1964

Histogenesis of Plasma Cells in Inflamed Oral Mucosa

Kenneth E. Nowlan
Loyola University Chicago

HISTOGENESIS OF PLASMA CELLS IN INFLAMED ORAL MUCOSA

by

Kenneth E. Nowlan

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

June

1964

LIFE

Kenneth E. Nowlan was born in Hammond, Indiana, January 26, 1916.

He graduated from Hammond High School in June, 1933.

From 1942 to 1945 he served as a bomber pilot instructor in the United States Air Force.

His predental education was received at Indiana, Roosevelt, and Loyola Universities from 1945 to 1950. In September 1950, he began his dental education at Loyola University School of Dentistry, Chicago, Illinois, and received the degree of Doctor of Dental Surgery in June 1954. Since that time he has served as a part time faculty member at the dental school.

He is married and has five children.

ACKNOWLEDGEMENTS

To Dr. Patrick D. Toto, my advisor, I extend my deepest appreciation for his supervision, assistance, and constant guidance in the construction of this thesis.

To Dr. John J. O'Malley I am indebted for his willingness to aid and advise on the form and content of this work.

To Dr. Joseph M. Gowgiel I am grateful for his many suggestions and for serving as a member of my advisory board.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Generally, the studies of plasma cells in the lymph nodes and spleen have dealt with the immunologic function of plasma cells. Antigens, such as horse serum and typhoid toxin have caused the production of large numbers of plasma cells in the reticulo-endothelial system. In such studies, the plasma cells were observed to arise from large lymphocytes or from stem cells. Furthermore, it was observed that plasma cells secrete gamma globulin.

Although many studies dealing with the histogenesis, maturation, and function of plasma cells in the spleen, lymph nodes, liver and intestinal mucosa have been reported, there are few reports in the literature dealing with the origin of plasma cells in the human inflamed oral mucosa. The oral mucosa is the site of plasma cell formation in gingivitis and chronic inflammatory hyperplasias. This report deals with the origin and function of plasma cells in a non-lymphoid connective tissue system.

CHAPTER II

REVIEW OF THE LITERATURE

The name plasma cell was introduced by Waldeyer (1875). From his work, it is not clear that he described a specific type of cell that later authors denote with the same word. A definite cell type was first recognized by Cajal (1890) in the condylomata of syphilis. He named these cells "cells cyanophils". The first detailed description of the plasma cells was made by Unna (1891) in a work on plasma cells in a case of lupus tuberculosis. His definition of the plasma cell was somewhat wider than that formulated later by Marshalko (1895). Marshalko's description more closely parallels our modern conception what is currently implied in the term plasma cell; that is, a round or, more commonly, oval cell with a strong basophilic cytoplasm, often containing a juxtanuclear light zone, and possessing an eccentrically situated nucleus with chromatin material somewhat grouped together and occasionally moulded into the shape of a cart-wheel. This form of the plasma cell is referred to quite commonly in the literature as the Marshalko cell type.

While Unna strongly emphasized the histiocytic origin of the plasma cell, the views of Marshalko were generally accepted.

He spoke of transition forms between lymphocytes and plasma cells regarding the plasma cell as a specific evolutionary form of the lymphocyte. Maximow's (1902) experiments with tissue culture seemed to establish the lymphocytic origin of the plasma cells. In tissue cultures he had observed transitions between lymphocytes and plasma cells though later his experiments were subject to much criticism. However, his opinion of the lymphocytic genesis of the plasma cell was shared by a number of subsequent authors such as Downey (1911) and Michels (1931). Unfortunately, this concept restricts the formation of plasma cells to the pre-existence of lymphocytes. Yet according to Cameron (1951) and Yoffey (1956), this conversion could not be demonstrated in viewing cells in the rabbit ear chamber.

In more recent years, investigations tend to support the histiocytic origin. Rohr (1931) has, in a number of works, disclosed numerous transitional forms between reticulum cells and fully developed plasma cells observable in smear culture from bone marrow. Parsons (1948), who studied the occurrence of plasma cells in lymphoid tissue of sarcoma-carrying mice, found no evidence of plasma cells being developed from lymphocytes. Parsons states that "the cell responsible for the plasmacytosis in the medullary cords of lymph nodes in experimental mice appears to be the fixed

reticulum cell of the stroma."

After extensive cytological investigations of punctuates from lymph nodes, Bessis and Scebat (1931) arrived at the same conclusion. They examined punctuates from normal lymph nodes and from such nodes that disclosed a more or less pronounced hyperplasia of plasma cells. In their opinion, the first sign of development from a reticular cell to a plasma cell is an increase in the basophilia of the cell and the somewhat eccentric position of the nucleus. This type of cell, in their opinion, called proplasmocyte by the authors, conformed to that of the Turk cell which they considered to be a young plasma cell.

In a study of the histogenesis of plasma cells, Miller (1931) reported finding plasma cells normally present in the subserosal connective tissue of the omenta of rabbits and the connective tissue cords of lymph nodes with an increase in number of these cells when the rabbits were injected with toxic irritants. He concluded that plasma cells arose near blood vessels from a primitive connective tissue cell. He described a maturation cycle in which he observed basically an increasing basophilia of the cytoplasm and a decreasing nuclear size as the stem cell developed into the mature or Marschalko type plasma cell.

Similar findings were reported by Fagraeus (1948) in a study

of antibody production in relation to the development of plasma cells. Fagraeus observed an increased production of plasma cells in the red pulp of the spleen of rabbits previously injected with horse serum and correlated a rising antibody titer with the differentation of reticulum cells into plasma cells. In her opinion, the development of the reticulum cell into the plasma cell is characterized by: (a) an increase in its cytoplasmic basophilia with a diminution of the volume of the cell and change of shape from a round into an oval type, (b) a decrease in nuclear size with an increase in nuclear stainability, (c) a relative increase in the cytoplasmic stainability with methyl green pyronine.

The development of plasma cells from reticulo-endothelial cells implies a gradual change in cellular morphology. Yet the wide variations of forms in the development of plasma cells observable in the same specimen reflect a maturation or biological process that may give an appearance of artificality to a strict classification or division into definite categories. However, for practical reasons, certain types have been defined outlining certain approximate stages in this continuous series of development within the limits of which one or the other type would belong. Fagraeus (1948) has classified four stages or categories: Undifferentiated mesenchymal cell or stem cells, proplasmoblast

(transitional), proplasmocyte (immature and plasmocyte (mature). This classification is based on the size, shape, and stainability of the nucleus and the stainability and quantity of the cytoplasm using hemotoxcylin-eosin stain.

Fagraeus describes these cells as follows: The undifferentiated mesenchymal cell or reticulum cell has a large pale staining nucleus and poorly defined cytoplasmic borders. These cells form a syncytium around capillaries and vascular channels.

She describes the transitional cell as showing some characteristics of the reticulum cell while in several respects differing from the ordinary lymphoid reticulum cell. The transitional cell has a free position being isolated from other cells in contrast with lymphoid reticulum cells, which appear with dim contour and, most often, in aggregations. Also the transitional cells, are, as a rule, larger than ordinary reticulum cells, their diameter sometimes measuring up to 20 microns. In the youngest cells the nucleus is large and round, or almost round. With increasing age, the nucleus diminishes in size assuming at the same time an oval shape. To begin with, it has a light color, is loose and foamy. The stainability increases as differentation proceeds. One, or more often, several big nucleoli are found. In Unna-Pappenheim staining, the cytoplasm, which in the youngest cells appears only

as a narrow band outside the predominating nucleus, first becomes faintly red, then takes on a deeper red. Often the cytoplasm contains small vacuoles. In more differentiated cells, a clearing is descernible in the cytoplasm close to one of the longitudenal sides of the nucleus.

The immature plasma cell, as observed by Fagraeus, is in its typical shape usually smaller and distinctly oval. In an earlier phase of the antibody formation when the immature plasma cells predominate, they are larger with a bigger nucleus. The cytoplasm stains a deeper red with Unna-Pappenheim stain for ribose nucleic acid (RNA) than the transitional cell. Yet, there are transitional cells that do not disclose any difference from immature plasma cells in stainability. She indicates that the main difference between the immature plasma cell and the transitional cell is to be found in the structure of the nucleus, that of the former being richer in chromatin and more oval in its configuration. The longitudinal axis of the nucleus, usually coincides with that of the cell, the nucleoli appear quite distinct. Along one of the longitudinal sides of the nucleus, a clearing in the cytoplasm is most often visible. In addition, a varying number of vacuoles, situated just below the cellular membrane or closer to the nucleus, are noticeable in the cytoplasm. Most often they are to be found on

the same side of the nucleus as the perinuclear clearing (halo). Vacuoles, however, have been observed on the other side.

Fagraeus describes the mature plasma cell as being pronouncedly oval and varying in size ranging from five to fifteen microns in diameter. The nucleus is smaller and, because of an increase in the cytoplasm in front of the peri-nuclear clearing in the immature cell, is eccentrically placed. Consequently, the long axis in the mature cell corresponds to the diameter that was shortest in the immature cell. In the mature cell, the longitudinal axis of the nucleus, which in the immature cell coincided with the longest cellular diameter, is perpendicular to the longitudinal axis of the cell. Also, cells with two nuclei are sometimes seen. In young mature plasma cells the cytoplasm becomes as red with Unna-Pappenheim stain for RNA as in the immature cells. Still, the small, older cells usually show a deeper tint. In young plasma cells vacuoles are as a rule, numerous, while in the old cellular forms they are sometimes totally absent. With increasing age, the nucleus gradually becomes more stainable. The chromatin often shows the typical cart-wheel arrangement. With ordinary methods of staining no nucleoli will appear in the small, most mature plasma cells.

Function

Ehrich, Drabkin, and Forman (1949) studied the relation of antibody formation and the change in the amount of nucleic acids in rabbit lymph nodes draining areas injected with typhoid vaccine. The increase in DNA was found to parallel the increase in weight of the nodes as might be expected from the active multiplication of cells. The peak of RNA increase occurred between the fourth and sixth days after vaccine injection when antibody formation was at its maximum.

In a histologic study of methyl green and pyronine stained sections of lymph nodes in rabbits injected with typhoid vaccine Ehrich (1949) found that during the first six days of the experiment the cellular reaction was chiefly one of plasma cells formation. During the first three days, plasmoblasts predominated; on the fifth and sixth days mature plasma cells were the prevailing cells. Most of the RNA was contained in the mature plasma cells. The lymphocytes began to proliferate in significant numbers on the third and fourth days, and germinal centers began to appear on the fourth and fifth days. They showed their greatest activity on the ninth day when RNA and antibody formation had passed their peaks. Ehrich interpreted these results as indicating that the plasma cell and not the lymphocyte is responsible for antibody

formation.

The relation of RNA to protein synthesis has been established by many investigators using different techniques. Harris and Harris (1949) stated that RNA accounts for a considerable degree of the cytoplasmic basophilia of plasma cells. They found that RNA appeared in developing plasmocytes in rabbits on the second day after injecting the antigen. Brachet (1951) concluded from studies in which cytochemical methods were used, that RNA had a function in protein synthesis. In a similar respect, Caspersson (1950), reported that protein synthesis occurs in cells with high concentrations of RNA. Kelshall and Crabb (1958) have concluded that plasma cells synthesize and store nucleo-proteins and amino acids in the intestinal mucosa between the lumen and blood and lymph. Also, lysis of plasma cells in the sinuses of the spleen, mesenteric lymph nodes, mucosa of intestines, and in other portal organs may be related to protein synthesis in the liver. Furthermore, the infiltration of plasma cells in chronic inflammation represents an intermediate process by which proteins and nucleic acids released from vascular exudates and from lysis of injured cells are retained locally. Such stored material subsequently is released and made available for use by other cells in regeneration and repair. Plasma cells then function as trephocytes making

available trephones necessary to the growth of cells. Plasma cells are found in areas of high protein concentration either in the gut or at sites of chronic inflammation.

As stated previously, Fagraeus (1948) and others advocated the view that plasma cells are derivatives of the reticulo-endothelium. Her results with tissue cultures led her to conclude that the formation of antibody takes place side by side with, and during, the development of the reticulum cells into plasma cells. With the appearance of mature plasma cells, the antibody titre in vitro declined. She concluded that antibody is formed within reticulo-endothelial cells. In case of intense antibody formation, a differentiation of these cells into plasma cells takes place. Thus, the mature plasma cell is to be regarded as the final link in a chain of developments, a cell which has already passed the stage of its greatest functional intensity.

Fagraeus, like many others, does not distinguish between immature reticulum cells (undifferentiated mesenchymal cells) (U.M.C.) and mature reticulo-endothelial cells (macrophages). She states that "there is no evidence to show that the highly differentiated macrophages have prospective potentialities. In fact, this assumption is contrary to all knowledge of ontogeny. There is little doubt, however, that the undifferentiated mesynchymal cells

if properly stimulated give rise to immature plasma cells. (Transitional cells of Fagraeus)."

In Ehrich's experiments, plasma cell production was well under way twenty-four hours after injection of typhoid vaccine. There were many plasmoblasts, many of which showed mitotic figures, indicating that multiplication of these cells was accomplished, like that of other blood cells, chiefly by mitotic division at the hemocytoblastic level. Maturation of plasmoblasts to plasma cells was associated, as in other blood cells, with reduction in the size of the nucleus and disappearance of the RNA in the nucleolus. These cellular changes were recognizable on the second and third days, were conspicuous on the fourth day, and were completed on the fifth day of the experiment. The maturation of the plasma cells thus paralleled the rise in RNA and antibody concentration in the lymph nodes. The concentration of both was greatest when the plasma cells had reached full maturity. Bing and Plum (1937) pointed out that patients with hyperglobulinemia have an increase of plasma cells in their tissues, and that the highest globulin levels are found in patients with plasma cell myeloma, whereas patients with lymphatic leukemia show no increase in globulins. Fagraeus found that tissue which contained abundant plasma cells formed larger amounts of antibody than tissue which included

Malpighian bodies. These observations suggest that the plasma cell, rather than the lymphocyte, is responsible for antibody production.

Using a fluorescent antibody technique, Ortega and Mellors (1957) conducted a study of the cellular sites of formation of gamma-globulin in lymphatic tissue of man. Their findings indicate that gamma-globulin is formed in the germinal centers of lymphatic nodules and in the cytoplasm of mature and immature plasma cells of two types -- those with and without Russell bodies, but both originating from a common stem cell, the plasmoblast. Also, their results suggested that maturation does not precede the formation of gamma-globulin in these cells but, rather accompanies it.

The Normal Oral Mucosa

As described by Orban and Sicher (1962), the morphologic structure of the mucous membrane and the mode of attachment vary in different areas of the oral cavity in accordance with the functions of specific zones and mechanical influences which bear upon them. Basically, the oral mucous membrane consists of a lamina propria of dense connective tissue and a covering of stratified squamous epithelium. The mucosa is bound to the deep structures (bone, muscles, etc.) by the submucosa which may be present or

absent as a separate and well defined layer.

Using this relationship of structure to function as a basis for classification, the oral mucosa may be divided primarily into three different types, the masticatory mucosa, lining mucosa, and specialized mucosa. The masticatory mucosa, consisting of the gingiva and covering of the hard palate, is subjected to strong forces of pressure and friction which is reflected in its structural arrangement. The epithelium is thick, cornified and contains a granular layer. The bulk of the epithelium consists of prickle cells which are connected to each other by intercellular bridges. The basal cell layer consists of regular, cuboidal cells which send fine cytoplasmic processes into the fibrous basement membrane of the lamina propria. The lamina propria is a dense connective tissue containing bundles of collagenous fibers, blood vessels, nerves, and lymphatics. The principal cell type is the fibroblast located in association with collagenous fiber production. Undifferentiated mesenchymal cells and macrophages are found adjacent to capillaries. The peri-vascular areas or spaces contain a stroma of delicate reticular fibers which are in close association with the capillary walls and the adjacent undifferentiated mesenchymal cells.

Clinically, the normal masticatory mucosa is pink in color and

firm in its consistency. The gingival portion presents a thin marginal contour and a pointed papillary contour. The attached gingiva is separated from the alveolar mucosa by a festooned line, the mucogingival junction. The alveolar mucosa which is non-keratinized, thin, and loosely attached to its base by collagenous and elastic fibers, is red in color.

Fibroblasts of the connective tissue are associated with the formation of collagenous fibers and mucopolysaccharides of the ground substance. Burstone (1962) describes the latter as hexosamine - containing polysaccharides. He states that ground substance also has proteins which contain carbohydrates, and these are referred to as glycoproteins. Mucopolysaccharides, the acid type of which is widely distributed in nature, are of primary significance in connective tissue and presumably act as binding and protective agents. The acid mucopolysaccharides of the ground substance are polymers containing acetylated amino sugars and hexuronic acids. Among the best known mucopolysaccharides are hyaluronic acid and chondroitin sulfuric acid.

CHAPTER III

MATERIALS AND METHODS

Plasma cell infiltration is characteristic of inflammatory hyperplasia of the oral mucosa as seen in gingivitis and vestibular and palatal inflammation beneath dentures. To determine the source of plasma cells of inflamed oral mucosa, several hundreds of oral mucosa biopsies and surgical specimens diagnosed as chronic inflammatory hyperplasia with the plasma cell as the principal inflammatory cell were studied.

Special staining was used on fresh frozen, carnoy, alcohol and formalin fixed material. The following staining procedures were used:

Hematoxcylin & Eosin	Routine Stain
Methyl-Green-pyronine	Ribose Nucleic Acid
Periodic acid Schiff (PAS)	Mucopolysaccharides
Toluidine blue	Acid mucopolysaccharide
Silver impregnation	Reticular fibers
Fluorescene isothiocyanate bound antihuman gamma-globulin rabbit serum	Gamma-globulin

CHAPTER IV

FINDINGS

Clinically, pre-surgical examination of some of the gingival and palatal areas of inflammation showed the typical characteristics associated with inflammation. These are listed as follows:

1. Change in color from the pinkish-white of normal gingiva to varying degrees of red.
2. Lack of stippling.
3. Expansion or swelling in the marginal and papillary regions.
4. Decrease in the firm consistency of the tissue.
5. Deepening of the gingival sulcus.

Stain	Results	Significance
Hemotoxcylin-Eosin	+	Presence and distribution of plasma cells
Methyl-Green pyronine	+	Ribose nucleic acid present
Periodic acid Schiff	-	No mucopolysaccharide
Toluidine blue	-	No acid mucopolysaccharide
Silver impregnation	+	Presence and relation of immature fibers to plasma cells
Fluorescent antibody	+	Gamma-globulin present

Histologically, the most salient feature is the presence of plasma cells grouped around capillaries and in close association with undifferentiated mesenchymal cells generally located adjacent to the capillary wall as seen in Figure II. These plasma cells vary in number from a few to many but, as the numbers of cells increase, they seem to blend with adjacent peri-vascular foci creating the diffuse type of infiltration characteristic of chronic inflammation of the oral mucosa (Figure IX).

Patent capillaries are present in all sections of the inflamed mucosa. Around these vascular components, delicate reticular fibers form a stroma and are in intimate contact with the capillary walls and undifferentiated mesenchymal cells adjacent to the capillary walls (Figure V). These fibers extend out into the peri-vascular areas and are in close association with the plasma cells. The peri-vascular areas occupied by plasma cells show a decrease in the mature collagenous fiber elements present. Furthermore, staining with PAS (Figure XI) and Toluidine blue show both a lack of mucopolysaccharides in the cytoplasm of plasma cells and a decrease in the amount of mucopolysaccharides in the ground substance. Also, staining with Methyl-green pyronine shows the presence of ribose nucleic acid in the cytoplasm of plasma cells (Figure VI).

Undifferentiated mesenchymal cells are seen adjacent to the capillary walls (Figure II, R). These cells are large cells with pale-staining oval nuclei and indistinctly defined cytoplasmic borders. Such cells are the reserve cells of the connective tissue. Peripheral to these reserve cells are immature and mature forms of plasma cells. Notabley absent from this concentration of cells is the lymphocyte. Two cell types appear to arise from the reserve cells; the reticulum cell which produces the reticular fibers and the plasma cells.

Cytogenesis of the plasma cell

Plasma cells are seen to arise from the syncytium of reserve cells. The first change in the reserve cell occurs in the nucleus which shows first a distribution of nuclear chromatin at the nuclear membrane (Figure II, R). This is associated with a reduction in the size of the nucleus which remains oval in shape. The cytoplasm still is pale staining and shows poor definition of the cell wall. The nucleus, at this time, is immediately surrounded by small quantities of ribose nucleic acid as seen by the methyl green pyronine stain in Figure VII, B. (This transitional phase may be compared to the plasmoblast as described by Fagraeus (1948).

In the second phase, the developing plasma cell shows an increase in the basophilic staining of the cytoplasm cell as seen in

hemotoxcylin and eosin stain with a beginning cartwheel distribution of the nuclear chromatin within the nucleus (Figure IV, B). The nucleus is smaller than that of the plasmoblast and appears more rounded. Evidence of a halo, beginning in the cell center, may be seen. The cytoplasm immediately adjacent to the nuclear membrane shows an increase in ribose nucleic acid synthesis as seen in methyl green pyronine stain (Figure VII, C). This phase represents the proplasmocyte stage of development.

Finally, the basophilic material of the cytoplasm concentrates around the periphery of the cell wall as the halo of the cell center enlarges and the nucleus moves to an eccentric position close to the cell wall (Figures II and IV). The nuclear chrometin assumes a more distinct and cartwheel distribution both in quantity of chromatin aggregates and intensity of staining qualities. The formation of RNA in the cytoplasm appears more intense than that observed in the proplasmocyte stage (Figure VII, D). This phase of development is the mature plasmocyte.

Mitotic figures are rarely seen in spite of finding large numbers of plasma cells present though mitotic activity is evident within the syncytium of reserve cells. Occasionaly, binucleate plasma cells are seen with the nuclei at opposite poles and common halo intervening (Figure VIII, B). Such evidence suggests the

possibility of atypical mitotic division.

In methyl green pyronine stain, ribose nucleic acid may be seen completely surrounding the nucleus though it is evident that there is a greater accumulation at one pole of the nucleus (Figure VII). At this pole of the nucleus, a clear non-staining slit develops between the nucleus and the ribose nucleic acid. The ribose nucleic acid fills the entire cytoplasm at this pole of the nucleus. The slit enlarges into a halo while the nucleus assumes the eccentric position of the mature plasma cell.

Though the results using a special stain for fluorescent antibody were incomplete because of laboratory techniques, the evidence suggests the presence of gamma-globulin production in plasma cells.

CHAPTER V

DISCUSSION

In inflammatory hyperplasia of the oral mucosa, the results seem to indicate that plasma cells arise from undifferentiated reserve cells in connective tissue around capillaries and not from lymphocytes. Though lymphocytes are present in some of the specimens, these cells seem to be in most instances in separate colonies of their own. Moreover, no direct change from lymphocyte to plasma cell is evident in any of the specimens studied. On the other hand, the evidence is such as to indicate that plasma cells arise from undifferentiated reserve cells. This assumption is based on the geographical relationship of the plasma cells to the reserve cells and the morphological and intra-cellular changes that occur as plasma cells develop. Specifically, these changes involve a decrease in nuclear size and an increase in nuclear chromatin formation and peripheral (cartwheel) aggregation. At the same time, there is an increase in both the quantity of cytoplasmic formation and basophilic staining properties. Also, the nucleus moves from a more central position to an eccentric location with the development of a halo-like area between the nucleus and the bulk of the cytoplasm.

The changes in the reserve cell begin with the aggregation of nuclear chromatin at the nuclear membrane. This appears to be an essential step prior to the synthesis of ribose nucleic acid (RNA) in the cytoplasm for, as the amount of nuclear chromatin increases in quantity and staining intensity, the synthesis of ribose nucleic acid in the cytoplasm also increases. Morphologically, the reserve cell assumes characteristics of the plasma cell by continued synthesis of RNA. RNA activity is slight in the cytoplasm of the plasmoblasts (transitional phase) but increases in intensity in the proplasmocyte and plasmocyte phases. Finally, the shift of RNA to one pole and the nucleus to the other pole with the intervening halo lends credence to the concept that the structure of the plasma cell is a function of RNA synthesis. Moreover, considering the inter-relation of RNA synthesis to protein synthesis and, in turn, to gamma-globulin and antibody synthesis, the plasma cell can be considered as the morphologic representation of a specific function of the reserve or undifferentiated cell. That is, when the reserve cell starts to form gamma-globulin, it develops into a specific cell type. As Fagreaus has shown, gamma-globulin production goes hand in hand with plasma cell formation and reaches its peak in the immature and mature stages of the development of plasma cells. Thus, structure and

function of the plasma cell are in fact one with the plasma cell being one of many structural and functional potentialities of the reserve cell.

It is observed that mitotic activity of the reserve cells, though evident, does not appear to account for the number of plasma cells produced. However, what needs to be taken into account is the number of reserve cells available for differentiation to plasma cells. Mitosis at least occurs to replace the reserve cells. The bi-nucleate plasma cells do suggest amitotic division but, this is not certain. However, many plasma cells have small nuclei which further suggests atypical mitotic division.

Plasma cells are always enmeshed by well developed reticular fibers that appear to fix or support plasma cells between capillaries. The relation of plasma cells to capillaries and the reticular fibers suggests the structure of a reticulo-endothelial system. This may explain the resistence to injury and excellent repairative quality of oral mucosa. Large numbers of plasma cells intervening between the oral epithelium and the capillaries may serve as a barrier against agents of an irritating nature by rendering them innocuous and by preventing their passage into the vascular system. Degenerating plasma cells showing large hyaline bodies are RNA and gamma-globulin positive. However, many smaller

spheriod particles of degenerating plasma cells are only slightly ribose nucleic acid positive. The liberation of RNA and gamma-globulin into the ground substance certainly should raise the plasma protein level of inflamed oral mucosa. Moreover, globulins could escape through minute ulcerations in the epithelium into the periodontal pockets or sulci and oral cavity. RNA could be picked up by reserve cells for the production of new plasma cells, reticulum cells, fibroblasts or other cells in repair or regeneration.

Physical changes that occur in the connective tissues can also be interpreted. In the areas of dense plasma cell infiltration, there is a decrease in the mucopolysaccharide content of the ground substance (as demonstrated in the PAS and Toluidine blue staining). This change in the ground substance can be associated with the relatively large volume of space occupied by the plasma cells and the change in the architecture of the collagenous fiber bundles. In association with the dense infiltration of cells and plasma fluid related to inflammation, the collagenous fiber bundles decrease in size and number. This breaking down or unraveling of the collagenous fiber bundles is related to the decrease in the mucopolysaccharide content as it is known that mucopolysaccharides act as a binding agent. Moreover, these physical changes in the ground substance and collagenous fiber architecture are manifested

clinically. These tissue effects are reflected clinically in the gingiva by a relative increase in tissue volume and a decrease in the firm consistency that normally are characteristics of healthy gingiva.

CHAPTER VI

SUMMARY

In order to determine the source of plasma cells of inflamed oral mucosa, several hundred surgical specimens and biopsies diagnosed as chronic inflammatory hyperplasia with the plasma cell as the principal inflammatory cell were studied. Most of the specimens were formalin fixed though several specimens were fixed in carnoy and alcohol solutions. Fresh frozen specimens were used for gamma-globulin determinations though these results were inconclusive. The specimens were imbedded in paraffin and sectioned five to six microns in thickness. Staining methods used included hematoxcylin-eosin, methyl-green pyronine, Periodic acid Schiff, Toluidine blue, silver impregnation, and fluorescent antibody.

Using the classification set forth by Fagraeus as a guide, it was observed that reserve cell differentiation into plasma cells involved basically four phases or stages listed as follows:

1. Reserve cell
2. Plasmoblast or Transitional phase
3. Immature plasma cell
4. Mature plasma cell

Identification and classification were based on observations of nuclear chromatin changes, changes in nuclear size, form and position, relative quantity, and basophilia of cytoplasm, and relative quantity and staining intensity of cytoplasm in RNA synthesis. However, it was noted that the typical plasmoblast forms were few in number. Though mitotic activity was apparent on a reserve cell level, mitotic figures were rare.

Associated with chronic inflammation of the connective tissue involving plasma cell infiltration, physical changes occurred within the ground substance and collagenous fiber elements which, in turn, were reflected in clinical changes in the papillae and attached gingiva.

CHAPTER VII

CONCLUSIONS

1. In inflamed oral mucosa, plasma cells arise directly from undifferentiated reserve cells and not lymphocytes.

2. The morphology of the plasma cell is a structural manifestation of the reserve cell activity synthesizing RNA which in turn synthesize gamma-globulin.

3. The relationship of the plasma cells to reserve cells, reticular fibers and capillaries suggests that the oral mucosa may contain a diffuse form of the reticulo-endothelial system.

ILLUSTRATIONS

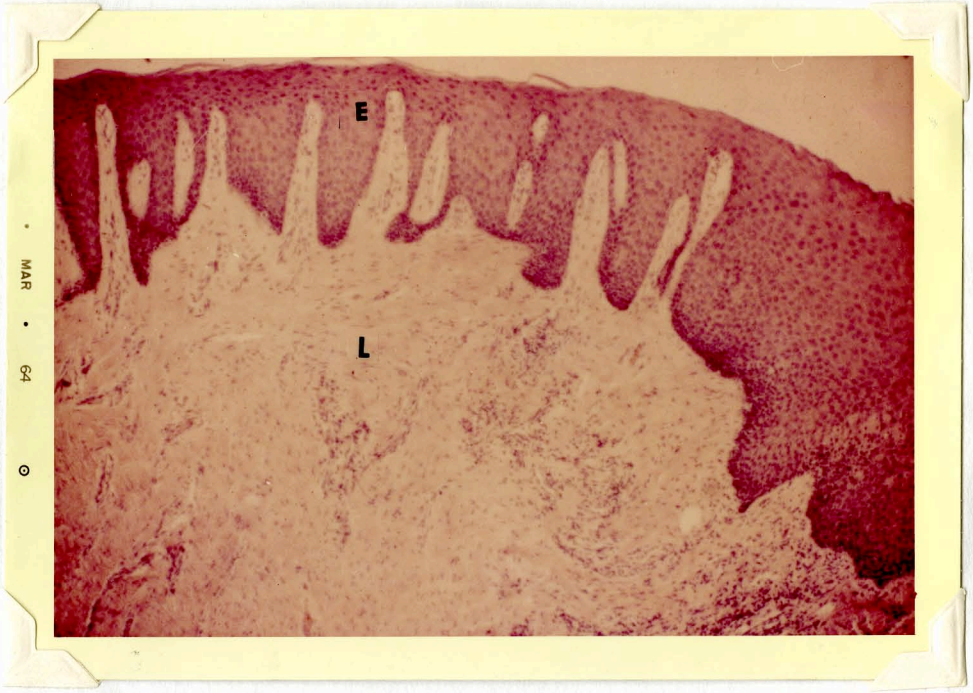

Figure I

Biopsy specimen of normal gingiva. E, epithelium; L, lamina propria.

H & E 100 X

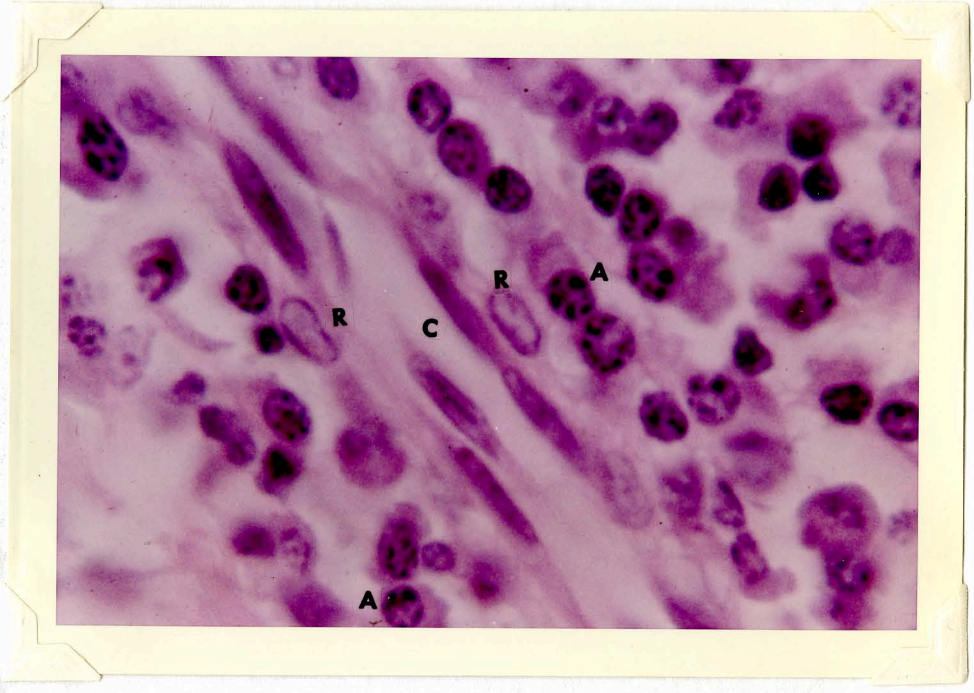

Figure II

Plasma cells grouped around a capillary, C; R, differentiating reserve cells; chromatin aggregates adjacent to nuclear membrane; A, mature plasma cell.

H & E 1000 X

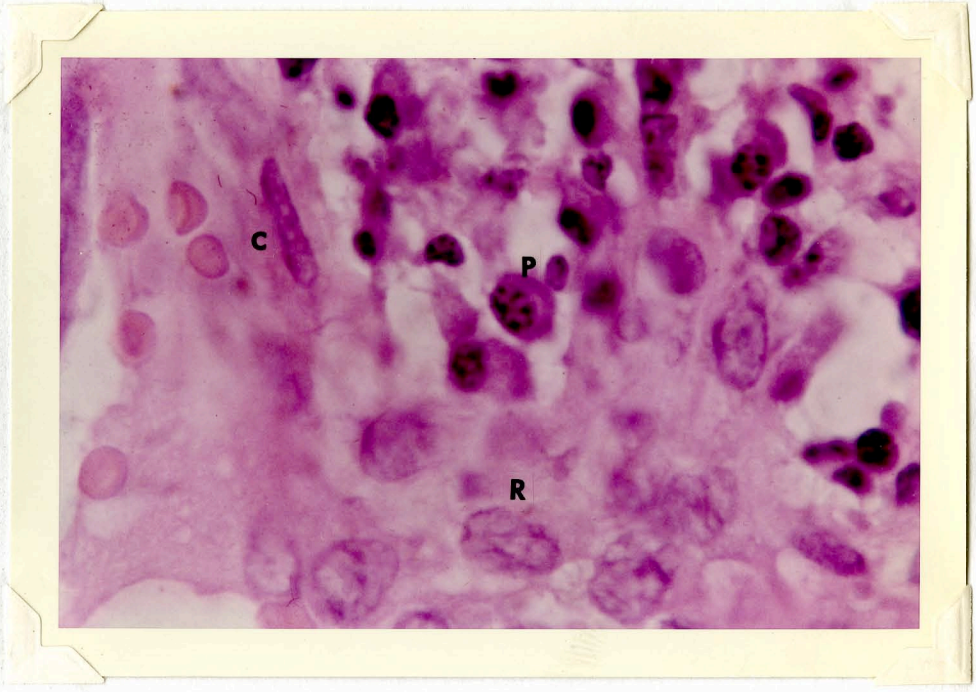

Figure III

C, tangential section through a capillary;

R, reserve cells; P, plasma cells.

H & E 1000 X

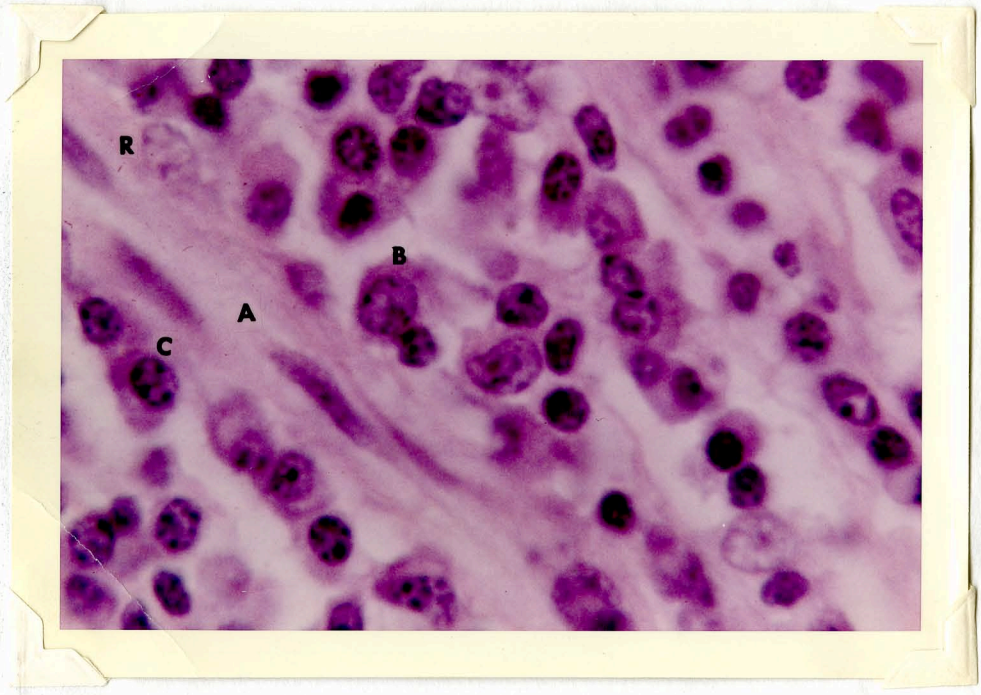

Figure IV

Plasma cells around capillary, A; Reserve cell, R; Immature plasma cell, C.

H & E 1000 X

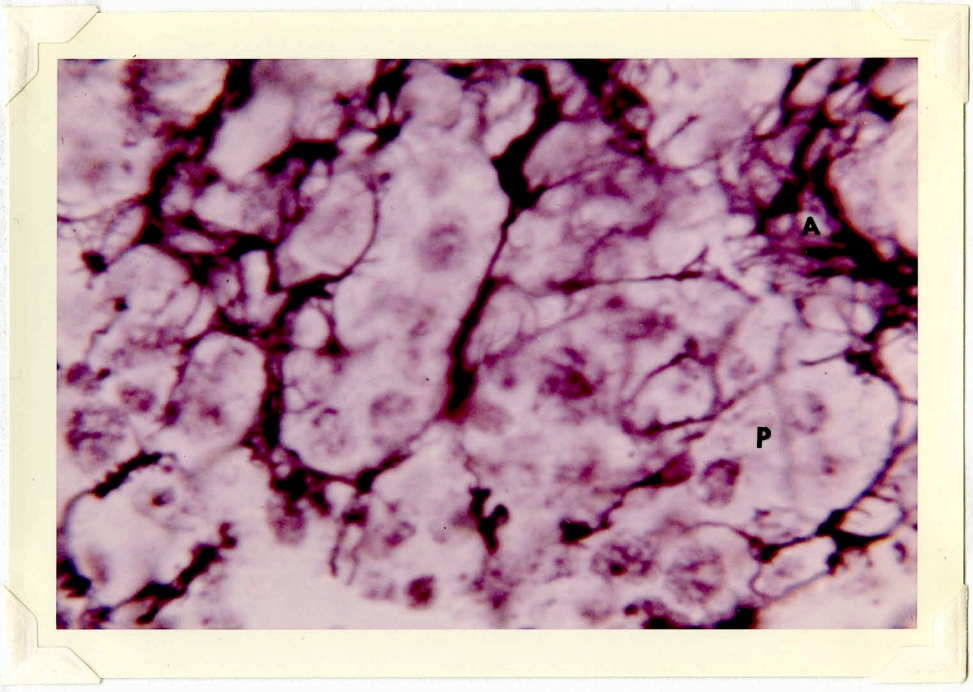

Figure V

Silver stained specimen containing plasma cells capillary wall, A; and plasma cells, P.

1000 X

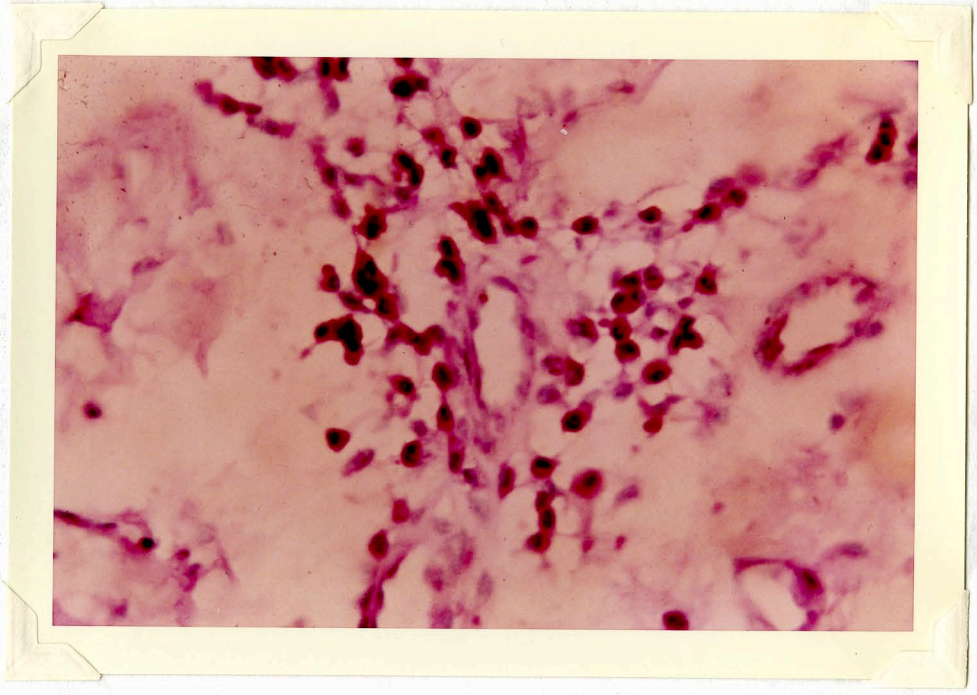

Figure VI

Plasma cells stained for RNA.

(Methyl-green pyronine stain) 450 X

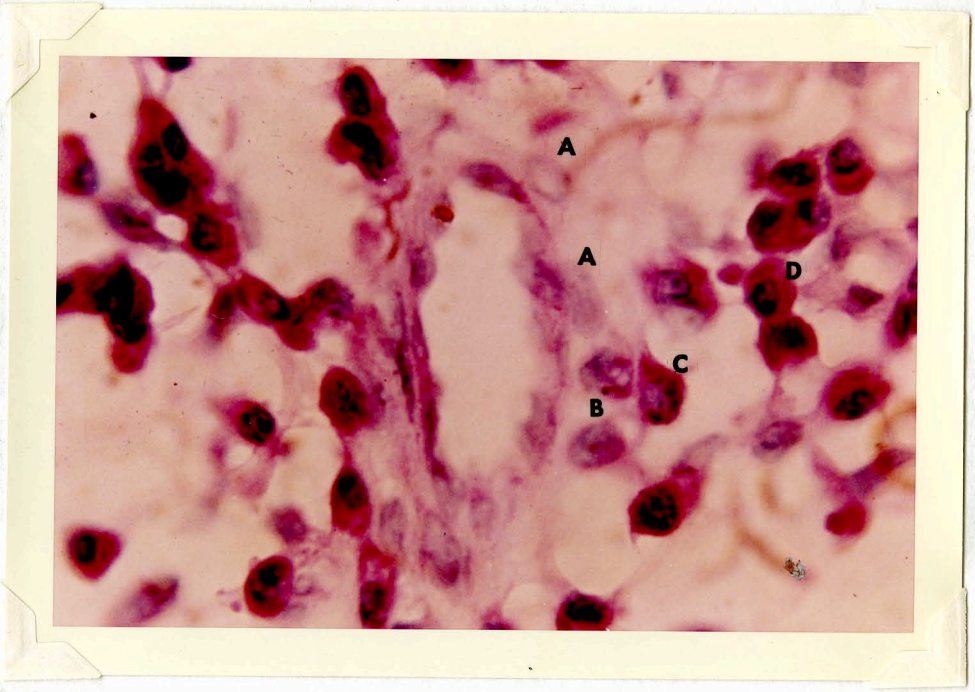

Figure VII

Higher power view of plasma cells as seen in Figure VI. Reserve cells, A; transitional form or plasmoblast, B; immature plasma cell, C; and mature plasma cells, D.

Methyl-green pyronine stain 1000 X

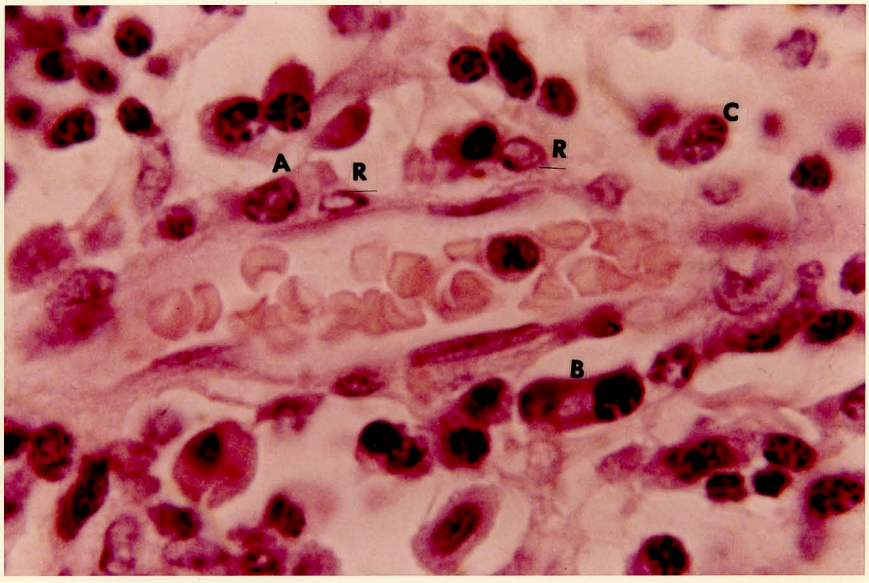

Figure VIII

Peri-vascular arrangement of plasma cells, reserve cells, R; immature plasma cell, A; and mature plasma cell, C, with halo or clear are adjacent to nuclear membrane. Bi-nucleate plasma cell, B, with intervening halo.

H & E 1000 X

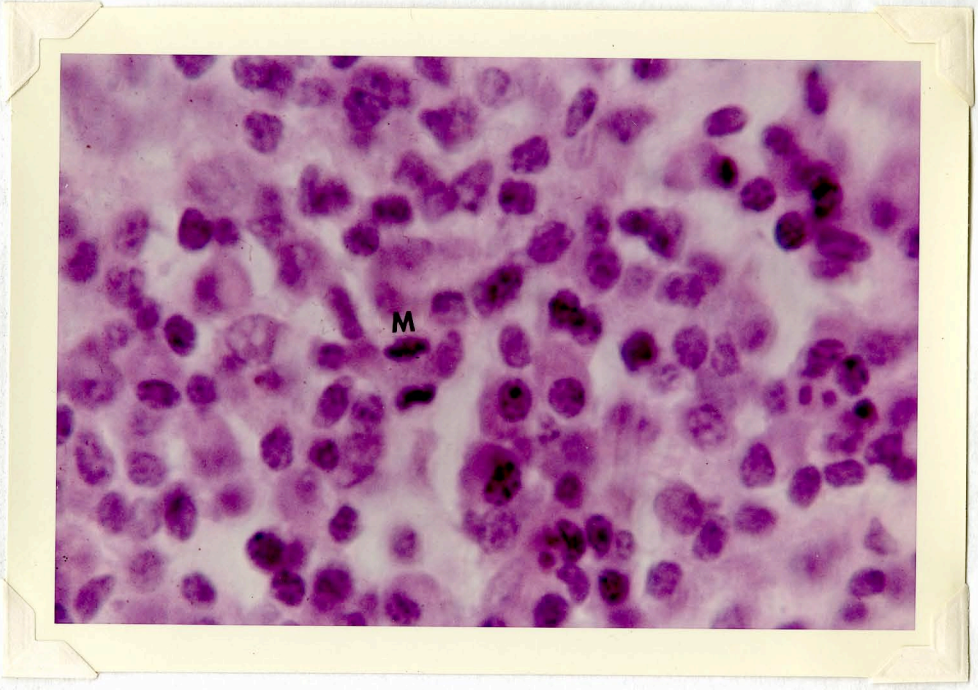

Figure IX

Dense infiltration of plasma cells, mitotic figure, M.

H & E 1000 X

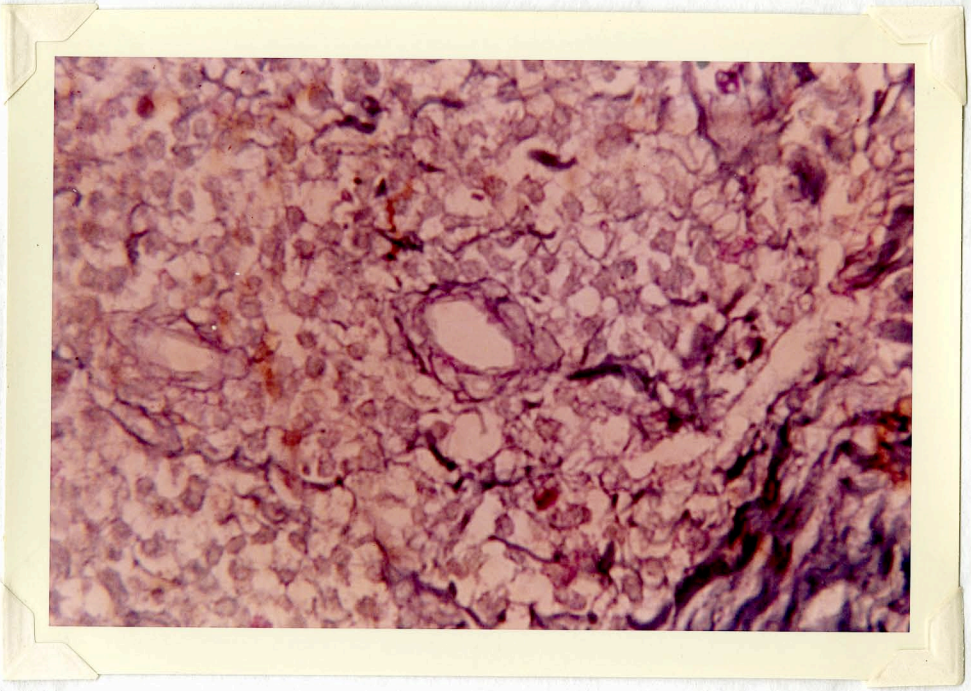

Figure XI

Area of dense plasma cell formation shows decrease in mucopolysaccharide content of ground substance.

(PAS) 1000 X

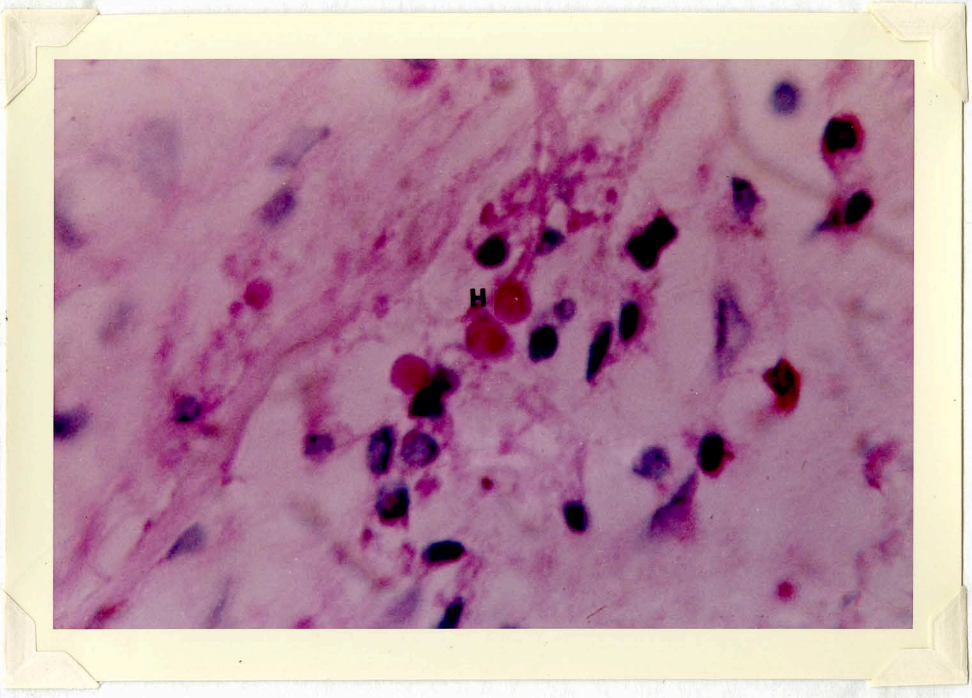

Figure XII

Degenerating plasma cells with Russell's bodies, H.

Methyl-green pyronine stain) 1000 X

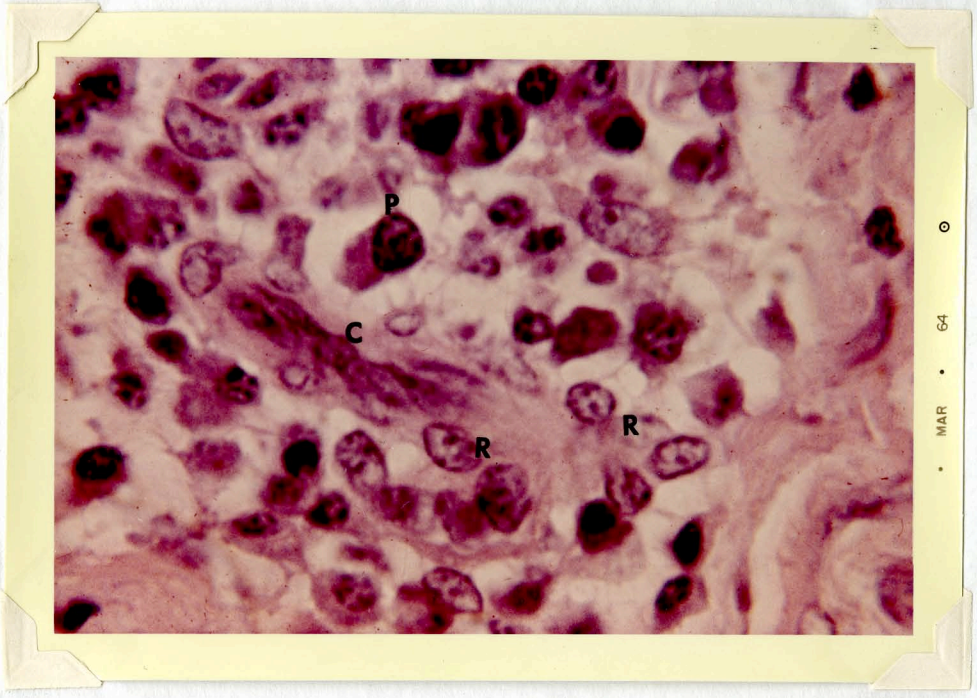

Figure XIII

Reserve cells, R; capillary, C; and plasma cells, P.

H & E 1000 X

BIBLIOGRAPHY

Bessis, M. 1956. Cytology of the Blood and Blood-Forming Organs (Translations with additions, from the French by Eric Ponder) Grune and Stratton. New York, N.Y.

Brachet, J. 1955. The Nucleic Acids. Chemistry and Biology. E. Chargaff and J. N. Davidson, Editions 2: 475-519 Academic Press, New York, N.Y.

Bruton, O. C. 1953. Medical Annual. District of Columbia 22;648 (Biography Abstract 28 #18183).

Cajal, R. Y. Manual of Anatomy and General Pathology, ed. 1 Barcelona, 1890 Cited by Michels (107).

Caspersson, T. O. 1950. Cell Growth and Cell Function. A Cytochemical Study. Norton, New York, N. Y.

Downy, H. Folia haematol., 1. Teil (Archiv.) 1911, 11, 275.

Ehrich, W. E., Drabkin, D. L. and Forman, C. Plasma Cells as Source of Antibodies. J. Exper. Med. 90:157 1949.

Ehrich, W. E. 1953. In the Mechanism of Inflammation G. Jasmin and A. Robert Edilun. 25-36. Acta, Inc., Montreal, Canada.

Fagraeus, A. Antibody Production in Relationship to the Development of Plasma Cells. Stockholm, Essette aktiebology 1948.

Good, R. A. 1955. Journal of Laboratory and Clinical Medicine 46:67.

Harris, T. N. and Harris S. 1949. Journal of Experimental Medicine 90:169.

Kelsall, M. and Crabb, E. 1958-59. Lymphocyte and Plasmocytes in Nucleoprotein Metabolism. Annals of the N. Y. Academy of Science, 72:293-338.

Marshalko, T. Arch. Dermat. u Syph. 1895, 30, 3, 241.

Maximow, A. Beitr. path. Anot. u. allg. Path., 1902 Suppl. 5, 1.

Maximow, A. and Bloom, W. 1949. A Textbook of Histology. W. B. Saunders Co., Philadelphia & London.

Michels, N. A. Plasma Cells. Archives Path. 11, 775, 1931.

Miller, F. R. Histogenesis of Plasma Cells. J. Exper. Med. 54:333 1931.

Newman, L. 1955. Acta Med. Scandinovia. 152:115.

Orban, B. Orban's Oral Histology and Embryology (Edited by Orban, B. and Wentz, F. and Sicher, H.) C. V. Mosby Co., St. Louis, Mo. 1962.

Orban, B. and Wentz F. 1960. Atlas of Clinical Pathology of the Oral Mucous Membrane. C. V. Mosby Co., St. Louis, Mo.

Ortega, L. G. and Mellors, R. C. Cellular Sites of Formation of Gamma-Globulin. Journal of Exper. Medicine. 106:672, 1957.

Sabin, F. R., Doan, C. A. and Foikner, C. E. J. Exp. Med. 1930, 52, Suppl. 3, 1.

Waldeyer, W. Ueber Bindegewebezellen. Arch. f. mikroskop Anat. II 176(1875) cited by Fagraeus.

Zbar, M. J. Cippers, K. E. and Alterman, S. I. 1956. American Journal of Clinical Pathology 26:1180.

APPROVAL SHEET

The thesis submitted by Kenneth E. Nowlan has been read and approved by three members of the faculty of the Graduate School.

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

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

May 22 1964
Date

Signature of Adviser