE FIBROBLASTS AND MAST CELLS WAS NOT ALTERED BY THE ENZYME TREATMENT.

THE METACHROMASIA FOUND IN THE DIFFERENT EPITHELIAL COMPONENTS WAS ALSO SEEN TO BE UNALTERED BY TESTICULAR HYALURONIDASE.

IT WAS NOTICED THAT CONTROL SLIDES OF BOTH THE ALVEOLAR MUCOSA AND GINGIVAL PAD INCUBATED IN BUFFER SOLUTION ALONE SHOWED A REDUCTION IN THE METACHROMASIA. THIS REDUCTION IN THE METACHROMASIA WAS NOT AS GREAT AS THE ONE OBSERVED IN THE SECTIONS INCUBATED IN BUFFER WITH ENZYME HYALURONIDASE IN IT.

#### PERIODIC-ACID SCHIFF:

THE ONLY PAS POSITIVE AREA FOUND IN THE EPITHELIUM WAS THE INTERCELLULAR SUBSTANCE, RECOGNIZABLE WITHIN THE EPITHELIAL LAYERS MORE TOWARDS THE BASAL CELL LAYER AS A THIN RED LINE, IN BOTH, THE ALVEOLAR MUCOSA AND GINGIVAL PAD. THE NUCLEI AND THE CYTOPLASM OF ALL THE EPITHELIAL CELLS WERE PAS POSITIVE.

THE BASEMENT MEMBRANE BETWEEN THE EPITHELIUM AND CONNECTIVE TISSUE WAS STRONGLY PAS POSITIVE AND STAINED INTENSELY RED IN COLOR.

THE CONNECTIVE TISSUE IN BOTH THE ALVEOLAR MUCOSA AND GINGIVAL PAD GAVE A SIMILAR PAS POSITIVE REACTION. THE GRANULES

WERE SEEN SCATTERED IN THE LAMINA PROPRIA OF THE ALVEOLAR MUCOSA. SUCH GRANULES APPARENTLY WERE MORE ABUNDANT IN THE GROUND SUBSTANCE OF THE LAMINA PROPRIA OF THE GINGIVAL PAD.

#### DIASTASE:

TREATMENT WITH DIASTASE SHOWED SLIGHT CHANGES IN THE PAS STAINABILITY OF THE EPITHELIUM OF THE ALVEOLAR AS WELL AS GINGIVAL PAD. THE EPITHELIAL CELLS IN THE GINGIVAL PAD WERE ONLY SLIGHTLY LIGHTER PAS POSITIVE. THE BASEMENT MEMBRANE MAINTAINED THE SAME INTENSE PAS POSITIVE REACTION AS DID THE BASEMENT MEMBRANE WITHOUT ANY TREATMENT WITH DIASTASE.

THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE IN THE DIASTASE TREATED SECTIONS OF THE ALVEOLAR MUCOSA AND GINGIVAL PAD STAINED ONLY SLIGHTLY LESS INTENSE WITH PAS THAN THOSE STAINED WITHOUT ANY TREATMENT WITH ENZYME.

IT WAS OBSERVED THAT MAST CELLS WERE DIFFICULT TO BE OBSERVED. THE GRANULAR CONTENT OF THE GROUND SUBSTANCE OF THE GINGIVAL PAD WAS OBSERVED TO BE INCREASED. DIASTASE TREATMENT CAUSED DISRUPTION OF THE CELL WALL AND EXPULSION OF THE GRANULES IN THE GROUND SUBSTANCE.

## DISCUSSION

CHEMICALLY, THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE OF THE ORAL MUCOSA IS PARTIALLY CHARACTERIZED BY THE PRESENCE OF MUCOPROTEINS AND GLYCOPROTEINS, AND BY THE MUCOPOLYSACCHARIDES. THE DISTRIBUTION OF THE GROUND SUBSTANCE CAN BE EFFECTIVELY DEMONSTRATED IN SECTIONS FROM TISSUES WHICH ARE FIXED BY THE FREEZE-DRY METHOD. THERE ARE SPECIFIC HISTOCHEMICAL PROCEDURES, TOLUIDINE BLUE AND PAS, WHICH COLOR THE HISTOLOGIC SECTIONS BY VIRTUE OF THE STAINABILITY OF CARBOHYDRATE CONTAINING COMPONENTS OF THE GROUND SUBSTANCE.

THE DETECTION OF ACID MUCOPOLYSACCHARIDES BY HISTOCHEMICAL METHODS IS BASED ON THE OBSERVATION OF THE METACHROMATIC SHIFT THAT BASIC DYES EXPERIENCE WHEN IN CONTACT WITH COMPOUNDS WITH A NET NEGATIVE ELECTRICAL SURFACE CHARGE (PEARSE 1960). IT HAS BEEN CLEARLY DEMONSTRATED THAT HYALURONIC ACID, HEPARIN AND CHONDROITIN SULPHURIC ACID STAIN METACHROMATICALLY. A DIFFERENTIATION BETWEEN THESE ACID MUCOPOLYSACCHARIDES CAN BE DONE BY TREATING THESE SUBSTANCES WITH ENZYMES WHICH WILL REACT SPECIFICALLY WITH SOME OF THEM ONLY. THUS, ON TREATMENT WITH TESTICULAR HYALURONIDASE, HEPARIN AND CHONDROITIN SULPHURIC ACID A. AND C. WILL BE HYDROLYSED (PEARSE 1960). SUBSEQUENT STAINING OF TISSUES WITH TOLUIDINE BLUE CAN LOCALIZE AND SEMIQUANTITATE ACID MUCOPOLYSACCHARIDES IN SITU.

A SATISFACTORY METHOD EMPLOYED FOR THE DEMONSTRATION OF

NEUTRAL POLYSACCHARIDES IS THE PERIODIC-ACID SCHIFF METHOD.

IT HAS BEEN DEMONSTRATED BY BRADEN (1955) AND HOOGWINKEL AND SMITS (1957) THAT ACID MUCOPOLYSACCHARIDES DO NOT STAIN POSITIVE WITH PAS METHOD. THIS OFFERS US AN OPPORTUNITY TO DIFFERENTIATE BETWEEN ACID AND NEUTRAL MUCOPOLYSACCHARIDES. GLYCOGEN, WHICH IS PAS POSITIVE, CAN BE REMOVED BY TREATING THE SECTIONS WITH DIASTASE AND A FURTHER DIFFERENTIATION OF NEUTRAL POLYSACCHARIDES CAN BE MADE.

THE MOST IMPORTANT FINDING WAS THAT SEEN AFTER TREATMENT WITH TESTICULAR HYALURONIDASE, THERE WAS A DIFFERENCE IN THE DEGREE OF METACHROMASIA IN THE GROUND SUBSTANCE OF THE ALVEOLAR MUCOSA AND THE GINGIVAL PAD. THE GINGIVAL PAD WAS LESS METACHROMATIC THAN THE ALVEOLAR MUCOSA AND IT WAS ALSO MORE MUCOID OR WATERY IN APPEARANCE. THE DECREASE IN THE METACHROMASIA OF THE ORAL MUCOSA AFTER INCUBATION WITH TESTICULAR HYALURONIDASE SUGGESTS THE PRESENCE OF ACID MUCOPOLYSACCHARIDES OF THE ORDER OF HYALURONIC ACID AND/OR CHONDROITIN SULPHURIC ACID A. OR C., IN THE GROUND SUBSTANCE; FURTHER, THE DIFFERENCE IN THE STAINING REACTIONS OF THESE COMPONENTS OF THE GROUND SUBSTANCE, OF TWO DIFFERENT BUT ANATOMICALLY CLOSE LOCATIONS, IS SUGGESTIVE OF THEIR PRESENCE IN A DIFFERENT DEGREE OF AGGREGATION. GERSON AND CATCHPOLE (1949) POSTULATED THAT THE INTENSITY WITH WHICH A TISSUE STAINED WAS RELATED TO THE CONCENTRATION OF THE REACTIVE ALDEHYDE GROUPS AND TO THE STATE OF AGGREGATION OF THE GROUND SUBSTANCE.

THIS CAN BE REGARDED AS AN EVIDENCE OF THE EXISTENCE OF WATER-RICH COLLOID-POOR PHASE IN THE GROUND SUBSTANCE OF THE GINGIVAL PAD. THIS RESULT GIVES SUPPORT TO THE PREVIOUSLY REPORTED FINDING THAT AS THE TEETH ERUPT THROUGH THE CONNECTIVE TISSUE OF THE ORAL MUCOUS MEMBRANE, THE GROUND SUBSTANCE BECOMES DEPOLYMERIZED AS EVIDENCED BY THE REDUCED METACHROMATIC REACTION (ENGLÉ 1951).

THERE WERE AREAS OF THE GROUND SUBSTANCE, ESPECIALLY IN THE LAMINA PROPRIA OF THE ALVEOLAR MUCOSA, THAT GAVE METACHROMATIC REACTION AND IN WHICH THE CELL PREDOMINANT WAS FIBROBLAST. THE CYTOPLASM OF FIBROBLASTS STAINS METACHROMATICALLY. THIS IS SUGGESTIVE OF THE ROLE OF FIBROBLASTS IN THE ELABORATION OF THE ACID MUCOPOLYSACCHARIDES OF THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE.

MAST CELLS WERE OBSERVED TO BE IN GREATER ABUNDANCE IN THE LAMINA PROPRIA OF THE GINGIVAL PAD THAN IN THE LAMINA PROPRIA OF THE ALVEOLAR MUCOSA. NONE OF THEIR INTRA OR EXTRACELLULAR GRANULES WERE AFFECTED BY TESTICULAR HYALURONIDASE. THEY MAINTAINED THE SAME DEGREE OF METACHROMASIA AFTER TREATMENT WITH TESTICULAR HYALURONIDASE. PEARSE (1960) STATED THAT THE CHONDROITIN SULPHATE B IS NOT HYDROLYSED BY TESTICULAR HYALURONIDASE. THE FACT THAT MAST CELLS MAINTAINED THE SAME DEGREE OF METACHROMASIA AS UNTREATED SPECIMENS AGREES WITH THE FINDINGS OF SCHULTZ-HAUDT (1958) WHO STATED THAT TESTICULAR HYALURONIDASE RESISTENT CHONDROITIN SULPHATE B IS PRESENT IN THE CONNECTIVE

TISSUE OF THE ORAL MUCOSA OF MAN. THE GRANULES ARE, THEREFORE, COMPOSED OF A SUBSTANCE DIFFERENT THAN HYALURONIC ACID AND CHONDROITIN SULPHATE A OR C.

THE RESULTS OBTAINED WITH THE PAS METHOD SHOWED DIFFERENCE IN THE CHARACTERISTICS OF THE EPITHELIUM OF THE ALVEOLAR MUCOSA AND GINGIVAL PAD. THE EPITHELIAL CELLS WERE LESS PAS POSITIVE AFTER TREATMENT WITH ENZYME DIASTASE. THIS INDICATES THE PRESENCE OF CARBOHYDRATES IN THE CYTOPLASM OF THE EPITHELIAL CELLS. THIS CARBOHYDRATE APPARENTLY HAD GONE INTO SOLUTION.

THE GROUND SUBSTANCE OF THE GINGIVAL PAD SHOWED A SLIGHT DECREASE IN THE STAINING WITH PAS AFTER TREATMENT WITH DIASTASE. THIS DIGESTION IS EVIDENCE THAT A CARBOHYDRATE-PROTEIN COMPLEX, GLYCOGEN, IS PRESENT IN THE GROUND SUBSTANCE. THE DIFFERENCE IN THE PAS STAINING OF THE GROUND SUBSTANCE OF THE GINGIVAL PAD AFTER TREATMENT WITH AN ENZYME AS COMPARED TO THAT OF THE ALVEOLAR MUCOSA AFTER TREATMENT WITH AN ENZYME, MAY BE AN INDICATION OF THE INCREASED AMOUNT OF WATER SOLUBLE GLYCO-PROTEIN MATERIAL IN THE GROUND SUBSTANCE OF GINGIVAL PAD. THIS IS SUPPORTED BY THE FINDINGS OF ENGLE (1956) AND GERSH AND CATCHPOLE (1949) WHO STATED THAT, AN ELEVATION OF THE WATER CONTENT OF THE TISSUE WILL CAUSE A REDUCTION IN THE INTENSITY OF THE PAS REACTION. THEY BELIEVE THAT THE GREATER DISPERSION OF TISSUE POLYSACCHARIDES IN A GIVEN AREA, CAUSED BY EDEMA, WILL REDUCE THE NUMBER OF REACTIVE GROUPS AVAILABLE FOR THE PAS STAIN.

THE DIFFERENCE IN THE STAINABILITY WITH PAS IN THE

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GINGIVAL PAD THAN THAT IN THE ALVEOLAR MUCOSA CAN THEREFORE, BE REGARDED AS AN EVIDENCE OF WATER-RICH COLLOID POOR PHASE OF THE GROUND SUBSTANCE OF THE GINGIVAL PAD.

THE MORPHOLOGIC, HISTOCHEMICAL AND CHEMICAL ASPECT OF CONNECTIVE TISSUE FORMED A BASIS FOR SOME UNDERSTANDING OF ITS PHYSIOCHEMICAL BEHAVIOR. THE ORGANIZATION INTO LARGE MOLECULES LENDS COLLOIDAL PROPERTIES TO THE MATRIX. SOME PARTS OF THE CARBOHYDRATE MOIETIES, NOTABLY THE CARBOXYL (COOH) AND SULPHATE ( $SO_4$ ) GROUPS, RENDERED A NEGATIVE CHARGE TO THIS COLLOID. THE PRESENCE OF WATER SOLUBLE AND WATER INSOLUBLE PARTS CHARACTERIZES THIS BIOLOGIC SYSTEM, AS A TWO PHASE SYSTEM, CONSISTING OF COLLOID RICH WATER POOR PHASE WHICH IS IN EQUILIBRIUM WITH COLLOID POOR WATER RICH PHASE. ENGLE (1958) HAS SHOWN THAT LOOSE, FAINTLY STAINED STRUCTURES WITH RELATIVELY GREATER AMOUNTS OF WATER SOLUBLE GROUND SUBSTANCE CONTAIN A LOW DENSITY OF NEGATIVELY CHARGED COLLOID. DENSER TISSUES MORE DEEPLY STAINED CONTAIN HIGHER CONCENTRATIONS OF CHARGED COLLOID AND RELATIVELY SMALLER AMOUNTS OF WATER-SOLUBLE MATERIAL. VERY DENSE CONNECTIVE TISSUES, SUCH AS MATURE BONE OR DENTINE, WHICH ARE VIRTUALLY UNSTAINED BY THE PAS METHOD AND WHICH CONTAIN VERY LITTLE SOLUBLE MATERIAL, SHOW THE GREATER DENSITY OF NEGATIVELY CHARGED COLLOID.

THE OBSERVED CHANGES OF THE GROUND SUBSTANCE, CAN BE REGARDED AS THE REMOVAL OF A KIND OF DERMAL BARRIER TO THE GROWING, ERUPTING TOOTH.



THE TENTATIVE IDENTIFICATION AND CHARACTERIZATION OF HYALURONIC ACID AND GLYCOPROTEINS HAS BEEN DEPENDENT UPON THE HISTOCHEMICAL EVIDENCE. AS THE TEETH APPROACH THE SURFACE OF THE ORAL MUCOUS MEMBRANE, THE GROUND SUBSTANCE IS ALTERED; IT IS THOUGHT TO BECOME DEPOLYMERIZED AS DEDUCED BY ITS STAINING REACTIONS WITH PAS AND TOLUIDINE BLUE AND ITS INCREASED CONTENT OF WATER SOLUBLE ALCOHOL INSOLUBLE CARBOHYDRATE MOIETY. IT IS SUGGESTED THAT THIS PROCESS IS AN INSTANCE OF THE LABILE NATURE OF THE CONNECTIVE TISSUE GROUND SUBSTANCE AS OBSERVED BY GERSH AND CATCHPOLE.

THE PRESENCE OF ACID MUCOPOLYSACCHARIDES IN THE GROUND SUBSTANCE OF THE GINGIVAL PAD IN A STATE OF AGGREGATION DIFFERENT FROM THAT OF ACID MUCOPOLYSACCHARIDES IN THE GROUND SUBSTANCE OF THE ALVEOLAR MUCOSA CAN BE REGARDED AS AN EVIDENCE OF THE DIFFERENT CONSISTENCY OF THE GROUND SUBSTANCE IN THESE TWO AREAS. IT CAN THEREFORE BE POSTULATED THAT THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE ABOVE AN ERUPTING TOOTH MAY UNDERGO CHANGES IN ITS CONSISTENCY DURING THIS PHYSIOLOGIC STATE. THE CHANGES IN THE GROUND SUBSTANCE MAY BE DUE TO DEPOLYMERIZATION OF THE CHEMICAL COMPONENTS OF THE GROUND SUBSTANCE WHICH MAY BE DUE TO MUCOLYTIC ENZYMES PRODUCED BY THE CELLS OF THE CONNECTIVE TISSUE, NAMELY THE FIBROBLASTS OR THE MAST CELLS OR THE EPITHELIAL CELLS OF THE REDUCED ENAMEL EPITHELIUM PRESENT IN THE LAMINA PROPRIA OF THE GINGIVAL PAD.

SUMMARY AND CONCLUSIONS

AN EXPERIMENT TO STUDY THE CHANGES OF THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE OF THE ORAL MUCOSA ABOVE AN UNERUPTED TOOTH WAS DONE. SPECIAL ATTENTION WAS GIVEN TO THE MUCOPOLYSACCHARIDES AND GLYCOPROTEIN COMPONENTS OF THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE. A COMPARATIVE EVALUATION OF THE GROUND SUBSTANCE OF ORAL MUCOSA FROM THE ATTACHED GINGIVA WAS DONE.

TWENTY-FOUR ALBINO RATS WERE USED. ANIMALS WERE SACRIFICED AT THE AGE OF 15 DAYS. TISSUE SPECIMENS OF THE GINGIVAL TISSUE WERE OBTAINED FROM THE MANDIBULAR AND MAXILLARY ALVEOLAR MUCOSA IN THE REGION OF THE MOLARS AND ALSO FROM THE GINGIVAL TISSUE OVERLYING THE UNERUPTED MOLARS. THE TISSUES WERE IMMEDIATELY TREATED BY FREEZE-DRYING METHOD.

THE HISTOCHEMICAL STAINING METHODS USED FOR THE DETECTION OF MUCOPOLYSACCHARIDES WERE: TOLUIDINE BLUE AND PERIODIC-ACID SCHIFF METHOD.

ENZYMAL ANALYSIS WAS ALSO DONE USING TESTICULAR HYALURONIDASE AND DIASTASE FOR THE FURTHER DIFFERENTIATION OF ACID MUCOPOLYSACCHARIDES AND NEUTRAL MUCOPOLYSACCHARIDES RESPECTIVELY.

THE RESULTS LEAD US TO THE FOLLOWING CONCLUSIONS:

1. THERE IS A CHANGE IN THE CONSISTENCY OF THE GROUND SUBSTANCE, A CHANGE FROM GEL STATE TO SOL STATE, OF THE CONNECTIVE TISSUE ABOVE AN ERUPTING TOOTH, AS SEEN BY THE DIFFERENCE IN THE STAINING REACTION WITH TOLUIDINE BLUE AND PAS AND COMPARING IT

WITH THE GROUND SUBSTANCE OF THE CONNECTIVE TISSUE OF THE ALVEOLAR MUCOSA.

2. THE CHANGE IN THE CONSISTENCY OF THE GROUND SUBSTANCE ABOVE AN ERUPTING TOOTH IS A LOCALIZED REACTION.

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FIGURE 1. ALVEOLAR MUCOSA, FREEZE DRIED STAINED WITH  
TOLUIDINE BLUE SHOWING METACHROMASIA IN THE  
CONNECTIVE TISSUE OF THE LAMINA PROPRIA  
X200

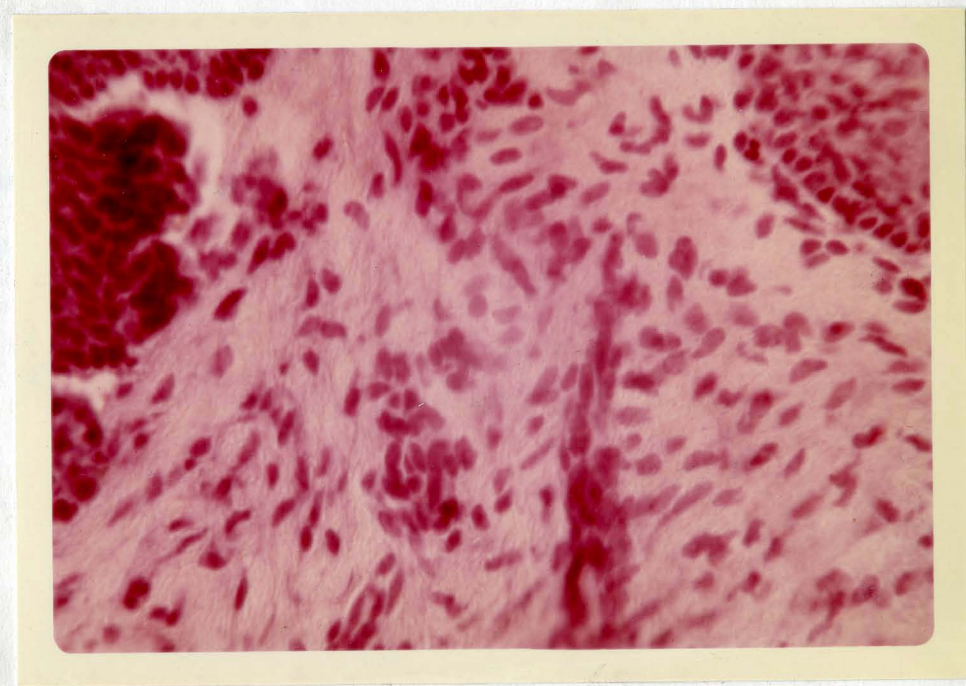


FIGURE 1.



FIGURE 2. ERUPTIVE MUCOSA, FREEZE DRIED STAINED WITH  
TOLUIDINE BLUE SHOWING METACHROMASIA IN THE CON-  
NECTIVE TISSUE OF THE LAMINA PROPRIA X100

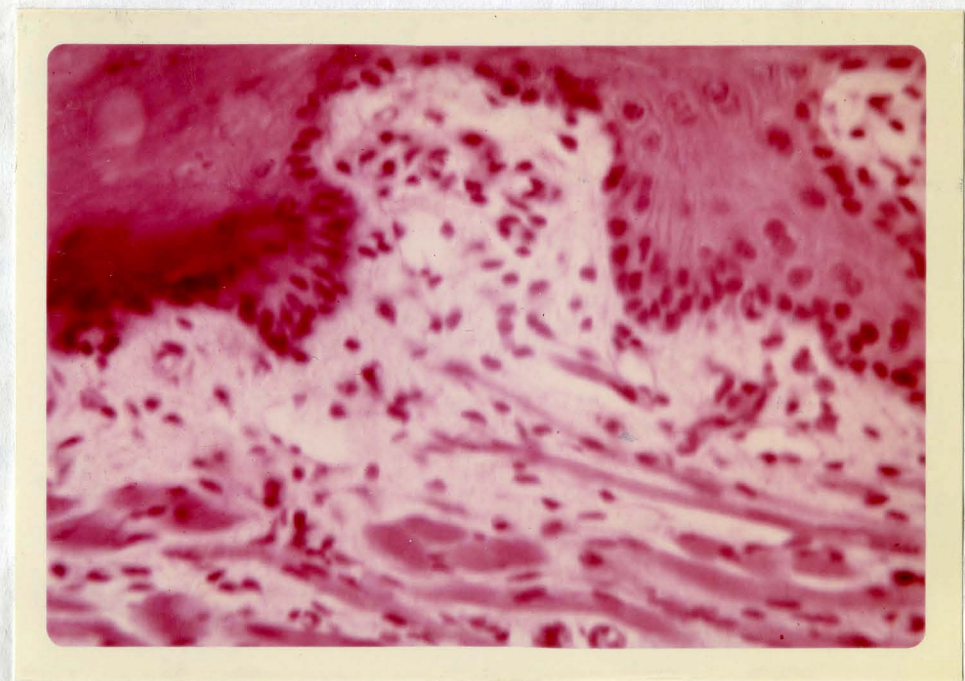


FIGURE 2.

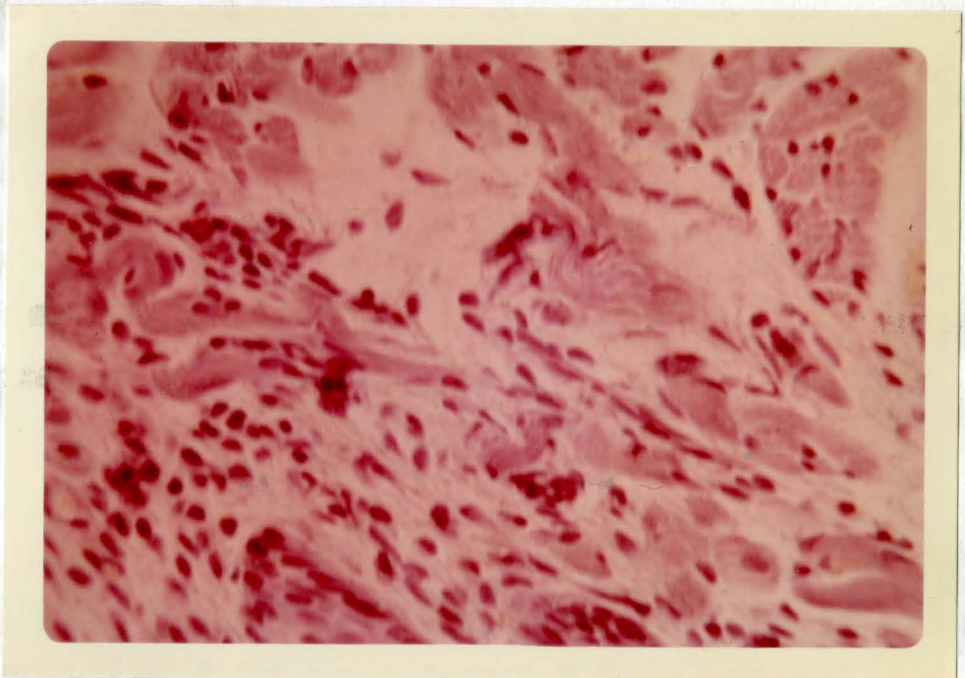


FIGURE 3. ALVEOLAR MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE.  
DECREASED METACHROMASIA OF THE GROUND  
SUBSTANCE X200

FIGURE 3.

FIGURE 4. ERUPTIVE MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE.  
DECREASED METACHROMASIA OF THE GROUND  
SUBSTANCE X200

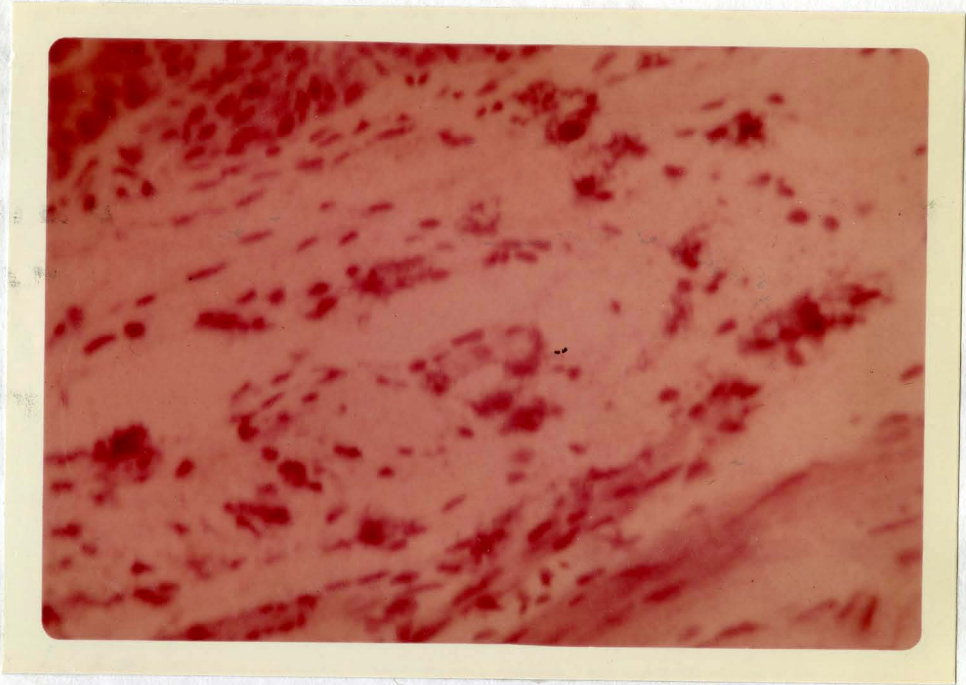


FIGURE 4.

FIGURE 5. ALVEOLAR MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE.  
DECREASED METACHROMASIA OF THE GROUND  
SUBSTANCE X970

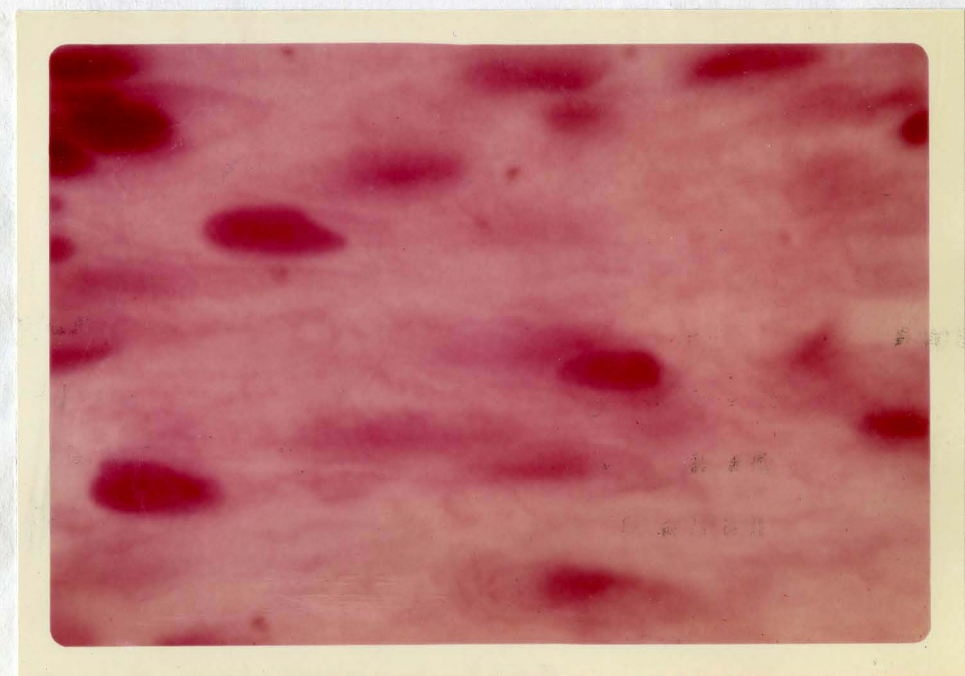


FIGURE 5.

FIGURE 6. ERUPTIVE MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE.  
DECREASED METACHROMASIA OF THE GROUND  
SUBSTANCE. GROUND SUBSTANCE SHOWS WATERY  
APPEARANCE. X970

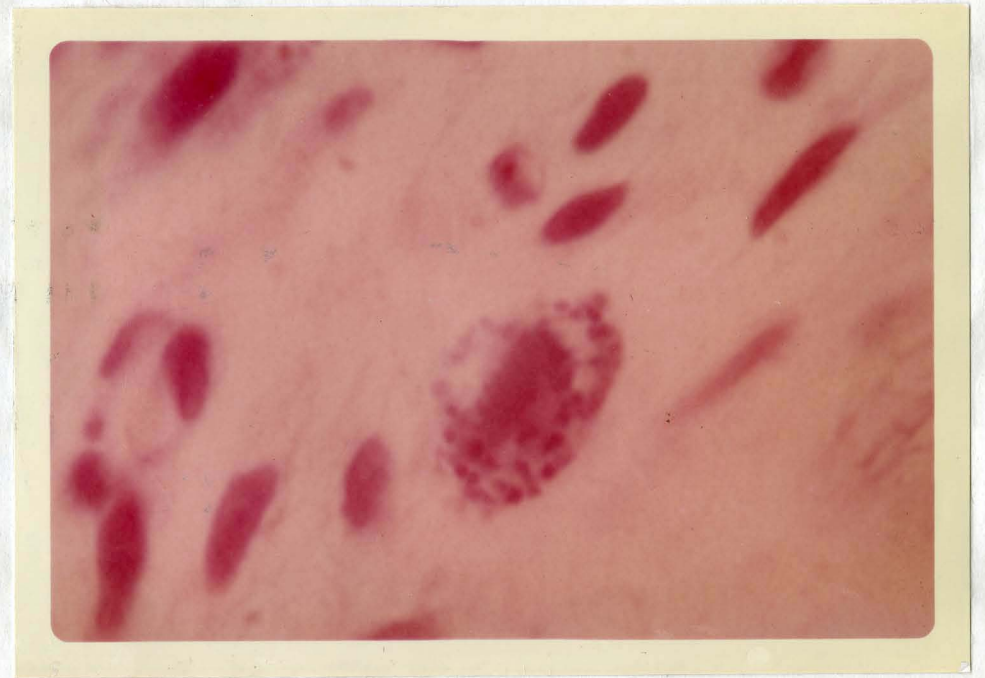


FIGURE 6.

FIGURE 7. ALVEOLAR MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE,  
SHOWING MAST CELLS IN THE LAMINA PROPRIA.

x970

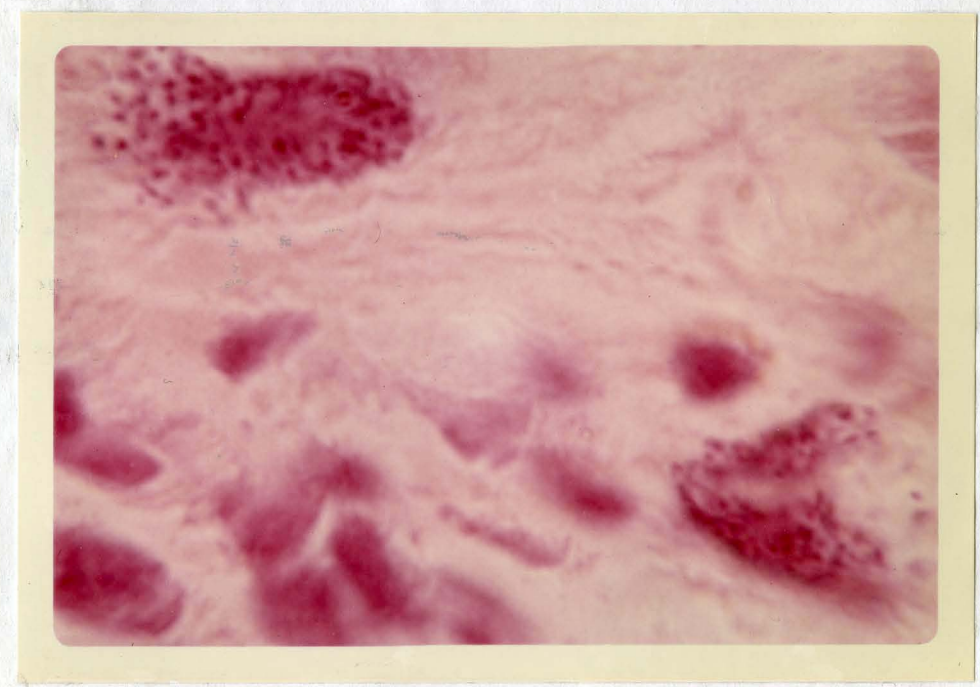


FIGURE 7.

FIGURE 8. ERUPTIVE MUCOSA, FREEZE DRIED, TREATED WITH  
HYALURONIDASE STAINED WITH TOLUIDINE BLUE,  
SHOWING MAST CELLS IN THE LAMINA PROPRIA

x470

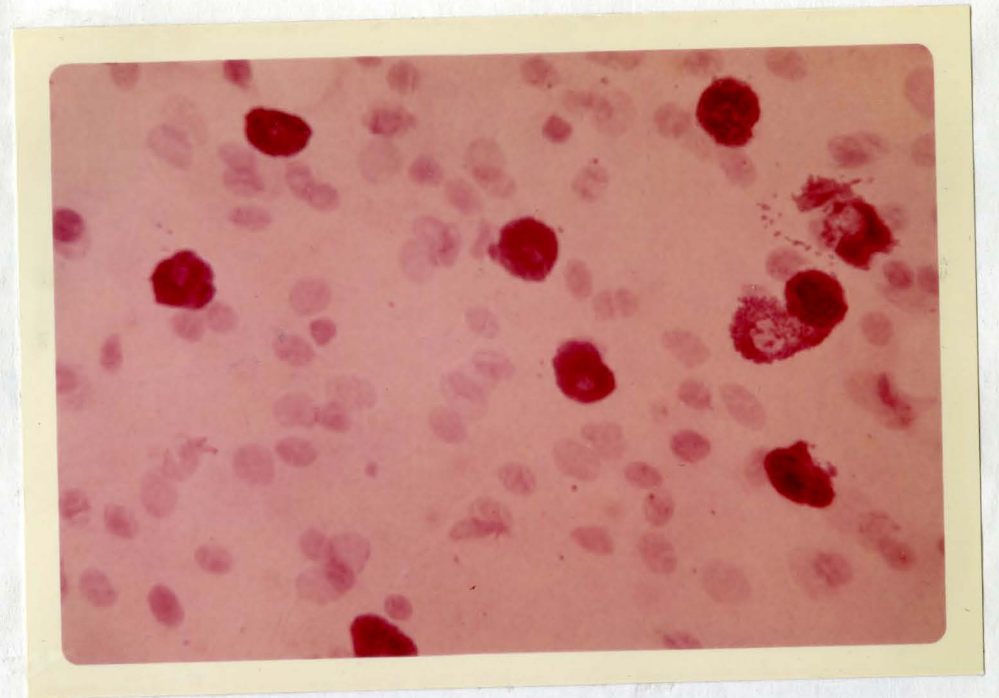


FIGURE 8.

FIGURE 9. ALVEOLAR MUCOSA, FREEZE DRIED, STAINED  
WITH PAS. SHOWING THE PROMINENT  
EPITHELIAL RIDGES. X35



FIGURE 9.



FIGURE 10. ERUPTIVE MUCOSA, FREEZE DRIED, STAINED WITH PAS. SHOWING THE ABSENCE OF PROMINENT EPITHELIAL RIDGES. X100

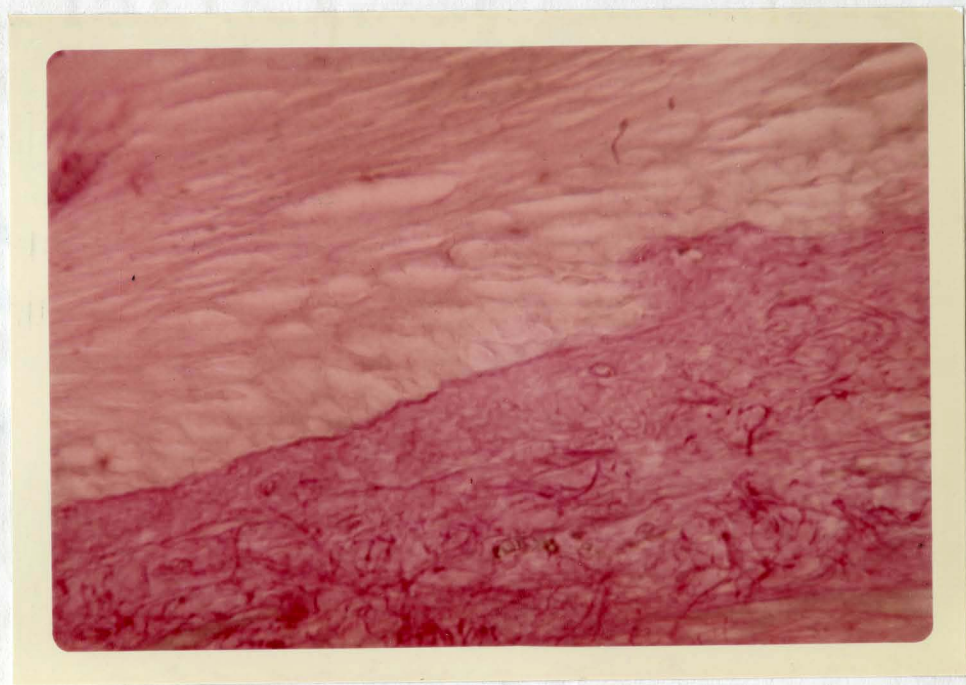


FIGURE 10.

FIGURE 11. ALVEOLAR MUCOSA, FREEZE DRIED, TREATED WITH ENZYME DIASTASE, STAINED WITH PAS. SHOWING THE REDUCED STAINING REACTION OF THE GROUND SUBSTANCE AFTER ENZYME TREATMENT.

X100

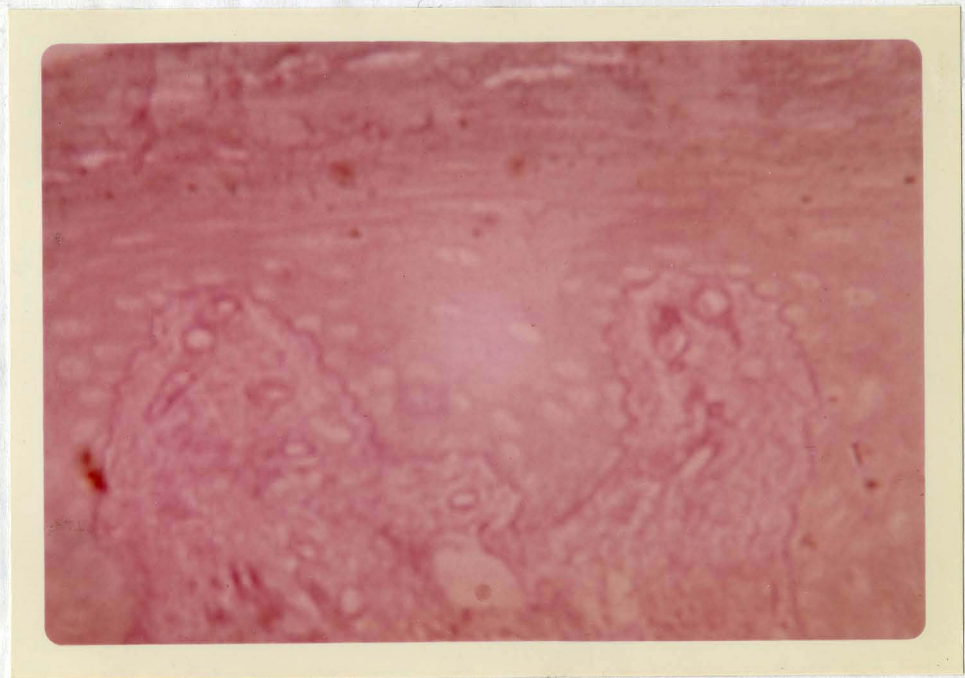


FIGURE 11.

FIGURE 12. ERUPTIVE MUCOSA, FREEZE DRIED, TREATED  
WITH ENZYME DIASTASE, STAINED WITH PAS.  
SHOWING THE REDUCED STAINING REACTION  
OF THE EPITHELIUM AND THE GROUND SUBSTANCE  
AFTER ENZYME TREATMENT. X200

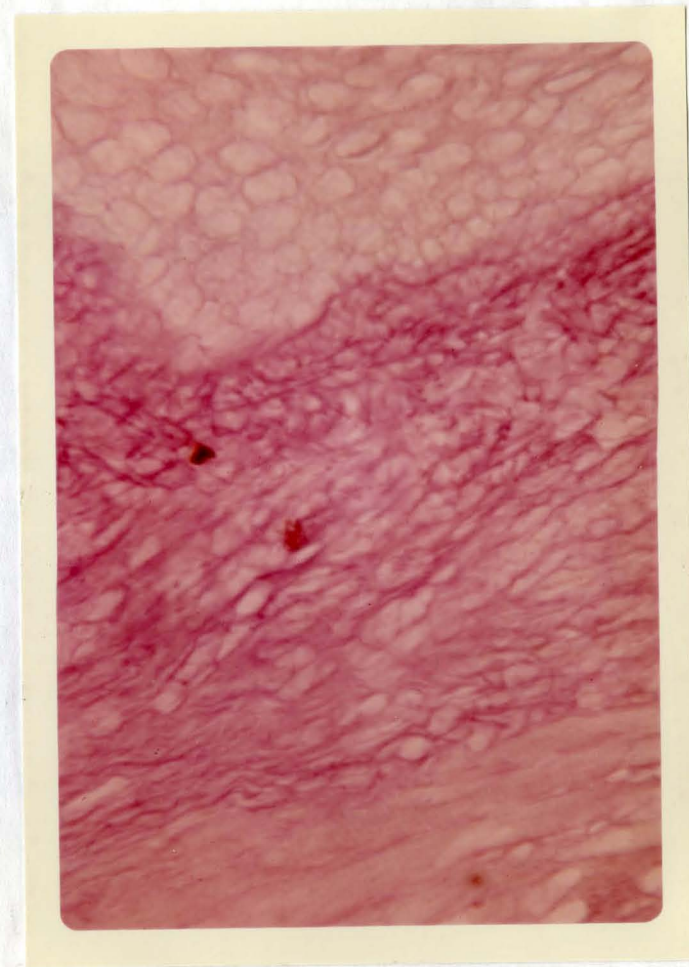


FIGURE 12.

FIGURE 13. ALVEOLAR MUCOSA, FREEZE DRIED, TREATED  
WITH ENZYME DIASTASE, STAINED WITH PAS.  
SHOWING THE GROUND SUBSTANCE X470

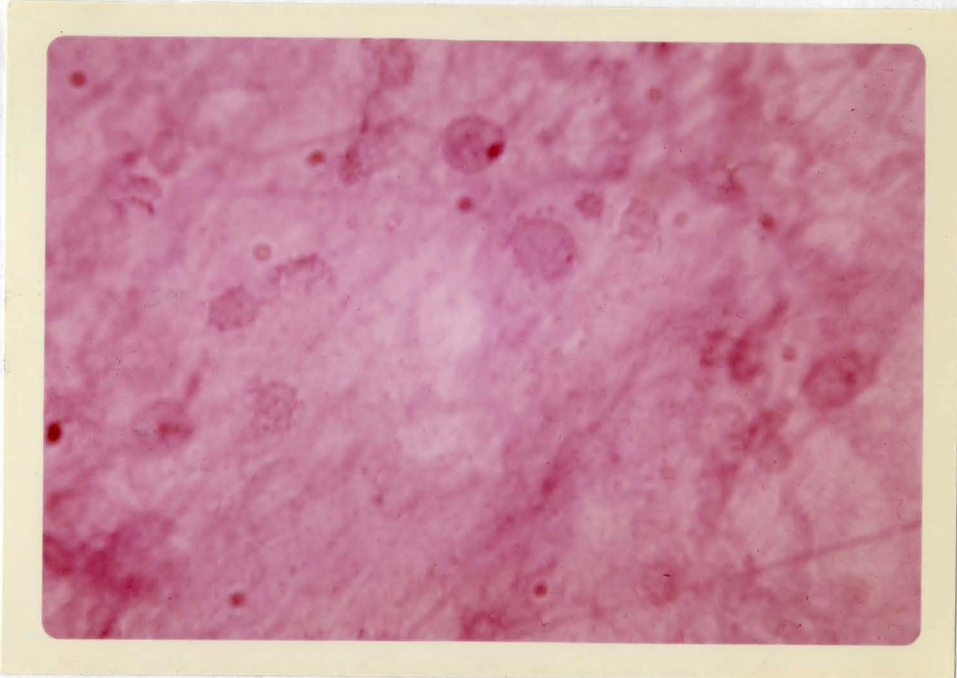
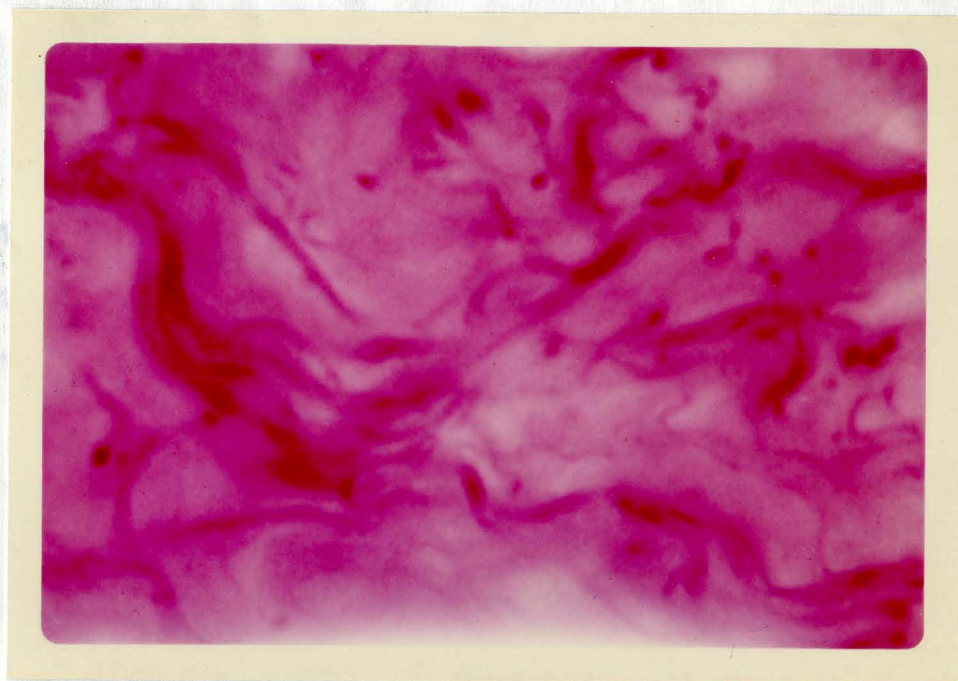


FIGURE 13.

FIGURE 14. ERUPTIVE MUCOSA, FREEZE DRIED, TREATED WITH ENZYME DIASTASE, STAINED WITH PAS. SHOWING THE GROUND SUBSTANCE. NOTE THE DISSOCIATED FIBRILS AND THE GRANULES IN THE GROUND SUBSTANCE. X970

APPROVAL SHEET

THE THESIS SUBMITTED BY DR. ANIL P. JULLIAR HAS BEEN READ AND APPROVED BY FOUR MEMBERS OF THE DEPARTMENT OF ANATOMY



May 22 1963

*[Signature]*  
Signature of the Approver

FIGURE 14.

APPROVAL SHEET

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May 25, 1963  
DATE

Antwick D. Toto  
SIGNATURE OF ADVISER