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The Histochemical Features of Mononuclear Phagocyte Population During Human Periodontal Disease

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THE HISTOCHEMICAL FEATURES OF MONONUCLEAR PHAGOCYTE
POPULATION DURING HUMAN PERIODONTAL DISEASE

by

Jacques Charon

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Master of Science

May

1980

DEDICATION

I dedicate this work to the members of my family
and especially to the memory of my late Mother.

ACKNOWLEDGMENTS

The author wishes to express his appreciation and gratitude to the following persons for their aid and assistance in preparation of this thesis:

Patrick D. Toto, D.D.S., M.S., Chairman of Oral Pathology Department, Committee Director, who was instrumental in the development and completion of this thesis.

Anthony W. Gargiulo, D.D.S., M.S., Chairman of Periodontics Department, Committee member, whose guidance was always available.

Joseph Keene, D.D.S., M.S., Director of the Postgraduate Program of Periodontics, who was influential and understanding in the processing of this manuscript.

I would like to express a special Thank-You to Lucille Flannagan, whose technical expertise was greatly appreciated.

VITA

The author, Jacques Andre Charon, is the eldest son of Paul Louis Charon and Raymonde Emilienne Charon. He was born on January 24, 1945, in Amiens, France.

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TABLE OF CONTENTS

	Page
DEDICATION	ii
ACKNOWLEDGMENTS	iii
VITA	iv
CHAPTER	
I. INTRODUCTION	1
II. REVIEW OF THE RELATED LITERATURE	2
1. The Mononuclear Phagocyte System	2
2. The Origin of the Tissue Macrophage	2
3. The Chemotactic Factors for the Monocyte and the Macrophage	4
a. The Complement Components	5
b. Bacteria	7
c. Lymphokines	7
4. The Activation of Mononuclear Phagocyte	9
a. Culture In Vitro	9
b. Purified Protein Derivative	10
c. Bacille of Calmette Guerin	10
d. Endotoxins	10
5. The Phagocytosis	11
6. Morphological Identification of Mononuclear Leukocytes in Gingiva	15
a. In Human	15
b. In Animal	19
7. Cytochemistry of the Mononuclear Phagocyte	25
a. Hydrolytic Enzymes and Their Intracellular Location	25
b. Histochemical Demonstration of Acid Phase and Esterase Activity	27
8. The Demonstration of Enzyme Activity in Gingiva	29
9. Conclusion	31

TABLE OF CONTENTS (Contd.)

	Page
III. MATERIAL AND METHODS	32
1. Selection of Patients	32
2. Clinical Criteria for Periodontal Disease	32
3. Biopsy Procedure	34
4. Staining Procedure	36
a. Acid Phosphatase	36
b. Non-Specific Esterase	36
c. Hematoxylin Eosin	37
d. Negative Controls	37
e. Positive Controls	37
IV. RESULTS	38
1. Examination of Inflamed Tissues	38
a. Hematoxylin Eosin Staining	38
Oral Epithelium	38
Sulcus Epithelium	38
Lamina Propria	38
b. Geimsa Staining	40
c. Naphtol Acetate Non-Specific Esterase	40
d. Acid Phosphatase Staining	45
2. Examination of Blood Smears from Inflamed Mucosa	50
a. Geimsa	50
b. Naphtol Acetate Non-Specific Esterase	50
c. Acid Phosphatase	51
3. Examination of Non-Inflamed Tissues	51
a. Hematoxylin Eosin Staining	51
b. Geimsa Staining	56
c. Non-Specific Esterase Staining	56
d. Acid Phosphatase	57
4. Examination of Blood Smears from Non-Inflamed Tissues	57

TABLE OF CONTENTS (Contd.)

	Page
5. Examination of Rat Liver	57
a. Hematoxylin Eosin Staining	57
b. Non-Specific Esterase Staining	60
c. Acid Phosphatase	60
6. Negative Controls	60
V. DISCUSSION	63
VI. CONCLUSION AND SUMMARY	78
VII. BIBLIOGRAPHY	79

CHAPTER I

INTRODUCTION

Monocytes and macrophages are present in clinically normal and inflamed gingival mucosa both in humans and animals. (Page, Simpson, Ammons and Schectman, 1971) (70), (Schroeder, 1970) (89) (Schroeder, Munzel-Pedrazzoli and Page, 1973) (92). However, because monocytes and macrophages have been infrequently reported in human gingiva and that there exists some confusion as to their identity by morphologic method alone, (Page, Davies and Allison, 1973) (65) this study was undertaken.

Two intracellular enzymes, acid phosphatase and non-specific esterase clearly identify both monocytes and macrophages in the epithelium, connective tissue and blood vessels in normal and inflamed gingiva. Furthermore, non-specific esterase also serves to differentiate positive T lymphocytes from negative B lymphocytes in normal and inflamed human gingiva. In addition, non-specific esterase also is seen in one population of plasma cells.

Gingival blood, similarly, contains monocytes and T lymphocytes which react positively for non-specific esterase.

Acid phosphatase is seen in blood monocytes. Additionally, neutrophils from tissue are also positive.

CHAPTER II

REVIEW OF THE RELATED LITERATURE

1. THE MONONUCLEAR PHAGOCYTE SYSTEM

Monocytes and macrophages are two cells which belong to the "Mononuclear Phagocyte System" (MPS). MPS was introduced to replace the old definitions in the literature such as "Macrophage-System" "Reticulo-Endothelial System," or even "Reticulo-Histio-cyte System" (Furth, Cohn, Hirsch and Humphrey, 1972) (29). However it has to be said that "Reticulo-Endothelial System" is still widely used to define the MPS. All the cells which compose the MPS are listed in Table I. It is readily evident that macrophages are widely distributed throughout the body whereas the term "monocyte" has to be referred to as a cell found either in the blood or in the bone marrow.

2. THE ORIGIN OF THE TISSUE MACROPHAGE

During inflammation the first wave of cells found in the site is the polymorphonuclear neutrophil leukocyte. This emigration is relatively brief and is immediately followed by a more prolonged emigration of mononuclear phagocyte cells (Hurley, Ryan, and Freidman, 1966) (38). Using the "skin-window" technique (Ryan, 1967) (84) and intradermal injections of various substances (Ebert and Florey, 1939) (24), (Galindo, Heise, and Myrvik, 1970)

TABLE 1

THE MONONUCLEAR PHAGOCYTE SYSTEM

Cells	LOCALIZATION
Precursor Cells	Bone Marrow
Promonocytes	Bone Marrow
Monocytes	Bone Marrow, Blood
Macrophages	Connective Tissue (Histiocytes)
	Liver (Kupffer Cells)
	Lung (Alveolar Macrophages)
	Spleen (Free and Fixed Macrophages)
	Lymph Node (Free and Fixed Macrophages)
	Serous Cavity (Pleural and Peritoneal Macrophages)
	Bone Tissue (Osteoclasts)
	Nervous System (Microglial Cells)

(30), it has been shown that blood monocyte is the precursor cell of the tissue macrophage. The later stages of inflammation show that mononuclear cells are predominant compared to the polymorphonuclear neutrophil cells. Spector and colleagues (Paz and Spector, 1962) (73), (Spector, Lykke, and Willoughby, 1967) (103), claimed that the development of mononuclear preponderance is not due to the later emigration of mononuclear cells but rather to a disappearance of the neutrophils but not the mononuclear phagocyte cells, by disintegration or by movement away from the inflamed area.

3. THE CHEMOTACTIC FACTORS FOR THE MONOCYTE AND THE MACROPHAGE

The presence of macrophages in the inflammatory site is not quite so surprising since many factors are chemotactic for mononuclear phagocyte cells in general and more especially for macrophages. Leukocytes show directional migration towards many different substances, such as polysaccharides, polypeptides, and proteins (Keller and Sorkin, 1968) (41), (Wilkinson, Borel, Stetcher-Levin, and Sorkin, 1969) (120). The action of some of these substances on mononuclear leukocytes has been thought to be direct and the term "cytotoxin" has been used to define these leukocyte direction acting agents. However, some of them, including immune-complexes, exert an indirect (cytotoxigenic) action on leukocytes through mediators present in the serum (Keller and Sorkin, 1968) (41). These cytotoxigen substances

include antigen-antibody complexes, zymozan, bacterial lipopolysaccharides, and plasminogen. Most of these substances are present in the inflamed gingiva especially bacterial endotoxins (Schwartz, Stinson and Parker, 1972) (93).

A. The Complement Components

Another group of substances which are chemotactic for macrophages are the products of the complement system. Three of them have been identified to be chemotactic for mononuclear phagocyte cells. They are:

- . C3a which is with C3b, the result of the cleavage of C3 during the classical pathway of activation of the complement system (Peltier, 1979) (75).
- . C5a which is more efficient (1,000 times in vivo) in terms of chemotactic activity than C3. Snyderman, Shin, and Dannenberg (1972), (99) showed that a leukocyte acid proteinase, with the properties similar to those of Cathepsin D, could also generate the chemotactic factors with physical properties similar to C5a when incubated with purified guinea pig C5.

This is a potentially important mechanism for the amplification of a chronic inflammatory response, whereby proteinase cleaves C5 which produces C5a. C5a attracts more macrophages into the inflammatory site. The same authors have shown that a single cleavage of C5, whether obtained as a result of a reaction of C5 with a proteolytic enzyme or with C5 convertase, has chemotactic activity for both the mononuclear phagocyte and polymorphonuclear leukocyte.

They stated that the mononuclear phagocyte might accumulate under conditions where the cleavage product of C5 would be found. These conditions are present with local antigen-antibody reactions, non-specific tissue trauma, and local cytolytic virus infections (Snyderman, Shin and Hausman, 1971) (100).

In addition, the complex C5b67 produced under the influence of C5 convertase both in the classic and alternate pathways, is chemotactic for macrophages. Besides those three chemotactic factors, the anaphylatoxins (AT) are not chemotactic until they are coupled to a crystallizable polypeptide called cocytotoxin. (Peltier, 1979) (75).

The presence of complement components has been demonstrated in the gingival sulcular fluid in human periodontitis (Attstrom, Laurell, Larson and Sjöholm, 1975) (4), (Shillitoe and Lehner, 1975) (97) and in the gingival connective tissue (Schenkein and Genco, 1977) (86), (Schenkein and Genco, 1977) (87). These findings all together strongly suggest that activated complement exists locally in the periodontium.

Bacterial enzymes, bacterial endotoxins and immune complexes have been demonstrated in the gingival connective tissue (Fullmer, Gibson and Lazarus, 1969) (28), (Genco, Mashimo, Krygier and Ellison, 1974) (31), (Ranney, 1970) (78), (Robertson and Simpson, 1976) (82). In vitro, such factors can activate the complement system and generate chemotactic factors for mononuclear phagocyte cells.

B. Bacteria

In addition to the chemotactic factors derived from complement, certain species of bacteria produce low molecular weight peptides that are directly chemotactic and do not require complement for activity. Their biochemical nature has been hypothesized by Schiffman, Corcoran and Wahl, 1975 (88).

C. Lymphokines

Lymphocytes activated by specific antigen produce lymphokines which are also chemotactic for macrophages. Ward, Remold and David, 1970 (118) have shown that lymphocytes from the sensitized guinea pig when stimulated *in vitro* by specific antigen, produce a factor which is chemotactic for mononuclear phagocytes. This factor is clearly distinguished from chemotactic factors for neutrophils. It has been called "chemotactic factor" (CF) and it is a lymphokine produced under the same conditions required for the production of migration inhibitory factor (MIF) (See below). This chemotactic factor has been demonstrated *in vitro* (David, Good and Fisher, 1973) (19) in using Boyden chamber.

In addition, Ward, 1968 (117) has shown that a mononuclear phagocyte chemotactic factor was present in lysates of neutrophils. This factor may be related to the cationic peptides of lysosomal specific granules. It becomes evident that many factors are chemotactic for macrophages and although their activities have been mainly demonstrated "*in vitro*," one could hypothesize that they are responsible for the presence of macrophages in the inflamed

gingival tissue.

Once the macrophage has migrated to the inflamed tissue by chemotaxis, it is prevented from escaping by another lymphokine called the migration inhibitory factor (MIF). Indeed, recent investigations suggest that migration inhibitory factor is secreted by both the T and B lymphocytes. The release of migration inhibitory factor from such lymphocytes sensitized by specific antigen is an early phenomenon, since it is disclosed as early as the sixth hour of culture in vitro. At this time, the in vitro lymphoblastic transformation and DNA synthesis at 37°C by the lymphoid cell occurs in the presence of antigen. However, migration inhibitory factor cannot be demonstrated in the acellular extracts of lymphocytes from the sensitized animal; that is to say, that migration inhibitory factor is distinguishable from CF (Chemotactic Factor) (Revillard, 1979) (79). Migration inhibitory factor is thought to be a non-dialyzable sialoglycoprotein although it has been succeeded to dialyze MIF (Possansa, Cohen, Yoshida and Cohen, 1979) (76).

Macrophage cell surface receptors for migration inhibitory factor contain fucose, since the addition of fucose competitively neutralizes migration inhibitory factor activity. Also, migration inhibitory factor is destroyed by proteases from macrophages so that its activity is self-limited (Revillard, 1979) (79). Although migration inhibitory factor activity is not dependent on the presence of an antigen, it has been shown to be more active in the

presence of antigen (Revillard, 1979) (79). Migration inhibitory factor exerts additional effects upon the biologic behavior of macrophages. Indeed, it increases the adherence of monocytes to the glass surface, movement of the plasma membrane, phagocytic activity, oxygen consumption, hydrogen peroxide production and lipid synthesis (Seravelli, 1973) (94).

4. THE ACTIVATION OF MONONUCLEAR PHAGOCYTE

When the monocyte has entered the site of inflammation, it becomes transformed to a macrophage. However, it does not remain in the same state during all its life span. In fact, under special conditions, it could undergo functional and morphologic changes called "activation" (Cohn, Fedorko and Hirsch, 1966) (15), (Cohn and Weiner, 1963) (16), (Mitchell, Pancake, Noseworthy and Karnovsky, 1969) (58), (Sutton and Weiss, 1966) (105), (Robineaux, Anteunis and Bona, 1971) (93).

A. Culture in Vitro

A simple culture in vitro of mononuclear phagocytes induces an increase in cell size, in the number of mitochondria, and forms more osmiophilic phase dense granules, and lipid droplets (Cohn and Benson, 1965) (14) as seen under phase microscopy.

Pantalone and Page, 1975 (71) have shown that a factor from non-specific mitogen stimulated lymphocytes induces a marked increase in the size of mononuclear phagocytes, when they are cultivated in vitro. This factor is compatible with macrophage

activating factor (MAF). In the same experiment, they showed that mononuclear phagocyte cell produces and releases large quantities of lysosomal enzymes into the extracellular environment.

B. Purified Protein Derivative (PPD)

Purified protein derivative is also able to activate macrophages in vitro. The macrophages undergo rapid and morphologic changes which are a 2 to 4 folds increase in cell size, an increase in ruffled membrane activity, and an increase in the number of lysosomes (Davies, Page and Allison, 1974) (20).

C. Bacille of Calmette Guerin (BCG)

Mackness in 1962 (49) has observed that after intravenous injections of BCG, the mouse peritoneal macrophages show an increase in the number of mitochondria and lysosomes, and increased pinocytotic activity as well. The same activated cell seen under the electron microscope shows an enlarged Golgi complex, increased free ribosomes, and more elaborated endoplasmic reticulum than the non-stimulated cell. Cohn, Fedorko and Hirsch, 1966 (15) also have shown that activated macrophages increase their synthesis in lysosomal acid phosphatase. Also, Gordon, Todd and Cohn, 1974 (32) have demonstrated the same phenomenon for activated peritoneal macrophages in the mouse.

D. Endotoxins

Endotoxins are able to activate macrophages from the peritoneal cavity when they are cultivated in vitro (Wahl, Wahl, Mergenhagen and Martin, 1974) (115). The macrophages synthesize

and release enzymes which are able to hydrolase collagen in vitro.

In addition, immune complexes have the capacity to activate macrophages in vitro and to induce the synthesis and release of Beta glucuronidase (Cardella, Davies and Allison, 1974) (12).

A summary of the features of activated macrophages is presented in Tables 2 and 3.

5. THE PHAGOCYTOSIS

The function of detoxication by macrophages is very important. For example, alveolar macrophages phagocytize the dusts of the respiratory tract and Kupffer cells of the liver "clean" the blood.

This function is made possible by the phagocytic ability of macrophages. The capacity to engulf particles is termed generally endocytosis and can be divided into two categories: the pinocytosis (substrate less than 10 nm. in diameter) and phagocytosis (substrate more than 10 nm. in diameter) (Fauve, 1979) (26). The particles engulfed during phagocytosis could be inert, i.e., silicium, viruses, or living cells such as bacteria and parasites. It is now well established that phagocytosis could be divided into 3 phases:

- . adhesion phase where the particle is in contact with the cell membrane,
- . ingestion phase where the particle is engulfed into the cytoplasm,
- . digestion of the engulfed particles.

TABLE 2 (G.B. MACKANESS, 1964)

MORPHOLOGIC CHANGES DURING MACROPHAGE ACTIVATION PHENOMENON

Increase in Cell Size

Increased Number of Mitochondria

Enlarged Golgi Complex

More Free Ribosomes

More Elaborate Endoplasmic Reticulum

Increased Number of Lysosomes

TABLE 3 (G.B. MACKANESS, 1964)

FUNCTIONAL CHANGES DURING MACROPHAGE ACTIVATION PHENOMENON

Increased Phagocytic Activity

Increased Bacteriostasis

Enhanced Bacteriocidal Capacity

Increase in Lysosomal Enzyme Synthesis

Increase in Lysosomal Enzyme Secretion

Increased Pinocytic Activity

Increased Metabolic Activity

Increased Protein Synthesis

Factors such as the electric charge of cells, presence of antibodies IgG1 and IgG3 (Hay, Torrigiani and Roitt, 1972) (33), temperature, pH, ionic force, presence of cations, lipids, complement, influence the adhesion, the first step of phagocytosis. Of particular interest is the role of the Fc portion of the antibodies IgG1 and IgG3 which is fixed on receptors of macrophages and facilitates the phagocytosis. However, it is known that the macrophage does not require serum factors for phagocytosis as compared to polymorphonuclear cells (Fauve, 1979) (26).

Opsinins, specific antibodies which bind to the bacteria facilitate the phagocytosis by the macrophages. On the other hand, splenic macrophages do not require opsonins to be able to phagocyte old erythrocytes (Pearsall and Weiser, 1970) (74).

The phase of ingestion consists of the engulfing of the particle. The macrophage folds its membrane around the particle to form an intracytoplasmic phagosome. This membrane activity is associated with a burst in the metabolism of macrophages with the activation of hexosemonophosphate pathway of glycolysis (Oren, Farnham, Sarto, Milofsky and Karnovsky, 1963) (64).

In the cytoplasm of the macrophages, the lysosome migrates and comes in contact with the phagosome and their membranes fuse forming a phagolysosome. The lysosomal hydrolytic enzymes are then liberated into the phagosome and the phagocytized particles are digested (Fauve, 1979) (26).

The killing mechanisms of bacteria by polymorphonuclear neutrophils during phagocytosis also occurs in the macrophages. The conditions for killing include a lower pH, lysosomal enzyme production, hydrogen peroxide, halogen, and myeloperoxidase and the synthesis of NADP oxidase.

Macrophages by the means of phagocytosis of immunogenic substances, present the antigen to the lymphocytes. Morphological and biochemical criteria have shown that the antigen is processed by the macrophages before being presented to the lymphocyte. The lymphocyte may respond by inducing antibody production and/or lymphokine effectors of immune response. This immune regulatory capacity of phagocytosis by macrophages is enhanced when the macrophage is activated (Pearsall and Weiser) (74).

6. MORPHOLOGICAL IDENTIFICATION OF MONONUCLEAR LEUKOCYTES IN GINGIVA

Most of the studies presented so far have dealt with mononuclear phagocytes either from the alveolar and peritoneal cavity in experimental animals or from in vitro studies. However, there are studies which have reported the presence of macrophages and/or monocytes in the gingiva.

A. In Human

Zachrisson, 1968 (123) has studied the cell population of clinically normal and inflamed human gingiva. The human gingivitis was the result of an experimental accumulation of plaque due to withdrawal of oral hygiene procedures for three weeks. Using the

light microscope and formalin fixed, paraffin embedded stained sections, he observed that the clinically normal gingiva contained mainly small and medium-large lymphocytes, macrophages, and, in the central region of the connective tissue, plasma cells. In the inflamed gingiva, greater numbers of mononuclear cells accumulated adjacent to the pocket sulcus epithelium. The cell population comprised small and medium-large lymphocytes, macrophages, and fewer polymorphonuclear leukocytes.

However, Zachrisson also reported difficulties in the differentiation of mononuclear cells by means of morphologic criteria in using fixed preparations.

Using also the experimental gingivitis design in humans, Payne, Page, Olgivie and Hall, 1975 (72) were interested in the histopathologic features of gingiva during the first week following the beginning of plaque accumulation. The patients in this study were younger than Zachrisson's patients and presented neither plaque accumulation nor clinical inflammation at the beginning of the experimental gingivitis.

Samples of buccal gingiva on the experimental side were taken at 0, 2, 4 and 8 days of plaque accumulation. Fixed preparations of specimens were used for study under the light and electron microscopes. Among other features, they measured the area of the leukocyte infiltrate in the connective tissue, and the number of various leukocytes and other cells found in the connective tissue.

They stated that while there was no difficulty to differentiate polymorphonuclear neutrophils, mast cells and small lymphocytes, "definitive identification of large lymphocytes, immunoblasts, macrophages, and fibroblasts was not possible. Although, a separation into large mononuclear cells and fibroblasts could be made with reasonable certainty on the basis of cytologic features." Examining the control biopsy specimens, they found that there always were signs of histologic inflammation. This was characterized by the presence of leukocytes within the junctional epithelium, especially in those regions near the base of the gingival sulcus. The connective tissue of the control specimens contained a small zone of leukocytic infiltration. This zone was located subjacent to the basement membrane region of the junctional epithelium near the base of the gingival sulcus. The infiltrative cell population in this basement membrane zone included polymorphonuclear neutrophils, small and large mononuclear cells, few fibroblasts, but no plasma cells. However, plasma cells were seen deeper in the connective tissue around blood vessels.

At day 8, mononuclear lymphoid cells were seen in the zone subjacent to the junctional epithelium at the base of the sulcus. Payne, Page, Olgivie and Hall, 1975 (72) identified such cells as lymphocytes. They reported that the only significant increased cell population was the lymphocyte population. From this study, it appears that the lymphocytes are evident only in the early lesion (8 days).

Freedman, Listgarten and Taichman, 1968 (27) have studied the chronically inflamed gingiva obtained from 8 human subjects. The authors did not make it clear if they were dealing with gingivitis or periodontitis. They observed in gingival crevicular epithelium, polymorphonuclear neutrophils, lymphocytes, monocytes, and cells of undetermined origin. They observed also, membrane bound dense bodies resembling the lysosomal granules of neutrophils freely suspended both in the intercellular spaces of crevicular epithelium and in the connective tissues. Interestingly enough, the neutrophils and monocytes that they observed rarely showed evidence of phagocytic activity. Concerning the connective tissue infiltrate, they reported that plasma cells were predominant. They observed also mononuclear monocytes derived from blood monocytes. They described those cells as containing a large, usually indented nucleus, a well-developed Golgi profile within a concavity and numerous lysosome-like granules of varying electron densities scattered throughout the cytoplasm.

Schroeder, 1970 (89) studied the histopathologic and electron microscopic features of the human gingiva. The patients were 10 children aged from 10 to 13 years old. Using clinical indices, the gingiva was assumed to be either normal or slightly inflamed. Under the electron microscope, the author assayed several quantitative parameters and, particularly the percentage volume of emigrating leukocytes in the volume of junctional epithelium. He reported difficulties in differentiating lymphocytes, or unknown

clear cells, from polymorphonuclear granulocytes. However, the reported percentage of neutrophils infiltrating the junctional epithelium ranged from 2 to 64%. There was considerable variation from individual to individual, and in the same individual, from site to site. The maximum number of neutrophils were encountered apical to the sulcus epithelium.

Concerning the connective tissue, the author observed mostly plasma cells (90%) with few other round cells and granulocytes.

In another study, Schroeder, Munzel and Pedrazolli, 1973 (90), using electron microscope reported that macrophages and monocytes were present both in normal and in slightly inflamed gingiva. They noticed that, in the inflamed gingiva macrophages and monocytes were absent in the non-infiltrated connective tissue, and very few of them were seen in the infiltrated connective tissue.

Schroeder, Munzel, Pedrazolli and Page, 1973 (92) reported that the frequency of macrophages and monocytes demonstrate the lowest percentage of the infiltrated cells when compared to other cells of human inflamed gingiva. Monocytes (1.6%) and macrophages (0.5%) were more numerous in the infiltrated connective tissue (ICT) than in the non-infiltrated connective tissue (NCT).

B. In Animal

The studies performed in animal periodontal tissues reported also the presence of macrophages on the epithelium and the

connective tissue during periodontal disease. Simpson and Avery, 1974 (98) observed in the baboon a large population of mononuclear phagocytes associated with lymphoid cells just below the basal membrane of the pocket epithelium and within the intercellular spaces of the epithelium. Interestingly enough, they noticed that the number of lymphocytes and monocytes was larger in a restricted inflammatory infiltration in the connective tissue than in the extensive infiltration.

In studying experimental gingivitis in the monkey, Listgarten and Ellegard, 1973 (46) also reported the presence of macrophages among other inflammatory cells in the infiltrated connective tissue.

Schectman, Ammons, Simpson and Page, 1972 (85), Page, Simpson, Alloms and Schectman, 1972 (69) have found that a colony maintained Marmoset developed a spontaneous, destructive periodontal disease. In this particular situation the histopathologic studies revealed that the predominant cells present in the infiltrate were the mononuclear phagocytes that they recognized as macrophages.

Lindhe, Schroeder, Page, Munzel, Pedrazolli and Hugoson, 1974 (43) reported that in experimental gingivitis in the dog, the number of neutrophils, monocytes and macrophages increased in the infiltrated connective tissue with increased severity of the inflammatory process. Interestingly, they also reported that the size of the macrophages and monocytes increased markedly at the

beginning of plaque accumulation but returned to normal size 4 days later.

In the dog, Attstrom, Graf-de-Beer and Schroeder, 1975 (3) reported the presence of mononuclear leukocytes both in the connective tissue and junctional epithelium in inflamed and clinically normal gingiva. They reported that the number of neutrophils, monocytes and macrophages was greater in the site of expected future infiltration of connective tissue than in the non-infiltrated connective tissue as developing in gingivitis.

As a summary, all the studies dealing with either animal or human, showed that during periodontal disease, monocytes and macrophages are present in the junctional epithelium and in the infiltrated connective tissue. However, except for the periodontal disease reported by Page and Ammons on Marmoset, no particular emphasis has been stressed upon the relation of macrophages and monocytes to periodontal disease. Since the macrophage is associated with chronic inflammation such as periodontal disease, it seems advisable to study more carefully this particular cell.

It has been well reported that morphologic criteria are lacking (Cowley, 1972) (17), (Page, Davies and Allison, 1973) (65) to ascertain that the particular cell in the connective tissue is a macrophage. For this reason, one of the most convenient ways to study the mononuclear phagocyte cell population in chronic periodontal disease is to use the histochemical techniques. A summary of the foregoing studies is presented at Table 4 and Table 5.

STUDIES WHICH REPORTED PRESENCE OF
MACROPHAGES IN HUMAN GINGIVAL TISSUE

TABLE 4

SUBJECT	DISEASE	LOCATION	AUTHORS & REFERENCES
Adult	Chronically inflamed gingiva.	Intercellular spaces of crevicular epithelium in tissue (ICT).	Freedman,et al J. Perio. Res. 3:313, 1968
Children	Normal or slightly inflamed.	Infiltrated connective tissue.	Schroeder,et al Arch. Oral Biol. 18:899 1973.
Children	Slight or moderate inflammation.	Infiltrated connective tissue.	Schroeder,et al. J. of Micr. 99:301, 1973
Adult	Experimental gingivitis.	Infiltrated connective tissue.	Zachrisson J. Perio. Res. 3:293, 1968.

STUDIES WHICH REPORTED PRESENCE OF
MACROPHAGES IN ANIMAL GINGIVAL TISSUE

TABLE 5

Cat	Chronic marginal gingivitis.	Intercellular spaces of crevicular epithelium in the basal lamina between epithelium and connective tissue.	Gavin J. Perio. Res. 5:19, 1970
Marmoset	Chronic periodontal disease and normal periodontium.	Lamina propria	Schectman, et al J.P.R. 7:195, 1972
Marmoset	Advanced periodontal disease.	Lamina propria	Page, et al. J.P.R. 7:283, 1972
Rhesus monkey	Experimental gingivitis.	Infiltrated connective tissue.	Listgarten J.P.R. 8:199, 1973

STUDIES WHICH REPORTED PRESENCE OF
MACROPHAGES IN ANIMAL GINGIVAL TISSUE

TABLE 5 (Contd.)

SUBJECT	DISEASE	LOCATION	AUTHORS & REFERENCES
Baboon	Inflamed gingiva.	Intercellular spaces of crevicular epithelium in the connective tissue.	Simpson J.P. 45:500, 1974
Dog (German Shepherd)	Early gingivitis.	Infiltrated connective tissue.	Lindhe,et al J.P.R. 9:314, 1974
Dog (Beagle)	Normal gingiva.	Infiltrated and non-infiltrated connective tissue.	Alztrom,et al J.P.R. 10:115, 1975

7. CYTOCHEMISTRY OF THE MONONUCLEAR PHAGOCYTE

The methods employed in cytochemistry and histochemistry give us more information about the cellular metabolism than does conventional histology. This is particularly true for mononuclear phagocytes. Besides, researchers are in agreement that histochemistry is a reliable method to individualize macrophages and monocytes either in the tissues and the blood smear (Seymour, Dockrell and Greenspan, 1978) (96).

We will report here, some of the information published in the literature about the cytochemistry of mononuclear phagocytes.

A. Hydrolytic Enzymes and Their Intracellular Location

Almost all the information available in the literature on the morphology and function of monocytes concerns the study of lysosomal enzymes.

Different approaches have been used to do so. Nichols and Bainton, 1973 (60) have studied the cytochemistry of mononuclear phagocytes using electron microscopy. They used two cytochemical markers for lysosomal acid phosphatase and arylphosphatase. They observed that the human monocyte was positive for acid phosphatase, being localized in most cisterns of the Golgi complex and rough endoplasmic reticulum. Therefore, they concluded that the reaction in those cells might be due to a non-lysosomal phosphatase. However, they reported that the reaction was much greater in rabbit peritoneal macrophages for acid phosphatase, than in their blood monocytes. Cohn and Wiener, 1963 (16) studied the cellular extrac-

tions of the hydrolases of rabbit alveolar and peritoneal macrophages. They revealed that 60% to 80% of the total cell content of acid phosphatase, cathepsin, Beta glucuronidase, acid ribonuclease, acid desoxyribonuclease, arylsulfatase, lysosome and lipase were located in the post-nuclear fraction. They observed that the enzyme content in macrophages varied from type to type of macrophage and from non-stimulated to stimulated activated macrophages. In a subsequent paper, they reported (Cohn and Wiener, 1963) (16) that BCG injection induced alveolar macrophages to liberate 50% to 60% of the total cell content of acid phosphatase, cathepsin, lysozyme, Beta glucuronidase, acid ribonuclease and acid desoxyribonuclease into the extracellular medium with a concomitant decrease in granule-bound enzyme. They further reported that this behavior also happened during phagocytosis by alveolar macrophages.

Bennett and Cohn, 1966 (6) have investigated the time-dependent activation of the horse blood monocyte simply cultivated in vitro. They reported that the level of acid phosphatase and arylsulfatase was markedly increased at 72 hours of culture compared to 18 hours level. Cohn and Benson, 1965 (14) also reported that during the in vitro differentiation of monocyte-like cell from the unstimulated mouse peritoneal cavity, there was an increased number of acid phosphatase positive granules. Also, they demonstrated that the same phenomenon happens in vivo, when the macrophages were stimulated by bacterial lipopolysaccharides.

Wahl, Wahl, Mergenhagen and Martin, 1974 (115), Wahl, Wahl, Mergenhagen and Martin, 1975 (116) reported that both endotoxins and lymphokines were able to activate guinea pig peritoneal macrophages, in vitro, with the release of collagenase in the extracellular medium. They demonstrated later (Wahl, Olsen, Sandberg and Mergenhagen, 1977) (114) that this release was dependent on prostaglandin regulation.

It appears that macrophages and monocytes are able to demonstrate, in vitro, evidence of acid phosphatase activity most probably from lysosomal structures. (Cohn and Wiener, 1963) (16).

B. Histochemical Demonstration of Acid Phosphatase and Esterase Activity

Carson, 1973 (13) was able to demonstrate the presence of macrophages from prostatic tissue specimens using frozen fixed sections. The substrate used in this study was Naphtol ASMX phosphate. The sites of acid phosphatase activity of macrophage appeared as red spots in the cytoplasm.

Using the Naphtol AS phosphatase azodye method of Burstone (Burstone, 1958) (10), Maramba, 1965 (52) was also able to demonstrate positive reaction to acid phosphatase in macrophages of prostatic tissue.

Beside acid phosphatase, esterase activity has been shown to be present in macrophages, by the means of histochemistry.

Burstone, 1957 (9) described a cytochemic technique using Naphtol AS ester as a substrate which is able to demonstrate non-

specific esterase activity in frozen tissue sections. Li, Yam and Crosby, 1972 (42) have studied the cell population of the human spleen in regards to acid phosphatase and non-specific esterase activity. They demonstrated that spleen macrophages were very strongly positive for both those enzymes. They observed that non-specific esterase activity of the macrophage was relatively stable and remained active after the specimen had been fixed and stored in the cold for several months. They concluded that Naphtol AS acetate esterase technique was useful in the identification and localization of macrophages in the spleen.

Dockrell, Seymour, Playfair and Greenspan, 1978 (22) have studied the cytochemical features of lymphoid tissues from the cat, rat and mouse. They demonstrated that T lymphocytes presented a positive single, characteristic, cytoplasmic dot-like granule when stained for non-specific esterase, while plasma cells showed a diffuse reaction for non-specific esterase (ANAE) activity. The macrophages were strongly positive both for ANAE and acid phosphatase.

Concerning the mononuclear phagocyte of blood, Tucker and colleagues (Tucker, Pierre and Jordan, 1977) (110) described a convenient method for rapid identification of the monocyte by non-specific esterase detection methods. They reported a so-called "wet technique" using naphtyl cutyrate as a substrate. In the monocytes, an insoluble precipitate formed demonstrating non-specific esterase.

Yam, Li and Crosby, 1971 (122) also reported that blood monocytes and histiocytes are very strongly positive for the non-specific esterase. Whereas granulocytes were not stained for the same enzymes.

Horwitz, Allison, Ward and Kight, 1977 (36), using the ANAE stain, have identified B lymphocytes, T lymphocytes and monocytes of human peripheral blood. They observed that 80% of the ANAE positive cells were T lymphocytes, whereas B lymphocytes were negative for ANAE. They reported that most blood monocytes show a diffuse cytoplasmic esterase reaction. Furthermore, they also noticed that 20% of the positive monocytes were not phagocytic and therefore, not activated, In addition to blood, they also stained the lymph node for ANAE and they showed that macrophages were diffusely, intensely stained.

8. THE DEMONSTRATION OF ENZYME ACTIVITY IN GINGIVA

The foregoing studies have shown that macrophages and monocytes are always positive for ANAE and acid phosphatase, and can be identified both in blood and tissue specimens.

The first studies dealing with enzyme activity in gingival studies were concerned with the relative distribution of enzymes in the connective tissue and epithelium. Cabrini and Carranza, 1961 (11) have used the Gomori technique to demonstrate the acid phosphatase activity within the inflamed and the non-inflamed human gingiva. They reported that acid phosphatase activity was found almost exclusively in the epithelium with some variations in

terms of intensity. Interestingly enough, they were not able to demonstrate enzyme activity in the connective tissue.

Lisanti, 1960 (44) has studied alkaline phosphatase, Beta D glucuronidase, galactosidase, glucosidase, esterase, cholinesterase and acid phosphatase activity in human inflamed periodontal tissues. They also reported that esterase activity was found on the epithelium associated with some activity on the inflammatory areas of the connective tissue. In agreement with Cabrini and Carranza, they revealed that acid phosphatase activity was found in the epithelium. However, they revealed an acid phosphatase activity in the area of inflammation of the connective tissue approximating the root surface.

Winer, O'Donnell, Chauncey and McNamara, 1970 (121) have reported that acid phosphatase activity was markedly evident on the inflammatory cells of the connective tissue. However, because of technical difficulties, they were unable to demonstrate non-specific esterase activity.

Seymour, Dockrell and Greenspan, 1978 (96) have studied the histochemical features of human chronically inflamed periodontal tissues. Investigating acid phosphatase and non-specific esterase activity, they observed that the bulk of cells was represented by a B lymphocyte which was negative for ANAE activity. They also reported the presence of large cells with intense activity for acid phosphatase and non-specific esterase that they interpreted to be representative of the macrophage population. The

location of such macrophages was immediately beneath the epithelium lining the periodontal pocket.

Cowley, 1972 (18) in a study of multiple enzyme activity also reported acid phosphatase and esterase in inflamed gingiva. However, he did not identify the mononuclear phagocytic cell.

9. CONCLUSION

The literature suggests that monocytes and macrophages are present in periodontal disease. However, no study has been reported clearly identifying and localizing monocytes and macrophages histochemically in human periodontitis.

As methods are available clearly to identify monocytes and macrophages by ANAE and acid phosphatase, they can be applied to tissue samples and blood in patients with clinical and histologic advance of periodontitis.

CHAPTER III

MATERIAL AND METHODS

1. SELECTION OF PATIENTS

Medical History

Fifteen patients, 8 males and 7 females, ranging from 30 to 88 years of age (mean \bar{X} = 60.2) participated in this study.

Parameters were used to select each patient in order to exclude patients with a positive medical history which could interfere with the mononuclear phagocyte system.

- . Any previous or present history of MPS diseases makes the patient non-suitable for this research.
- . Any medication taken on a regular basis (amidopyrine, aspirin, corticoid) known to alter the MPS.
- . Any known immune disease.
- . Any recent bacterial or viral infection within the past six months.
- . Periodontosis as determined by (BAER J. Perio. 49: 153, 1978).
- . Any oral disease other than periodontal disease and caries.

2. CLINICAL CRITERIA FOR PERIODONTAL DISEASE

All patients chosen for this study were diagnosed as

Type IV ADA Periodontitis patients according to the ACA Classification.*

Although the subjects were not willing to undergo any periodontal therapy, they presented themselves with all clinical features of advanced chronic periodontal disease. Therefore, they presented a group of patients untreated for their periodontitis. The clinical features were as follows:

- . Missing teeth.
- . Inflamed gingiva.
- . Tooth mobility.
- . Pain.
- . Bad breath.
- . Poor oral hygiene.
- . Bone loss as seen on the radiographics.

In order to diagnose the periodontal disease, the following parameters were used:

- . Gingival Index (Loe, 1967) (47).
- . Plaque Index (Loe, 1967) (47).
- . Full mouth dental radiographic examination.

Once examined, the patients were classified in two categories: gingivitis patients and periodontitis patients. Only periodontitis patients were selected for this study. Only patients

* Type IV ADA Advanced Periodontitis: Deep pockets, severe bone loss, advanced mobility pattern (usually cases involving missing teeth and reconstruction)

not willing to undergo prescribed periodontal therapy but who were elected to have extraction of the involved teeth were selected for this study. No initial preparation such as oral hygiene instructions, scaling, curettage or root planing was performed before the day of extraction of the teeth.

If the patient was administered antibiotics before oral surgery, he was rejected from the study.

The tooth selected for collecting the mucosa sample was examined and presented at least one of the following clinical features:

- . Redness and edema of the gingiva.
- . Mobility from 1 to 4 on a mobility scale of 1, 2, 3, and 4.**
- . Evidence of infrabony defect upon radiographic examination.
- . Bleeding upon probing.
- . Supra and subgingival accumulation of plaque on one surface.
- . Presence of gingival fluid.
- . Pain upon axial and horizontal percussion.

3. BIOPSY PROCEDURE

On the day of the surgery, under local anesthesia, the

- ** Mobility scale: 1 - no detectable mobility
2 - mobility less than 1 mm.
3 - mobility more than 1 mm.
4 - axial mobility

inflamed gingival tissues were excised with a 15 C blade before extraction of the tooth.

Care was taken not to destroy the tissue during the biopsy procedure. Immediately after incision, before removing the tissue, five drops of blood from the wound were taken with a sterile pipette and each deposited on a separate histologic slide and prepared as a smear.

Marginal and papillary gingiva and non-inflamed edentulous ridge mucosa were then removed and cut in two parts, each specimen measuring approximately 3 x 3 mm., in a plane corresponding to the long axis of the tooth.

One specimen was put into a formol sucrose solution (10% formalin and 5% sucrose) at pH 6.8.

The other specimen was immediately fresh-frozen at -20°C using the IEC cryostat.* The frozen sample was embedded with OCT compound.** The tissue was oriented in a plane parallel to the long axis of the tooth and sectioned between 2 and 6 microns. The sections were positioned on glass slides and placed in the freezer at 0 degree centigrade for brief storage.

* IEC Cryostat: International Equipment Co., Needham, Mass.

** OCT Compound: (Optimum Cutting Temperature Compound) Lab-Tek Products, Naperville, Ill. 60540

4. STAINING PROCEDURE

A. Acid Phosphatase

The slides were removed from the freezer, allowed to air dry for one hour, then stained for acid phosphatase detection by a modified procedure of Kaplow and Burstone, 1964 (40), as follows:

- . Incubate for 1 hour at 37°C in the following medium: 4 mg. of Naphtol AS-BI dissolved in 0.1 ml. of Dimethyl Formamide added to 25 ml. of 0.2 acetate buffer pH 5.2-5.6. Then added to 12 mg. of fast violet B (Sigma Chemical Cig.). Then shake and filter.
- . Rinse in running water.
- . Counterstain 5 minutes in a 1% buffered methyl green at pH 4.0 (0.2M sodium phosphate, 0.1M citric acid at pH 4.0)
- . Rinse in running water.
- . Mount in water or mounting medium gums***

B. Non-Specific Esterase (ANAE)

Specimens that first were fixed in formol sucrose for 24 hours at 4 degrees centigrate were then stored in Holt's solution. Holt's solution consists of 30 gr. of sucrose and 1 gr. of gum arabic, mixed dry, and hydrated to a volume of 100 ml. of distilled water. The specimens were then embedded in OCT compound and frozen sectioned in an IEC cryostat at 2 to 6 microns thickness.

Activity of ANAE was ascertained by incubation of the sections for 3 hours at room temperature in a medium container

*** Gurr Mounting Medium, Searle Diagnostic, High Wycombe Bucks, England

consisting of 40 ml. 0.067M phosphate buffer, pH 5.2. 4 mg. of hexazotized pararosaniline; and 10 mgr. of Naphtol acetate (Sigma St. Louis) in 0.4 ml. acetone, adjusted to pH 5.8 using 2 normal NaOH. This was followed by 2 rinses in distilled water. The sections were counter-stained with 1% methyl green in 0.1M acetate buffer, pH 4.2, hydrated, and permanently mounted with permount.

C. Hematoxylin-Eosin and Geimsa

Tissue sections were also processed for classical hematoxylin-eosin and Geimsa methods. The blood smears were stained by Geimsa method.

D. Negative Controls

Blood smears and tissue sections simultaneously were processed but without substrate, in order to be used as a negative control.

E. Positive Control

In addition, rat liver tissue also was simultaneously processed with all staining procedure of control and inflamed gingiva and used as positive control. Tissues and blood smears were examined under the light microscope AO 10** at 40, 100 and 250 magnifications including 400X oil immersion.

** AO RO Rochester, New York

CHAPTER IV

RESULTS

1. EXAMINATION OF INFLAMED TISSUES

A. Hematoxylin and Eosin Staining

Oral Epithelium

The oral epithelium was always present and seen as a keratinized stratified squamous epithelium. In all instances, it presented with histologic signs of inflammation which included the following: Intra-epithelium vacuoles, edema, and intercellular infiltration by polymorphonuclear and mononuclear cells.

Sulcus Epithelium

The sulcular epithelium demonstrated micro-ulcers at its surface. In most instances, it presented either as a few fragments or a very few layers of cells. Intracellularly, the cells demonstrated a hydropic degeneration, spongiosis, and edema. Also, the intercellular spaces were enlarged due to necrosis of some epithelial cells. Variable numbers of polymorphonuclear neutrophil cells were always seen intercellularly in the sulcus epithelium at the site of necrosis and edema. The basal membrane supporting the sulcus epithelium was thin, fragmented and, in some instances, undetectable.

Lamina Propria

The most striking feature was the apparent dissolution

of collagen and the disappearance of collagen fiber bundles. The lamina propria was edematous and contained only residual degenerating collagen.

This residual collagen was seen separating the perivascular spaces which were devoid of collagen. There was a hypervascularization with the presence of dilated capillary loops, sometimes seen at the very surface of the sulcular epithelium. The lumen of the capillaries contained numerous polymorphonuclear leukocytes. The leukocytes demonstrated diapedesis, marginating and emigrating, as some were seen adjacent to the external wall of blood vessels. Such leukocytes were scattered throughout the connective tissue, as well.

However, the most numerous cells were plasma cells. They formed a perivascular collar or cuff around the blood vessels. Some of these plasma cells were seen to contain spheroid, homogeneous eosinophil material, Russel's bodies. Also, following the course of the blood vessels, immature plasma cells and binucleated plasma cells were noted.

Mononuclear cells including monocytes and macrophages could be seen in the epithelium and the lamina propria. However, in some instances, it was difficult to ascertain their nature with hematoxylin eosin stains. Few lymphocytes always were seen distributed unevenly in the cellular infiltrate of the perivascular spaces throughout the connective tissue.

B. Geimsa Staining

The cellular infiltrate was observed to be identical to that seen with the hematoxylin eosin stain. However, the morphology of the inflammatory cells was easier to distinguish. Furthermore, polymorphonuclear eosinophils showing the characteristic eosinophilic granules were clearly evident, in contrast to other cellular elements. They were unevenly distributed and the less numerous of the infiltrating cells. The mast cells containing basophilic granules in their cytoplasm were clearly demonstrated along the course of blood vessels and in the perivascular spaces.

C. Naphtol Acetate Non-Specific Esterase (ANAE)

Several types of cells were readily distinguishable according to the way they were stained. However, while esterase activity was clear, the morphology of the cells was not always quite evident. Nevertheless, in each case, the cell morphology could be determined.

- Some cells, the lymphocytes, presented with a single positive red dot located in the cytoplasm at the periphery of the cell nucleus. This cell was interpreted as a T lymphocyte. (Fig. 1)
- Another ANAE positive cell was the plasma cell. They have more than 1 ANAE positive granule located at the junction between the nucleus and the cytoplasm. (Fig. 1) The number and size of such granules were variable. Generally, the numbers ranged from 2 to 7 positive granules. In many cases, the staining

granules appear to overlay the halo of the plasma cell which contain the Golgi apparatus.

As seen by the hematoxylin and eosin stain, the plasma cells form the most common cell type seen in the connective tissue. They were located in the area beneath the lamina propria, around blood vessels occasionally accompanied by Russel's bodies. Very rarely, a plasma cell was seen intercellularly in the necrotic epithelium at the basal layer of the sulcus epithelium.

In addition to ANAE positive plasma cells, it was observed that there were both ANAE negative plasma cells and negative lymphocytes, as well. The ANAE negative lymphocyte was interpreted as a B cell lymphocyte (Fig. 1)

The most striking feature was the presence of strongly ANAE positive mononuclear cells, located in the lamina propria. They were observed usually coursing along with the blood vessels in the perivascular spaces. (Fig. 2 & Fig. 3)

They were intensely stained with large, either elongated or ovoid deposits in the cytoplasm, frequently obscuring the nucleus. Such cells were large, ranging in size from 20 to 30 microns in diameter. Considering the size and other features, they had appeared as tissue macrophages. In the inflammatory infiltrate, usually, they were adjacent to T and B lymphocytes. Both longitudinal and cross sections of blood vessels also showed the presence of such large, positively stained macro-

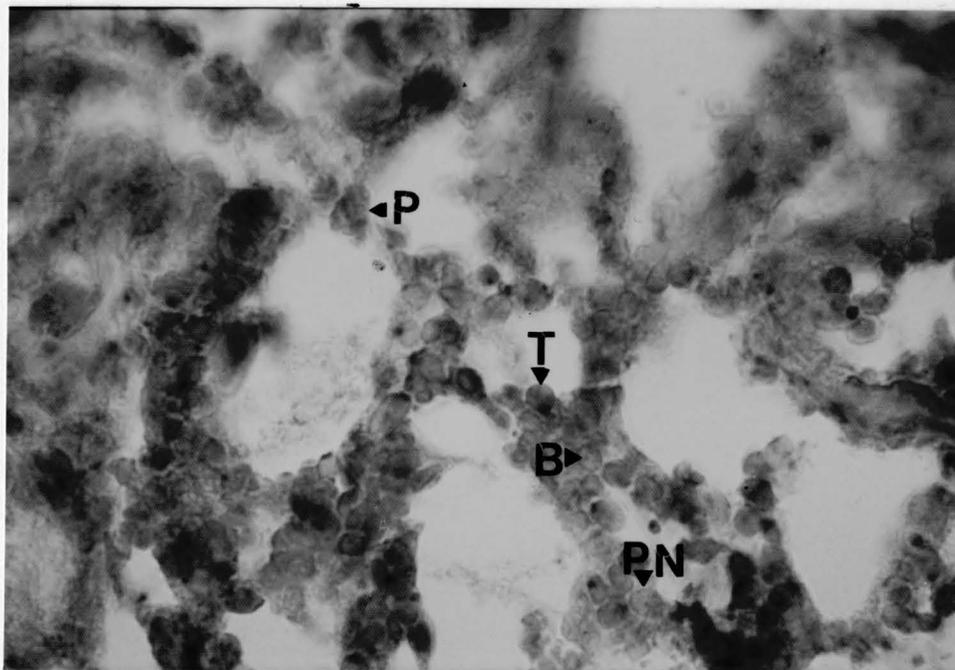


FIGURE 1

High magnification of inflamed gingival connective tissue stained for ANAE. Note T lymphocyte with a single positive red dot located in the cytoplasm at the periphery of the cell nucleus, plasma cell with several positive red dots located at the junction between the nucleus and the cytoplasm, ANAE negative plasma cell, and B lymphocyte negatively stained for ANAE.

(Original Magnification 1000X)

T: T Lymphocyte

P: Plasma Cell

PN: Negative Plasma Cell

B: B Lymphocyte

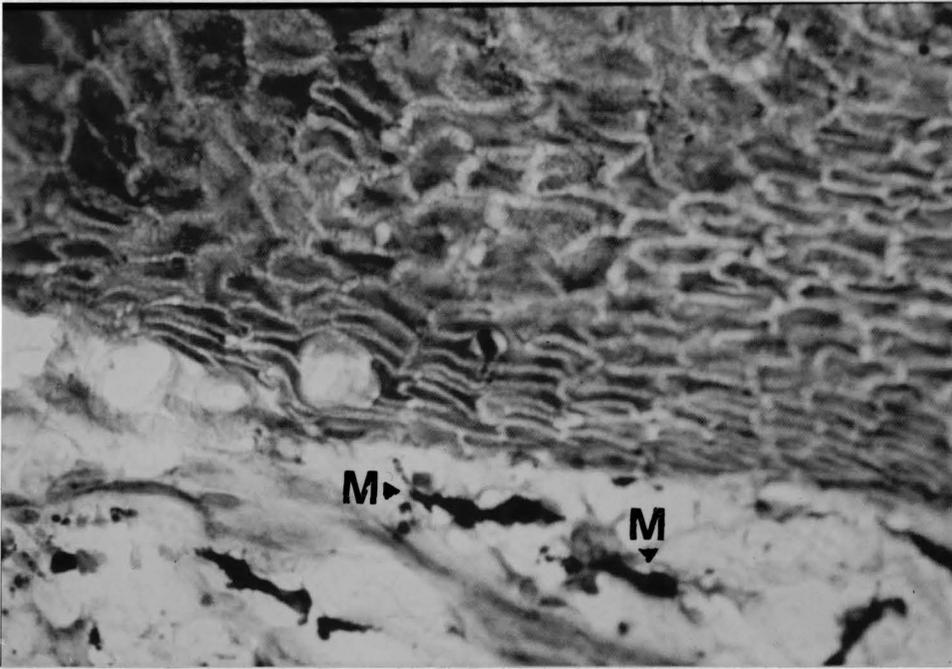
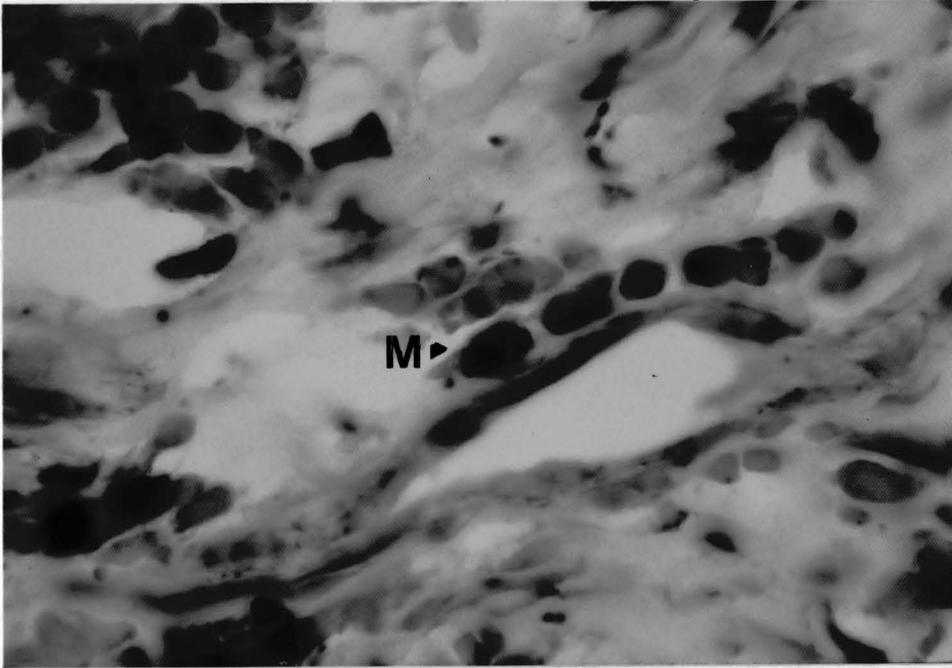


FIGURE 2

High magnification of inflamed gingival connective tissue showing a strongly ANAE positive macrophage located in the lamina propria.

(Original Magnification 1000X)

M: Macrophage



negative.

D. Acid Phosphatase

FIGURE 3

Several features are readily seen upon acid phosphatase staining. High magnification of inflamed gingival connective tissue showing ANAE positive macrophages coursing along with blood vessels.

(Original Magnification 1000X)

M: Positive Macrophage

phages. In the lamina propria, such cells were morphologically characteristic of blood monocytes. Frequently, such monocytes were marginating in the walls of blood vessels.

Positive large macrophages also were seen intercellularly in the sulcus epithelium, either in the basal layer or the stratum spinosum. (Fig. 4)

Interestingly, it was occasionally noted that some fibroblasts, collagen, intercellular spaces of the epithelium of the stratum granulosum, and stratum corneum also were ANAE positive.

- Polymorphonuclear cells were seen clearly in the inflammatory cellular infiltrate. However, they all appeared to be ANAE negative.

D. Acid Phosphatase

Several features are readily seen upon acid phosphatase staining. First, in the epithelium, the intercellular spaces were filled with positive red granules from the basal layer to the superficial layer. Remarkably, in the superficial layer, the acid phosphatase reaction was more diffuse and more intense than in the subjacent layers.

In addition to the staining of the intercellular spaces with the red granules there were some large, intensely stained mononuclear cells located between the epithelial cells. Usually, such cells had cytoplasmic processes extending between several cells giving the aspect of stellate shape. (Fig. 5)

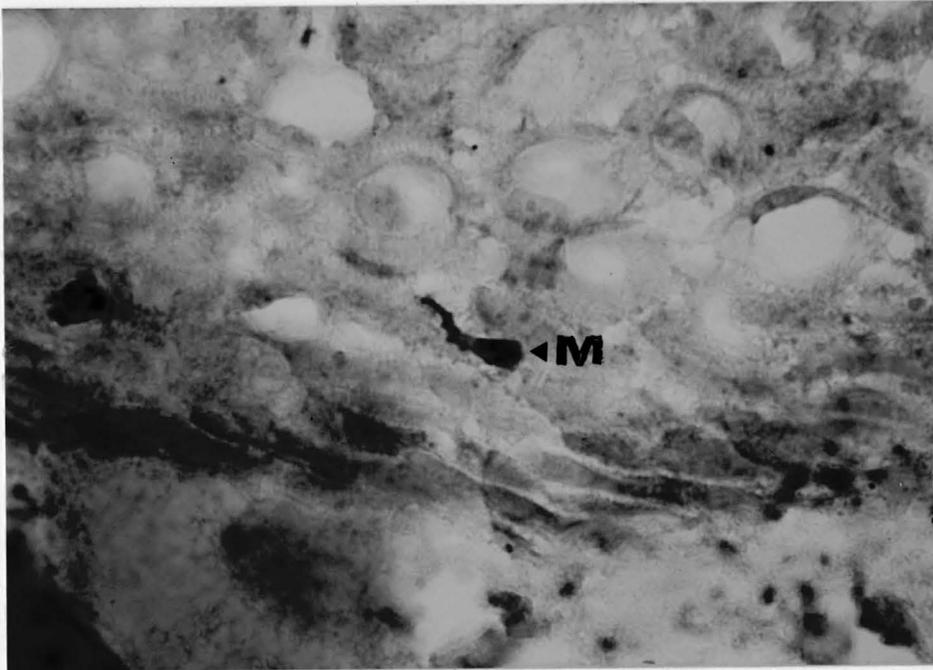


FIGURE 4

High magnification of inflamed sulcular gingival epithelium showing ANAE positive macrophage located in the intercellular space.

(Original Magnification 1000X)

M: Macrophage

Polymorphonuclear cells, less intensely positive than the mononuclear cells and without detectable cytoplasmic processes also were found between the epithelial cells. (Fig. 5)

In several instances, the cytoplasm of epithelial cells stained positively for acid phosphatase. (Fig. 5) This staining was diffuse, although occasionally it was observed that single epithelial cells contained granules overlapping the nucleus. However, it was not difficult to differentiate positive epithelial cells from the infiltrating mononuclear and polymorphonuclear cells which are easily recognizable because of their more intense staining to acid phosphatase.

In the lamina propria of the inflamed connective tissue, either immediately beneath the destroyed basal membrane or around the adjacent blood vessels, there were many positive cells. The most intensely positive ones were large mononuclear cells, either round, ovoid, or irregular in shape. Occasionally, such cells were seen infiltrating the basal layer immediately at the basement membrane. (Fig. 6)

At this point, it was observed that due to the thickness of frozen sections and unfixed preparations, the morphology of some cells of the connective tissue, and the surrounding collagen fibers, either destroyed or intact, is not clearly defined. In particular, the cell mononuclear contours were difficult to define. However, the characteristic ways these large mononuclear cells were stained were either diffuse or granular.

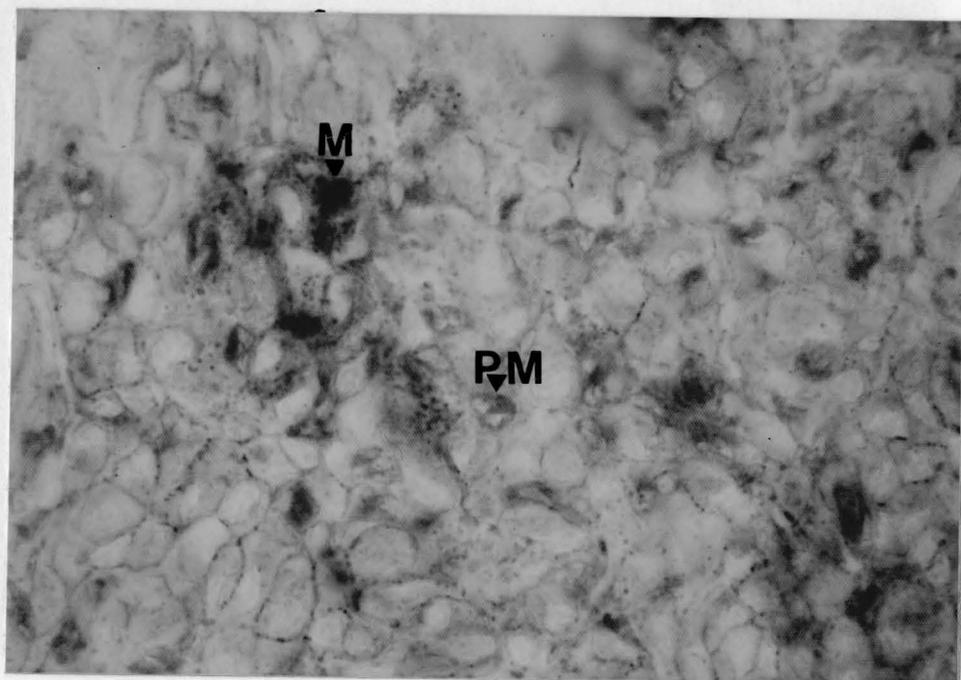


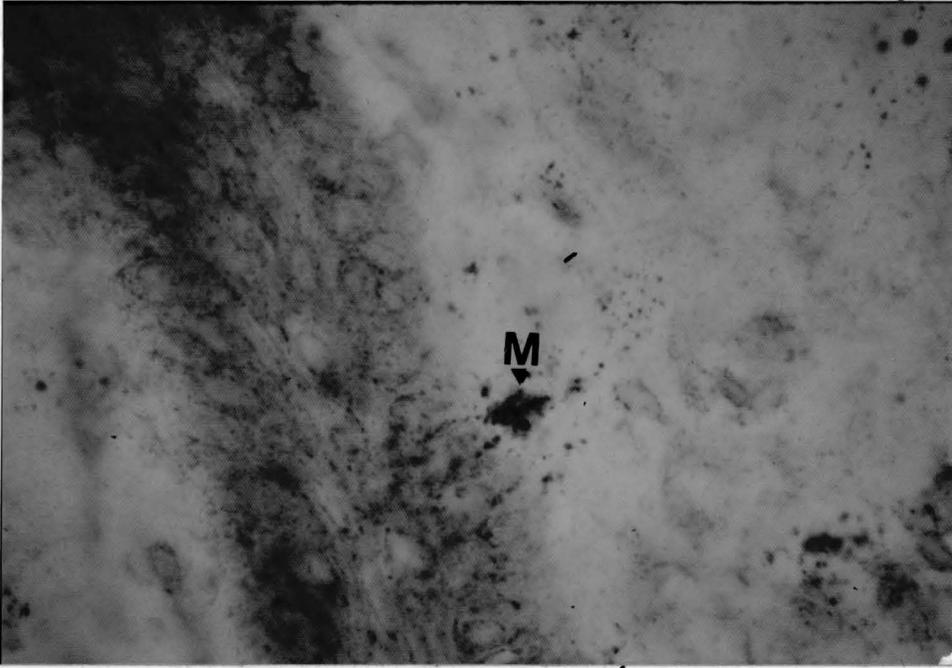
FIGURE 5

High magnification of inflamed oral epithelium showing intercellular spaces positive for acid phosphatase activity and positive macrophage located between the epithelial cells. A positive polymorphonuclear leukocyte can be seen in the intercellular space. Some epithelial cells show evidence of cytoplasmic acid phosphatase activity.

(Original Magnification 1000X)

PM: Positive Polymorphonuclear Leukocyte

M: Positive Macrophage



2. EXAMINATION OF BLOOD SMEARS FROM INFLAMED MUCOSA

A. Giemsa

FIGURE 6

All the blood smears examined showed the usual mature blood cells of the hematopoietic system. The polymorphonuclear leukocyte appears as a round cell containing a faintly stained positively for acid phosphatase and located beneath the basal eosinophilic cytoplasm and a dense characteristic multilobed nucleus. All of the lymphocytes were seen as round cells containing round basophilic nucleus surrounded by a thin crown of cytoplasm. Also, monocytes presented themselves as a more or less round cell with containing an indented basophilic nucleus and eosinophilic cytoplasm.

(Original Magnification 1000X)

M: Macrophage

B. Naphthol Acetate Non-Specific Esterase

The morphologic appearance of the blood smears treated for non-specific esterase presented with good morphology. The

Other acid phosphatase positive cells with multilobed nuclei were seen throughout the connective tissue. However, they are less intensely stained than that of the larger mononuclear cells. Morphologically, they appeared as polymorphonuclear leukocytes.

Small cells, 6 to 8 microns in size appeared as lymphocytes. They contained round nuclei and little cytoplasm. All such cells were negative to the acid phosphatase reaction. In addition, the acid phosphatase technique reveals the presence of a few positive reacting plasma cells.

2. EXAMINATION OF BLOOD SMEARS FROM INFLAMED MUCOSA

A. Geimsa

All the blood smears which were examined showed the usual mature blood cells of the hematopoietic system. The polymorphonuclear leukocyte appears as a round cell containing a faint eosinophilic cytoplasm and a dense characteristic multilobed nucleus. All of the lymphocytes were seen as round cells containing round basophilic nucleus surrounded by a thin crown of cytoplasm. Also, monocytes presented themselves as a more or less round cell with containing an indented basophilic nucleus and eosinophilic cytoplasm.

B. Naphtol Acetate Non-Specific Esterase

The morphologic appearance of the blood smears treated for non-specific esterase presented with good morphology. The

polymorphonuclear neutrophils presented the same morphologic features as seen by the Geimsa method but all of them were negative to non-specific esterase.

The non-specific esterase technique differentiated two types of lymphocytes. One type which apparently is the less numerous is made up with non-specific esterase negative round cells. The second type is composed of the round cell with one positive red granule at the junction of the cytoplasm and the nucleus. (Fig. 7)

Monocytes clearly were seen. They measured 12 to 15 microns in diameter and were clearly larger than other cells in the blood. The monocytes were seen to react faintly, but few also strongly reacted to ANAE. (Fig. 8)

C. Acid Phosphatase

Polymorphonuclears and lymphocytes were seen to be negatively stained. Furthermore, distinction could not be made between the lymphocytes as compared to the ANAE reaction as they present themselves with identical cytologic features. (Fig. 9)

However, some monocytes could be seen positively stained. (Fig. 10)

3. EXAMINATION OF NON-INFLAMED TISSUES

A. Hematoxylin and Eosin Staining

Under low power, the tissue appears morphologically normal. The mucosa was composed of stratified squamous keratinized

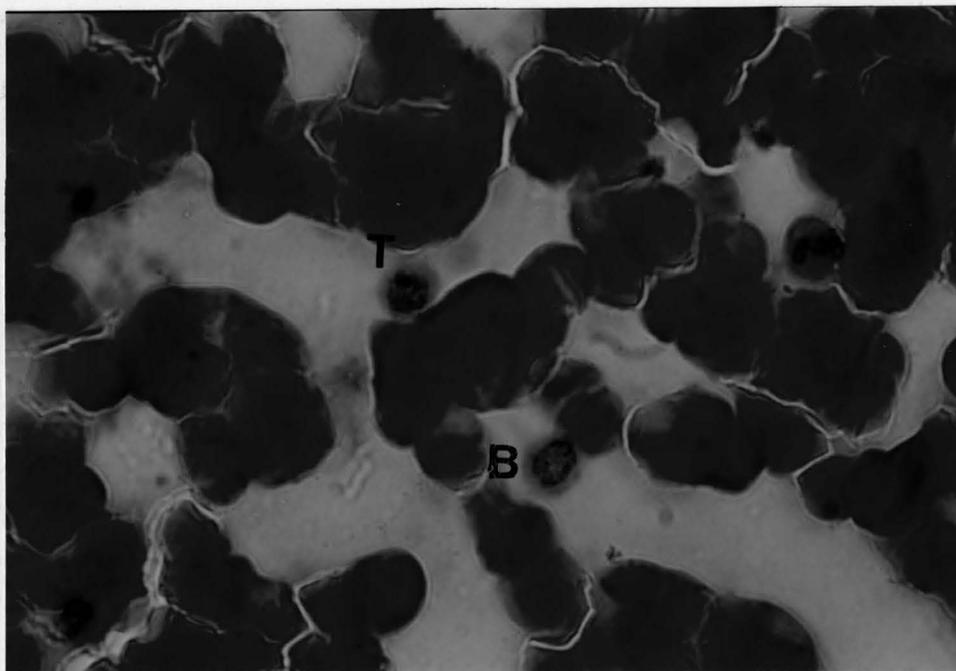


FIGURE 7

High magnification of the blood smear from inflamed gingiva stained for ANAE showing T lymphocyte with a single positive dot, and a negative B lymphocyte.

(Original Magnification 1000X)

T: T Lymphocyte

B: B Lymphocyte

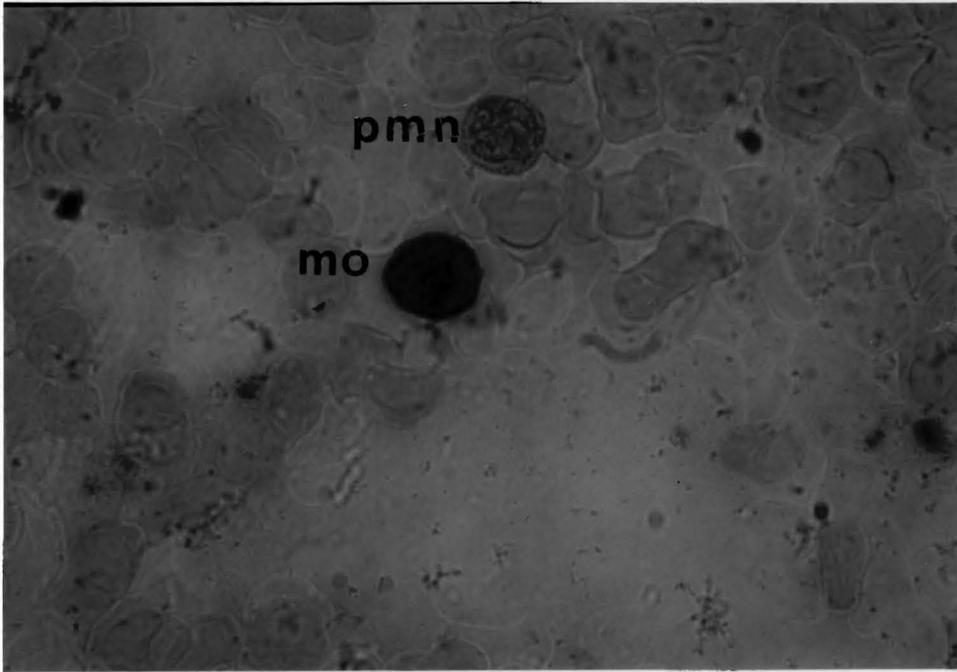


FIGURE 8

High magnification of a blood smear from inflamed gingiva stained for ANAE showing a positive monocyte and ANAE negative polymorphonuclear leukocyte.

(Original Magnification 1000X)

MO: ANAE Positive Monocyte

PMN: ANAE Positive Polymorphonuclear Leukocyte

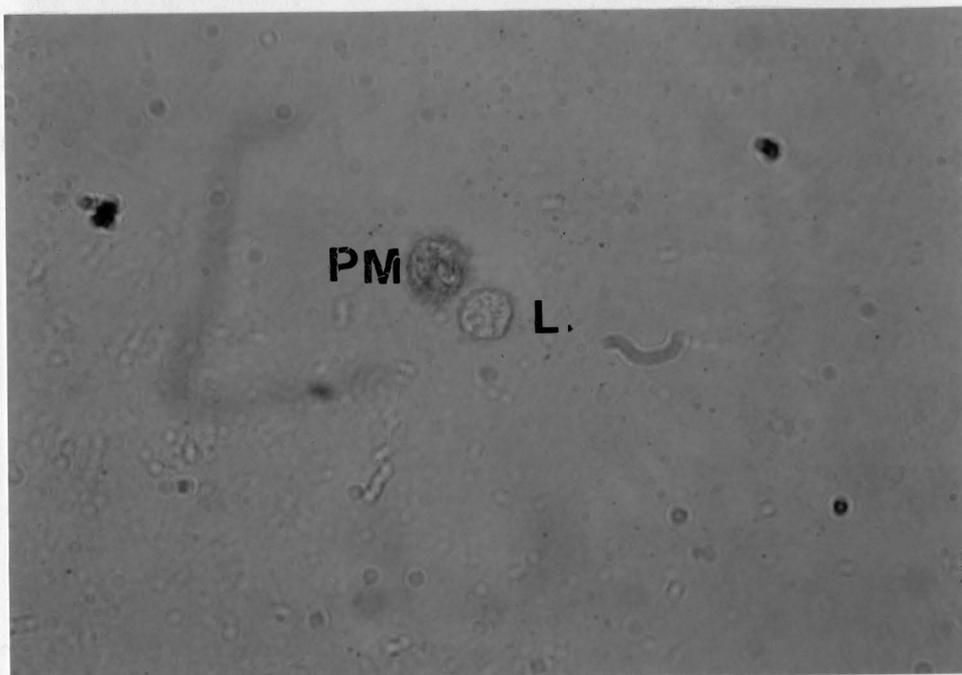


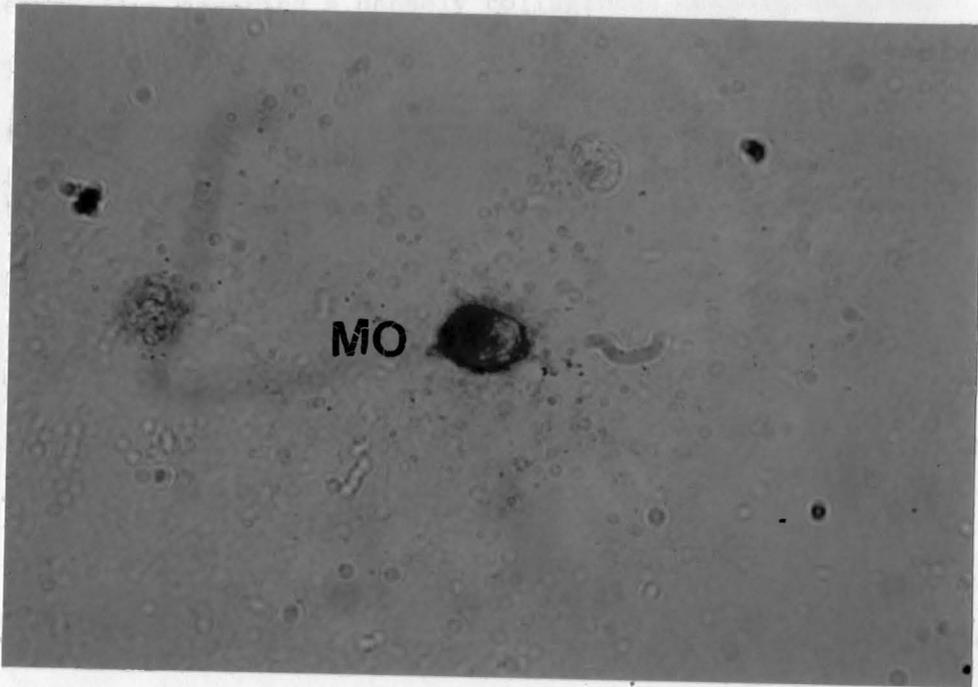
FIGURE 9

High magnification of a blood smear from inflamed gingiva showing negative polymorphonuclear leukocyte and negative lymphocyte for acid phosphatase staining.

(Original Magnification 1000X)

PM: Negative Polymorphonuclear Leukocyte

L: Lymphocyte



B. Guinea Staining

The same morphologic features as were noticed with hematoxylin and eosin stained control tissues also

FIGURE 10

High magnification of blood smear from inflamed mucosa showing a positive monocyte to acid phosphatase staining.

(Original Magnification 1000X)

MO: Positive Monocyte for Acid Phosphatase Staining

The intercellular spaces of the epithelium were ANAE positive as observed in the inflamed tissue. However, no ANAE positive infiltrative cells were seen in the epithelium. However, few ANAE positive large nonnuclear cells were seen in the lamina propria of the connective tissue, immediately beneath a non-disrupted basement membrane. Other large poorly defined cells were seen occasionally as slightly positive. The ANAE reaction

epithelium covering a densely collagenous connective tissue. There were very few polymorphonuclear neutrophils seen infiltrating the epithelium, intercellularly, but generally it was normal.

The lamina propria of connective tissue was normal in appearance without notable presence of inflammatory cells. Neither plasma cells nor lymphocytes were seen. The vascularization was normal and appeared as many small capillaries with well developed adventitia. Collagen fibers were intact with fibroblasts and appeared in dense woven bundles. Fibrocytes appeared to be parallel to the long axis of the collagen fibers.

B. Geimsa Staining

The same morphologic features as were noticed with hematoxylin and eosin stained of non-inflamed control tissues also were seen with Geimsa method. As above noted, few polymorphonuclear neutrophils could be seen intercellularly in the epithelium. Otherwise, all features appeared normal.

C. Non-Specific Esterase Staining

The intercellular spaces of the epithelium were ANAE positive as observed in the inflamed tissue. However, no ANAE positive infiltrative cells were seen in the epithelium. However, few ANAE positive large mononuclear cells were seen in the lamina propria of the connective tissue, immediately beneath a non-disrupted basement membrane. Other large poorly defined cells were seen occasionally as slightly positive. The ANAE reaction

appeared to select out for observation those sparse ANAE positive and negative lymphocytes, in the connective tissue. Furthermore, few positive plasma cells were made evident by ANAE reaction and seen around the blood vessels. Moreover, even a few fibroblasts appeared to be ANAE positive. (Fig. 11)

D. Acid Phosphatase

Intercellular spaces and superficial layers of the epithelium were positive. However, no positive infiltrative positive cells were seen intercellularly in the epithelium. The lamina propria of the connective tissue contained very few large positive mononuclear cells with the expected positive red granule.

4. EXAMINATION OF BLOOD SMEARS FROM NON-INFLAMED TISSUES

They presented exactly the same features as observed in the blood smears from the inflamed tissues. Lymphocytes with single red dot ANAE positive stain were seen among negatively reacting ones. Also, monocytes presented positively to ANAE.

Only some monocytes showed a positive stain for acid phosphatase reaction. (Fig. 12) Other monocytes, lymphocytes and neutrophils were negative.

5. EXAMINATION OF RAT LIVER (POSITIVE CONTROL)

A. Hematoxylin Eosin and Geimsa Staining

Classical hepatocytes, sinuses, and Kupffer cells were seen. The hepatocytes were clearly divided into lobules separated by a fibrovascular stroma.



FIGURE 11

Low magnification of non-inflamed mucosa stained for ANAE.
Few large positive cells can be seen in the lamina propria of the
connective tissue.

(Original Magnification 1000X)

M: Positive Macrophage

L: Acid Phosphatase
Negative Lymphocyte

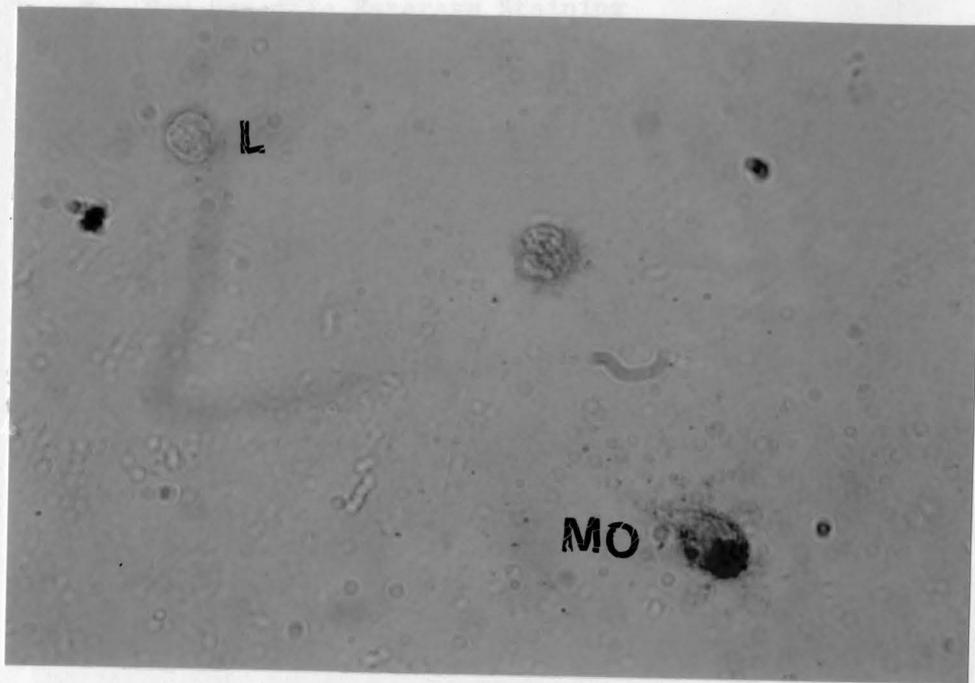


FIGURE 12

High magnification of a blood smear from non-inflamed gingiva stained for acid phosphatase showing a positive monocyte and a negative lymphocyte.

(Original Magnification 1000X)

- MO: Acid Phosphatase
Positive Monocyte
- L: Acid Phosphatase
Negative Lymphocyte

B. Non-Specific Esterase Staining

Irregularly shaped, large strongly positive cells were seen throughout the sample among positive hepatocytes. Such large positive cells were seen coursing along with the sinusoidal blood vessels and in the stroma. They appeared as Kupffer cells in their sinusoidal distribution and as macrophages in the stroma. (Fig. 13)

C. Acid Phosphatase Staining

The hepatocytes presented as polygonal cells with positive granules in the cytoplasm. Large mononuclear cells were also present with positive granules in the cytoplasm. They were observed also to follow the course of the sinusoidal blood vessels and in the stroma of the liver. Such cells could be identified as Kupffer cells and macrophages of the stroma, respectively. (Fig. 14)

6. NEGATIVE CONTROLS

The tissue samples incubated without substrate were all negative when reacted without substrate for ANAE and acid phosphatase.

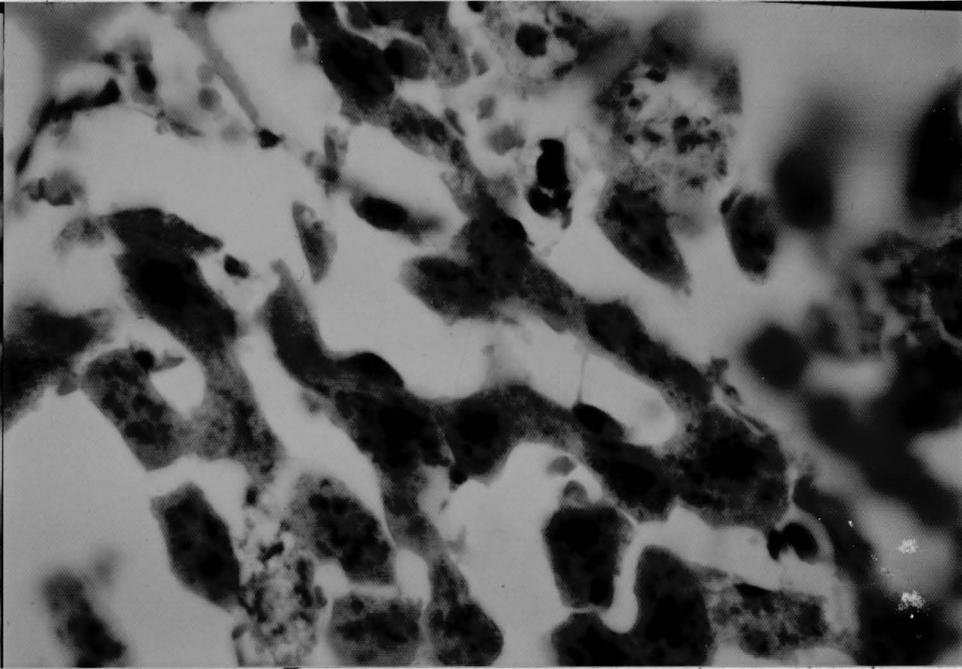


FIGURE 13

High magnification of rat liver tissues stained for ANAE showing positive hepatocytes and positive Kupffer cells.

(Original Magnification 1000X)

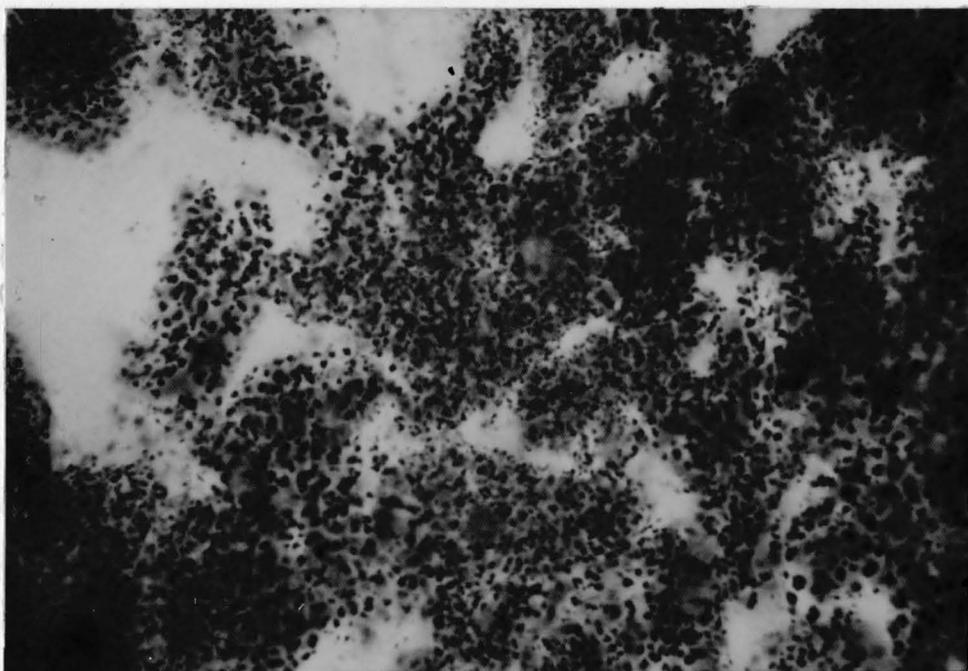


FIGURE 14

High magnification of rat liver tissue stained for acid phosphatase showing positive hepatocyte and positive Kupffer cells.

(Original Magnification 1000X).

CHAPTER V

DISCUSSION

The histologic findings within inflamed biopsy specimens of the gingiva confirmed the observed clinical inflammation since all of the tissue samples exhibit features of chronic inflammation. The most common cell type found in the infiltrated connective tissue lining the periodontal pocket was the plasma cell. This is in agreement with the findings reported by other investigators (Melcher, 1967) (55), (Freedman, Listgarten and Taichman, 1968) (27).

According to the classification of Page and Schroeder, 1976 (68), the observation of plasma cell predominance in this study would be classed as "advanced lesion." In this study, the epithelium of human gingiva as found both in inflamed and non-inflamed specimens was found to be positive for acid phosphatase activity. This observation has been also reported by Cabrini and Carranza, 1951 (11), Lisanti, 1960 (44), Winer, O'Donnell, Chauncey, and McNamara, 1970 (121). However, Zander, 1941 (124) and Tynelus-Bratthall, and also Attstrom, 1972 (112) have failed to demonstrate this enzyme in the gingival epithelium. The human gingiva shows increasing reaction to acid phosphatase from the basal cell layer to the superficial layer where the reaction is the most intense. This observation has also been reported by Ten Cate, 1963 (106). Acid phosphatase activity is associated with lysosomal hydrolases (Weissman, 1965 (119). Oral

epithelial cells have such lysosomes in their cytoplasm (Olson and Nordquist, 1966) (63). Acid phosphatase appears to be secreted into the intercellular spaces. This supports the observation which has shown that acid phosphatase also is present in the membrane coating granules of gingival epithelial cells (Squier and Waterhouse, 1970) (104). During keratinization, acid phosphatase is extruded in the intercellular spaces of stratum spinosum and stratum granulosum.

In few instances, as acid phosphatase is diffusely distributed inside the epithelial cells both in inflamed and clinically normal gingiva, it may be considered as naturally active. Squier and Waterhouse, 1970 (104) have also reported the same observation. In oral epithelium, acid phosphatase participates in the enzymatic breakdown of certain organelles within the granular layer. Lysosomal hydrolases in the intercellular spaces may participate in the modification of the intercellular matrix as well as the cell membrane and desmosomes which in turn, facilitate the desquamation of the superficial layers of cells. The disorder in the epithelium as seen in this study, could, in part, be attributed to excessive release of potent hydrolase activity simultaneously with acid phosphatase activity. Non-specific esterase activity in the inflamed and non-inflamed gingival epithelium are prominent in the superficial layer as compared to its relative absence in the spinous and basal layers. However, while esterase activity does not appear evident in the intercellular spaces, such activity occurs in the cytoplasm. This observation was also reported by Ten Cate, 1963 (106). ANAE activity in inflamed gingival epithelium may be variable. In one instance, this activity was very strong

as seen as a band beginning in the basal layer and extending to the superficial layer, appearing as a column of cells. The most common cell found in the infiltrated connective tissue of inflamed gingiva is the plasma cell. The cytoplasm of some plasma cells is positive for ANAE. However, this reaction varies in quality and quantity from cell to cell. Dockrell, Seymour, Playfair and Greenspan, 1978 (22) have also reported the occurrence of non-specific esterase positive plasma cells within the medullary cords of mouse lymph nodes and in the red pulp of the mouse spleen. However, they observed a diffuse pattern of the ANAE activity in the plasma cell. In inflamed and rarely in clinically normal gingiva, ANAE is present as variable numbers of discrete dots in the cytoplasm of some plasma cells. The differences between the diffuse distribution reported by Dockrell, Seymour, Playfair and Greenspan, 1978 (22) and the dot-like staining reported here may be explained by the fact that, in this study, a different time of incubation and a different pH were used. However, another important consideration is that human samples of gingiva were studied and not mouse tissue. It should be noticed, however, that mouse and man share a rather similar immune system.

A diffuse staining of plasma cells for ANAE may suggest a non-lysosomal enzyme, whereas a dot-like granule distribution may suggest lysosomal-containing enzymes. While the use of the light microscope does not permit the investigator to ascertain the origin of non-specific esterase enzymes in plasma cells, the variability

in staining, numbers and, distribution may indicate different stages of maturation of the plasma cell in the gingival connective tissue as reported in mouse lymphoid tissue by Dockrell and Greenspan, 1978 (21). Some lymphocytes negatively react to non-specific esterase and acid phosphatase activities. Such cells are present in the intercellular spaces of the epithelium whereas the majority are seen infiltrating the connective tissue away from the epithelium. This clearly indicates that such cells migrate through the connective tissue to enter the epithelium; presumably, they may migrate into the sulcus. Negative lymphocytes appear in greater numbers in the inflamed tissue and in the blood as well.

Other studies (Horwitz, Allison, Ward and Knight, 1977) (36), (Seymour, Dockrell, Playfair and Greenspan, 1978) (96) have presented evidence that ANAE negative and acid phosphatase negative lymphocytes belong to the B cell lineage since the thymo-independent follicular area of peripheral lymphoid organs show predominance of this type of cell. In addition, it has been shown that after T cell elimination, the remaining cells were negative for esterase and acid phosphatase (Dockrell, Seymour, Playfair and Greenspan, 1978) (96).

The ANAE negative and acid phosphatase negative lymphocytes in inflamed and clinically normal gingiva probably represent B cells. Moreover, such B cells have their origin in the lymphoid tissues and reach the gingiva by the blood circulation. It is known that B cells are the precursors of plasma cells. It is likely that B cells emigrate from the capillaries, they remain in the perivascular

space where they differentiate to plasma cells and assume the collar or cuff frequently observed (Toto, 1961) (108). The fact that we observed B cells to be negative for ANAE activity and plasma cells to be either negative or positive for ANAE activity makes us hypothesize that some of the positive lymphocytes could be lymphocytes of the B lineage.

The local perivascular differentiation of B cells in the gingiva provide also a continuous population of plasma cells. The fact that others have demonstrated the presence of immunoglobulins in the inflamed gingival tissues (Von Swol, Gross, Setterstrom and D'Allessandro, 1980) (113), (Setterstrom, Gross, D'Allessandro and Godat, 1980) (95), Marttala, Toto, Gargiulo, 1974, (53), Toto, Li-Min-Lin, Gargiulo, 1978 (109), added to the fact that B cells and plasma cells are also found in the inflamed tissue provide strong evidence for humoral type of immune response to occur in the inflamed gingival tissue involved with periodontal disease.

It is interesting to note that other oral disease which stimulate or complicate periodontal disease shows different types of response such as a cell mediated response reported in lichen planus (Dockrell, 1979) (21).

In addition to these negative lymphocytes, other lymphocytes were observed which were positive for esterase and phosphatase activities. They were found sometimes in the intercellular spaces of inflamed epithelium and more often in the infiltrated connective tissue. The same type of positive cells were observed in the blood

smears. Upon esterase staining, such positive lymphocytes presented a characteristic single dot at the junction between the nucleus and the cytoplasm. The ANAE positive lymphocytes seen both in the inflamed and the clinically normal gingiva clearly indicate that T cells participate in periodontal disease. The evidence for such T cell marker was presented by Dockrell, Seymour, Playfair and Greenspan, 1978 (22) which showed that the single dot lymphocyte belongs to the T cell lineage. Furthermore, it is reasonable to say that the dot-like granule staining reveals the lysosomal enzyme as suggested by Turk (1967) (111).

The function of the T lymphocytes in human periodontal disease is yet to be understood. T cells now are known to participate in the immune response serving as helper cells stimulating B cell differentiation to plasma cells, and the production of antibodies. Also, T suppressor cells may prevent B cell differentiation suppressing antibody formation. Furthermore, T cytotoxic cells can identify and kill B cells regulating the humoral response.

The production of lymphokines such as Osteoclast Activating Factor (OAF), or cytotoxin from cytotoxic T cells could explain part of the tissue destruction during human periodontal disease.

Among inflammatory cells of inflamed gingiva, the macrophage shows a striking positive reaction, both for non-specific esterase and acid phosphatase activity. Indeed, they are so strongly stained, that they are readily differentiated from the B lymphocyte, the T lymphocyte, polymorphonuclear leukocyte, and plasma cells. The

macrophages were intensely stained for non-specific esterase, whereas they exhibit diffuse cytoplasmic positive granules for acid phosphatase staining. This is in agreement with Li, Yam and Crosby, 1972 (42) who reported the same finding for human spleen macrophages. Seymour, Dockrell and Greenspan, 1978 (96) have also reported the presence of heavy esterase positive activity in macrophages of human inflamed gingiva during periodontal disease. The ability of lymphoid organ macrophages to exhibit strong acid phosphatase and non-specific esterase activity has been widely reported (Horwitz, Allison, Ward and Kight, 1977) (36), (Seymour and Greenspan, 1978) (96) (Dockrell, Seymour, Playfair and Greenspan, 1978 (22), (Li, Yam and Crosby, 1972) (42), (Yam, Li and Crosby, 1971) (122). The reliability of the non-specific esterase technique to identify macrophages was reported as early as 1957 by Burstone (9). Specimens showed esterase positive macrophages located in the lamina propria, around blood vessels and sometimes between epithelial cells of inflamed gingiva. Additional studies dealing with histopathology of human inflamed gingiva also have reported the same location of macrophages (Zachrisson, 1968) (123), (Freedman, Listgarten and Taichman, 1968) (27), (Payne, Page, Ogilvie and Hall, 1975) (72), (Schroeder, 1970) (89), (Thilander, 1968) (108), (Brandtzaeg, 1966) (8), (Schroeder and Munzel-Pedrazzoli, 1973) (90), (Schroeder and Munzel-Pedrazzoli, 1973) (92). However, only one study (Seymour, 1978) (96) has reported the presence of non-specific esterase positive macrophages in human inflamed gingiva during periodontal diseases. This observation is confirmed in

this report since Kupffer cells and macrophages were identically stained for acid phosphatase and non-specific esterase as reported by Seymour, Dockrell and Greenspan, 1978 (96). Furthermore, the morphology of the macrophages as observed in the inflamed and clinically normal gingiva and the blood also is compatible with prior data (Nichols, Bainton, 1973) (60).

The presence of chemotactic factor for macrophages and monocytes in the gingival sulcus (Attstrom, 1975) (1), (Shillitoe and Lehner, 1972) (97) and inflamed gingiva (Schenkein and Genco, 1977) (86) (87), (Fullmer, 1969) (28), (Genco, Mashimo, Krygier and Ellison, 1974) (31), (Ranney, 1970) (78), (Robertson and Simpson, 1976) (82) explains the presence of the observed macrophages in the gingiva. Furthermore, the in vitro demonstration of macrophage inhibitory factor (MIF) synthesized and released by antigen stimulated lymphocytes (Galindo, Heise and Myrvik, 1970) (30), (Seravelli and Taranta, 1973) (94), (Ward, 1970) (118) also suggests that gingival macrophages are retained in the inflammatory site. In this study, the greater aggregation of macrophages in the lamina propria of connective tissue immediately beneath the basal membrane of the gingiva suggests that macrophages may aggregate on their way from the blood vessels of the connective tissue to the gingival sulcus.

All mononuclear phagocyte cells are positive for non-specific esterase, but there is a difference in their staining intensity as seen between monocytes of blood smears, monocytes observed within blood vessels of histologic sections and macrophages found in the epithelium and connective tissue of inflamed gingiva. Indeed, the

blood smear monocytes stain less for non-specific esterase than monocytes observed inside the lumen of the blood vessels of inflamed gingiva. (Fig. 15) Such monocytes react equally as well as the tissue macrophages for non-specific esterase. The only difference between blood monocytes and tissue macrophages is the phenotypic appearance. It has been said that peripheral blood monocytes are relatively immature cells en route to tissue sites for further maturation (Pearsall and Weiser, 1970) (74). Although they may contain a variety of enzymes, the number and size of enzymes containing lysosomes are characteristically less in blood monocytes than those in more mature macrophages found elsewhere (Pearsall and Weiser, 1970) (74). This would explain the different intensity of positivity observed in this study. The blood smear monocytes could be the less mature cell which did not adhere on the blood vessel endothelium at the time of tissue preparation. On the other hand, monocytes which were undergoing diapedesis and thus seen in the histologic sections, inside the blood vessels, could be more mature cells as indicated by increased intensity of the stain, more positive for non-specific esterase; additionally, large non-specific esterase positive macrophages observed in the connective tissue and epithelium of inflamed gingiva are in the final stage of differentiation.

Monocytes seen in the blood smear could have lost part of the enzyme activity due to the histologic processing explaining their reduced stain since Yam, Li and Crosby, 1971 (122) reported that

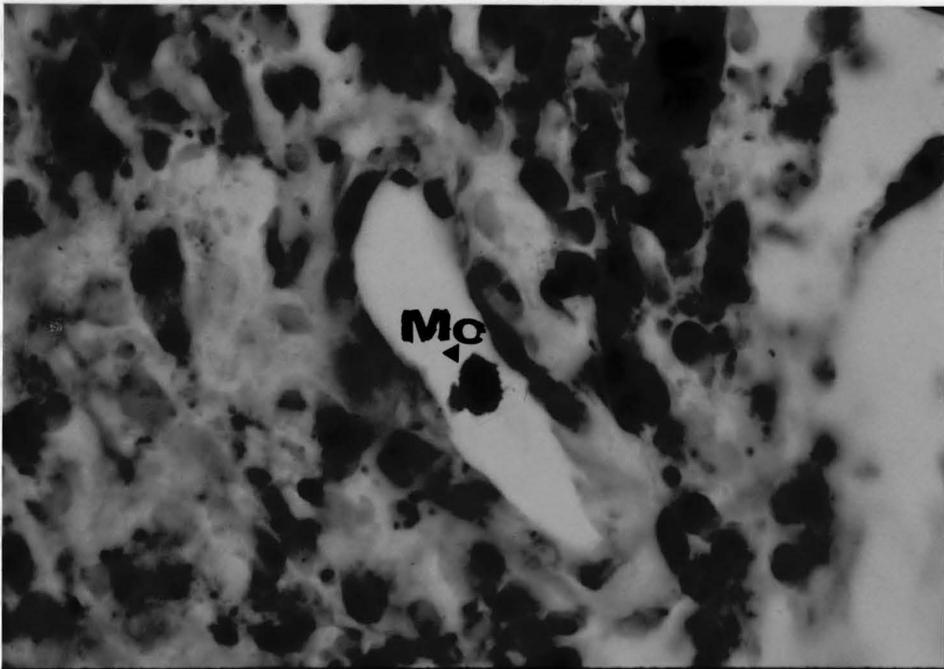


FIGURE 15

High magnification of inflamed gingiva showing a strongly ANAE positive mononuclear cell against the inner part of a blood vessel.

(Original Magnification 1000X)

MO: Positive Monocyte

blood smear monocytes were strongly positive for non-specific esterase activity.

The macrophages in the liver (Kupffer cells) are constantly in an activated state (Matter, Forssmann and Rouiller, 1968) (54). It was interesting to compare the activated Kupffer cells of the rat liver with gingival macrophages observed in this study. Both showed heavy staining for acid phosphatase and non-specific esterase. This observation strongly suggests that heavy stained gingival macrophages are activated. Page, Davies and Allison, 1973 (65), and Page, Davies and Allison, 1972 (67) have already made this hypothesis since they succeeded to activate mouse peritoneal macrophages with human dental plaque in vitro. They observed that activated mouse peritoneal macrophages increased in size with an increased synthesis of lysosomal and non-lysosomal enzymes. Moreover, they found that the activated macrophages released up to 80% of their hydrolases during activation by minute plaque concentration. Based upon the previously noted investigations and our observation, it is reasonable to suggest that gingival macrophages in human inflamed gingiva are not only activated but also could release their lysosomal enzymes in the extracellular spaces of connective tissues and epithelium. Moreover, studies performed in vitro have shown that cultured macrophages are activated by lymphokines (Wahl, Wahl, Mergenhagen and Martin, 1972) (116), (Mooney and Waksman, 1970) (59), (Pantalone and Page, 1975) (71), (Mackaness, 1969) (50), endotoxins (Wahl, Wahl, Mergenhagen and Martin, 1974) (115), complement (Sorber, 1978) (101), immune

complexes (Cardella, Davies and Allison, 1974) (12), streptococcal wall substances, (Davies, Page and Allison, 1974) (20) actinomyces viscosus (Richman, Robertson, Trummel and Patters, 1979) (80), and histamines (Pearsall and Weiser, 1970) (74). The presence of such macrophage activating substances in the periodontal tissues has been frequently suggested in reports by Mergenhagen, 1971 (57), Brandtzaeg, 1973 (7), Genco, Mashimo, Krygier and Ellison, 1974) (31), Horton, Oppenheim and Mergenhagen, 1974 (35), or demonstrated (Schwartz, Stinson and Parker, 1972)(93), (Hay, Torrigiani and Roitt, 1972) (33), (VanSwol, Gross, Setterstrom and D'Allessandro, 1980) (113), Setterstrom, Gross, D'Allessandro and Godat, 1980) (95), (Attstrom, 1975) (1). These reported findings are compatible with the hypothesis that macrophages observed in the inflamed gingival tissues are activated. The finding of strongly positive reaction for acid phosphatase and non-specific esterase inside blood vessels suggests that the process of activation begins before the blood monocyte entered the extravascular spaces of the inflamed gingiva. Furthermore, wherever activated macrophages were observed, they stained strongly for non-specific esterase and acid phosphatase. Therefore, macrophages could be activated in the blood vessels before they migrate to the gingival sulcus.

The role of macrophages in the pathogenesis of chronic inflammatory diseases has been discussed extensively (Page, Davies and Allison, 1974) (66), (Spector, 1970) (102), (Mackness, 1962) (51), (Page, Davies and Allison, 1972) (67), (Nelson, 1976)(62), (Mac-Cluskey and Cohen, 1974) (48), (Dunlop, 1970)(23). Other researches have

suggested the role of macrophages in the pathogenesis of human periodontal disease (Page, Davies and Allison, 1973) (65), (Cowley, 1972) (17), (Page, Simpson, Ammons and Schectman, 1971) (70), (Mergenhagen and Snyderman, 1970) (56). Macrophages at the chronic inflammatory site are primarily responsible for the elimination of irritants by means of either endocytosis or phagocytosis. However, previous studies which have reported the morphologic features of macrophages in inflamed gingiva did not report evidence of phagocytosis inside the tissue (Zachrisson, 1968) (123), (Freedman, Listgarten and Taichman, 1968) (27), (Schroeder, Munzel-Pedrazzoli and Lindhe, 1972) (91), (1973) (92). Morphologic evidence of phagocytosis by macrophages has been reported in the gingival sulcus (Attstrom, 1975) (1), (Brandtzaeg, 1966) (8), (Attstrom, 1970) (2). Since bacteria are not seen inside the inflamed tissue during periodontitis it is possible that they are phagocytized in the gingival sulcus by polymorphonuclear leukocytes and/or macrophages. If so, their capacity to phagocytize must be tremendous. It has been shown that macrophages are able to phagocytize cellular debris whereas polymorphonuclears are not (Pearsall and Weiser, 1970) (74). The response of mononuclear phagocytes to endocytosable substances changes with the nature of the substance. For example, inert particles such as silk suture material does not induce changes in cell morphology nor the levels or distribution of various energy-producing or hydrolytic enzymes (Page, Davies and Allison, 1974) (66). On the other hand, mouse peritoneal macrophages phagocytizing erythrocytes or aggregated

gamma-globulins exhibit dose dependent and transient increases in the levels of acid phosphatase, Beta glucuronidase and acid protease (Axline and Cohn, 1970) (5). However, in the latter case, there is no release of hydrolase enzymes. Studies performed in vitro have shown that macrophages are activated by endotoxins with the concomitant synthesis and release of lysosomal enzymes (Wahl, Wahl, Mergenhausen and Martin, 1974) (115). Furthermore, as reported earlier in this discussion, (Page, Davies and Allison, 1973) (65) have demonstrated that macrophages being activated in vitro with human dental plaque release lysosomal enzymes in the extracellular medium. The nature of these lysosomal enzymes could explain the soft tissue destruction during human periodontal disease. Houck, Johnston and Jacob, 1968 (37), suggested that the breakdown of collagen during inflammation is a consequence of the release of lysosomally derived enzymes into the extracellular spaces which activate collagenase. Other enzymes such as Beta glucuronidase or N Acetyl-Beta galactosidase could hydrolyze the mucopolysaccharides of the connective tissue ground substance and extracellular matrix of the epithelium as well. Furthermore, it has been recently suggested that monocytes could play a role in bone resorption since human circulating monocytes in tissue culture appear capable of resorbing devitalized adult bone and fetal bone (Kahn, Stewart and Teitelbaum, 1978) (39) and monocyte derived cells would phagocytize collagen fibrils exposed after osteoclastic activity. The latter findings have been reported in vivo by Rifkin and Heijl, 1979 (81).

Therefore, the presence of activated macrophages within the human inflamed gingiva probably indicates an attempt to remove the irritants causing gingival inflammation. On the other hand, macrophages could participate in the destruction of periodontium through the release of lysosomal enzymes during activation.

CHAPTER VI

SUMMARY AND CONCLUSION

Fifteen patients, 8 males and 7 females, ranging from 30 to 88 years of age and having periodontitis were selected for this study.

Biopsies were taken of both normal and inflamed gingival tissues. In addition, samples of blood also were taken from the blood of normal and inflamed gingiva. Blood and tissues were processed for detection of non-specific esterase (ANAE) and acid phosphatase activity.

Examination of the specimens revealed that:

- . Macrophages stained positively for ANAE and acid phosphatase. The comparison of such macrophages with the blood smear monocytes and with Kupffer cells suggests that they are activated.
- . Plasma cells presented a diffuse cytoplasmic positive staining reaction for ANAE.
- . Lymphocytes containing a single dot-like ANAE positive granule in the cytoplasm at the nuclear margin and lymphocytes negative to ANAE are found in all specimens of normal and inflamed gingiva as well as in blood from normal and inflamed gingiva. ANAE positive and negative lymphocytes are respectively interpreted as T cell and B cell.

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The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science in Oral Biology.

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