1969

Immune Reactions in the Gingiva of the Pregnant and Non-Pregnant Human Female

Ronald C. Hartzer
Loyola University Chicago

IMMUNE REACTIONS IN THE GINGIVA OF THE PREGNANT AND NON-PREGNANT HUMAN FEMALE

by

RONALD C. HARTZER

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

May

1969

I dedicate this thesis to my wife, Ginny, whose infinite patience, constant encouragement, and sincere understanding have made this work possible.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Patrick D. Toto for his guidance and constant advice in preparing this thesis.

The author further wishes to express his gratitude to Dr. Anthony Gargiulo for his continuing interest in this work and also for the opportunity to have studied under him in the field of Periodontics for the past two years.

To Dr. John W. Wittwer, an unselfish educator and friend, the author gives his thanks, for his countless helpful suggestions in all areas of this thesis.

Finally, the author wishes to thank the laboratory technicians for their assistance in preparing the tissue sections.

ACADEMIC LIFE

Ronald C. Hartzer, second of two children, was born in Chicago, Illinois, October 19, 1941. He was educated in the Chicago parochial school system, attending St. Pascal Elementary School, and St. Patrick High School, where he was graduated in 1959.

In September, 1959, he enrolled in the pre-dental curriculum of John Carroll University, Cleveland, Ohio. Transferring to Loyola University of Chicago in June, 1961, he continued his pre-dental studies. He received a Bachelor of Science degree in biology from Loyola in June, 1963.

He entered Loyola University School of Dentistry, Chicago, Illinois, in September, 1963. In June, 1967, he was graduated with the degree of Doctor of Dental Surgery.

In September, 1967, he entered the graduate school of Loyola University of Chicago to obtain a Master of Science degree in the field of Oral Biology, while at the same time doing postgraduate study in the dental specialty of Periodontics.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

The question as to why the maternal organism tolerates the placenta and fetus in her body during pregnancy has defied solution for many years. Since the fertilized ovum from which the placenta is derived is made up of both maternal and paternal germ cells, the placenta and fetus have paternal components, and as such must be considered homografts in the uterine tissue according to Medewar (1953). Thus we could expect a homograft rejection reaction which would result in the destruction of the fetus and placenta. Of course, this does not happen in a normal pregnancy.

One of the possible explanations for the survival of this homograft could lie in the immune response of the mother during her term of pregnancy, especially in the first trimester. If the maternal immune mechanism is altered in some way to protect the fetus from rejection, the host resistance of the mother to certain diseases could also be altered.

Specific immunity is a consequence of the reaction of the host to an antigen, and provides the basis for prophylaxis against a disease or infection. Its development during the course of the disease may be one of the primary determining factors in the outcome of the infection.

In recent years, Schultz-Haudt and Stig (1956), MacDonald and Gibbons (1962), and Schneider, Toto, Gargiulo, and Pollock (1966), it

has become apparent that microorganisms play a role in inflammatory periodontal disease, either in initiating the disease process, or, at the very least, contributing to the progression of the disease. According to Toto (1961) it is supposedly the immunologically competent cells, and in particular the plasma cells, that fight the inflammatory process in inflamed gingiva. The antibodies produced by these cells react with the bacterial antigen to produce an antigen-antibody reaction, in an attempt to destroy the toxic products produced by the vast numbers of bacteria in the gingival sulcus.

The inflammation present in the gingiva of a person with inflammatory periodontal disease can be traced directly to the presence of bacterial plaques or other local irritants about the teeth. However, the gingiva of the pregnant human female sometimes shows pathology that is not directly related to the amount of local irritants present about the teeth. There apparently are other mechanisms involved, one of which may be an immune mechanism, to account for the gingival inflammation that is seen during pregnancy.

It is the purpose of this study to determine if certain specific immune reactions in the gingiva of the pregnant human female are altered during the course of chronic gingival inflammation.

CHAPTER II

REVIEW OF LITERATURE

Immunologic Aspects of Pregnancy

It was not until 1953 that the immune mechanisms during pregnancy were looked upon with any great interest. It was Medewar's (1953) thoughts on the placenta acting as a homograft which stimulated interest in this subject.

The fact that the placenta does not maintain absolute integrity of the fetus and maternal circulations is documented by many studies of the passage of cells in both directions between mother and fetus. Zarrow, Lichtman, and Hellman (1964) have labeled maternal erythrocytes with radioactive chromium^{51} and found them in the fetal circulation in some apparently normal pregnancies. Desai and Creger (1963) have labeled maternal leukocytes and platelets with atabrine and found that they also crossed the placenta. Zipursky, Pollack, Chown, and Israels (1963) have shown that there may be 0.1 to 3.0 ml of fetal blood in the maternal circulation normally, and much more in cases of fetal anemia. Fetal cells other than those found in blood have also been identified in the maternal circulation. Douglas, Thomas, Carr, Cullen, and Morris (1959) have found cells morphologically identified with trophoblast in the uterine venous blood.

Eastman and Hellman (1961) have mentioned several hypotheses that attempt to explain the survival of the fetus as a homograft. These

hypotheses are: 1) the fetus is immunologically immature,; 2) the uterus provides an unusually favorable location for homograft survival; 3) there is a physiologic barrier between mother and fetus; 4) pregnancy alters the immunologic capability of the mother.

Billingham (1964) has reviewed the evidence for the above hypotheses and has come to the following conclusions: Hypothesis 1 - He has stated that the fetus is not immunologically immature, and this is supported by evidence from Billingham and Silvers (1963) that transplantation antigens are present very early in birds and mammals. Hypothesis 2 - According to Billingham, the uterus is not a privileged site, although there are certain sites in the body where tissue grafts can survive for long periods, for example, the brain and the anterior chamber of the eye. These sites have one feature in common that accounts for the absence of immunologic response, and that is the lack of lymphatic drainage from the grafts. However, the uterus has lymphatics available and also, there is a good blood supply. Another reason for Billingham discarding this hypothesis is the success of ectopic pregnancies. When fetal death does occur in these instances, it is due to lack of adequate blood supply and not homograft rejection. Hypothesis 3 - Billingham has given most support to this hypothesis because of the animal experiments of Lanman, Dinerstein, and Firkig (1962) which demonstrated that all the necessary factors were present for homograft rejection, but it did not occur. Billingham has postulated that this is because of the immunologic pecularities of the

trophoblast. Supporters of this concept feel that the only hypothesis capable of accounting for these findings is that the mother and fetus are completely separated by an anatomic barrier which prohibits sensitization of the mother to fetal transplantation antigens. Pirofsky (1966) has identified the trophoblast as this anatomic barrier. The trophoblast is interposed between the fetal and maternal circulations, but it has also been thought to be non-antigenic, based on the findings of Simmons and Russell (1962). In a later work, Simmons (1967) has stated that the trophoblast does not owe its privileged immunologic behavior to an extracellular barrier, but is probably due to a relative intrinsic deficit of transplantation antigens on the cell surface. Hypothesis 4 - This concept will be discussed in greater detail even though Billingham has discarded it.

Based on the literature none of the hypotheses has provided a clear cut answer, and several factors may be important contributors to maternal tolerance of the fetal and placental homografts.

In order to discuss hypothesis 4, that there is an altered immune state in the mother, we must first know what factors produce immunologic changes in man.

The thymolymphatic system, that is, the thymus, spleen, lymph nodes, and lymphoid tissue that is found in the gastro-intestinal tract, is known to produce lymphocytes which are capable of responding to foreign antigens with the production of antibodies. DePetris, Karlsbad, and Perni (1963) have shown that plasma cells have this same function, but

their origin is disputed. Nelson and Hall (1965) have said that the small lymphocyte is no longer considered an end-stage cell, but may develop into a medium or large lymphocyte or a plasma cell. However, Toto (1961) has shown that plasma cells arise directly from undifferentiated mesenchymal cells or connective tissue reserve cells in inflamed oral mucosa. He has shown that when the reserve cells begin to make RNA and then gamma globulin, the cell changes in structure into a plasma cell.

There are certain factors that influence or affect the function of the thymolymphatic system. Changes in the endocrine system, radiation, and certain drugs such as azaserine and 6-mercaptopurine are known to affect this system. The endocrine system is the only factor in pregnancy which may cause regression of the thymolymphatic structures and a possible alteration in immune function.

Nelson (1967) has shown that estrogen depressed thymic function in small mammals when administered over a 21 day gestation period at doses calculated to simulate hormonal levels seen during human pregnancy. In the same study a similar result was obtained for progesterone, while the administration of both hormones depressed thymic function more so than each one separately, and the greatest decrease in thymic function was obtained upon the administration of estrogen, progesterone, and HCG, with two animals having no detectable thymus tissue remaining.

It is also firmly established that adrenal cortical steroid levels are increased in pregnancy. These hormones have been shown to produce acute atrophy of all lymphoid tissue when administered intramuscularly

into rats, as documented by Dougherty, Berliner, Schnelbeli, and Berliner (1964) and by Angervall and Lundin (1965). A study by Doe, Zinneman, Flink, and Ulstrom (1960) has further shown that the total plasma 17-hydroxycorticosteroid level is elevated in pregnancy, and that the amount of nonprotein bound 17-hydroxycorticosteroids in pregnancy is 7.5 times that present in nonpregnant human plasma.

Goodlin and Herzenberg (1966) have demonstrated a defect in antibody production in pregnant mice by injecting fetal erythrocyte antigens into pregnant and nonpregnant mice. Thirty-four percent of the nonpregnant animals had positive antibody titers while only eight percent of the pregnant animals had positive titers.

Conclusive studies are lacking in humans with regard to the effects of pregnancy on lymphoid tissue. Nelson and Hall (1964) have shown an absence of germinal centers in pelvic lymph nodes removed from women undergoing elective caesarian section, while women undergoing hysterectomies in the same age group showed an average of eight germinal centers. This finding indicated marked depression of small lymphocytes. In a later study Nelson and Hall (1965) have shown that there was almost complete absence of germinal centers in lymph nodes during the first four weeks post-partum, while at 28-40 days post-partum most germinal centers were still absent, but evidence of regeneration could be seen.

The foregoing studies have shown that there is a possibility of depressed immune function during pregnancy. The possibility of immune reactions occurring in the inflamed gingiva and its relationship to

gingival disease in pregnancy will now be discussed.

Bacteria As Etiologic Agents of Periodontal Disease

In recent years it has become evident that one of the etiologic factors, if not the main factor, in inflammatory periodontal disease may be bacterial action, resulting in tissue destruction. This may occur through the release of bacterial enzymes or toxic products in the gingiva, resulting in an immune reaction.

Among the researchers supporting this supposition are Rosebury (1947), Waerhaug (1952), Ramfjord (1952), Bibby (1953), Schultz-Haudt (1956), MacDonald (1960, 1962), Wentz (1960), Arnim (1964), Schneider (1966), and Toto (1968). The hypothesis implicating bacteria as etiologic agents does not apply to every situation however. Baer, Newton, and White (1964) have demonstrated the development of periodontal pockets in germ-free mice, even if the process was very slow. These mice were of a strain previously observed to be susceptible to periodontal disease. MacDonald (1960) was not entirely satisfied though, that the periodontal destruction seen in the germ-free mice of Baer's study was comparable with that seen in man. However, the studies of Cohn (1960) have shown a pattern of periodontal destruction in rats similar to that seen in Baer's mice, and at the same time revealed many similarities to the histopathology of pocket formation seen in humans.

The existence of bacterial enzymes capable of destroying tissues has been reported by several investigators. Schultz-Haudt, Bibby, and Bruce (1964) have found that collagenase from Clostridium welchii in

gingival plaque was released after two to three hours. They were also able to show hyaluronidase activity from filtrates of seven individuals with chronic generalized marginal gingivitis, while activity against condroitin sulfate was shown in only one of five filtrates, and against histamine in none of the filtrates. Dewar (1958) has collected gingival debris from 38 individuals with varying degrees of periodontal disease, and found that hyaluronidase, condroitin sulfate hydrolase, and collagenase were always present in unfiltered material. However, in filtered material the enzymes were detected only infrequently. Dewar (1958) was also able to show that pure cultures of spirochetes and fusiforms had only slight enzyme activity. Since Dewar's work showed little enzyme activity with pure cultures, Mergenhagen and Scherp (1960) have attempted to confirm this, and also have attempted to give a better understanding of the activity of mixed cultures. They showed that reconstituted collagen is moderately susceptible to digestion by mixed cultures started with gingival plaque. Exposure to these cultures for seven days affected from 20 percent to 30 percent reduction in hydroxyproline content of the collagen samples tested. Longer exposures increased the digestion. They suggested that the slow and incomplete activity of these cultures on collagen is consistent with the chronic nature of inflammatory periodontal disease. They also isolated a number of pure cultures from gingival plaque and tested their action on reconstituted collagen. There were seven strains of veillonella, six strains of fusiform bacilli, four strains of streptococci, and two strains of diphtheroid bacilli. Singly

or in various combinations of strains, these organisms failed to give any indication of collagenolysis after incubation for seven days.

The results of these investigators support the notion that more than one species of bacteria is probably responsible for the degradation of the collagen substrate seen in periodontal disease.

Although pure cultures taken from human dental plaque failed to lyse collagen, pure cultures of certain oral microorganisms have been shown to produce experimental periodontal disease in animals.

Howell and Jordan (1967) have isolated a filamentous organism, Odontomyces viscosus from the plaque of hamsters, and showed that this organism could produce periodontal disease in the hamster by the production of plaque material. They also suggested that the production of an extracellular levan by this organism could be related to its ability to form the plaque.

Jordan, Fitzgerald, and Stanley (1965) have been able to use aerobic actinomyces isolated from hamsters and rats with periodontal disease to monoinfect germ-free rats maintained on high sugar diets. Plaque formation occurred mainly around the molar teeth. Periodontal pathology including alveolar bone loss could be seen in histologic sections.

How the bacteria reach the internal tissues, or even if they reach the tissues at all, is open to speculation. Bibby (1953), and Wentz (1960) have analyzed the possibility of actual invasion into the tissues and separately concluded that the bacteria exert their pathologic effects by the diffusion of tissue-destructive bacterial products into the periodontium. If bacterial products penetrate the gingival tissues they would

act as foreign antigens and the body would be expected to reject them. The large numbers of plasma cells seen in inflamed gingiva along with their known function of antibody production as documented by DePetris, et al (1963) and Toto (1961) has led several investigators to the possibility that there may be antigen-antibody reactions taking place in the gingiva of a person with inflammatory periodontal disease. These investigators are Toto (1961), Schneider, Toto, Gargiulo, and Pollock (1966), and Toto, Wittwer, and Dickler (1968).

Schneider and colleagues (1966) have shown the presence of specific bacterial antibodies in inflamed human gingiva by the use of a fluorescent antibody technique.

Toto and co-workers (1968) have shown the presence of Streptococcus mitis antigens in the inflamed human gingiva by the same fluorescent microscopic technique.

Gingivitis in Pregnancy

The fact that pregnancy can have profound effects on the gingival tissue has been known for a long time. Quoting from Ziskin and Nesse (1946):

> Oakley Coles, in 1874, described fairly accurately the condition which has subsequently been called pregnancy gingivitis. In 1877, Drs. A. and D. Pinard stated that the gingivitis was induced by pregnancy although the proximate cause was not known. At the close of the nineteenth century this disturbance was fairly well accepted, since the condition of pregnancy gingivitis and palliative treatment is included in the fifth edition of A System of Oral Surgery by Garretson.

Although the altered gingival condition during pregnancy has been known for a long time, little progress has been made in defining the etiology. Monash (1926) has stated that the condition was entirely inflammatory in nature and merely represented an exaggerated degree of generalized gingivitis. As for etiology, little was said except that there was some gingivitis before pregnancy which was intensified during pregnancy.

The earliest work that attempted to correlate endocrine function with gingival inflammation during pregnancy was by Ziskin, Blackberg, and Stout (1933). They made clinical and histologic observations and found some form of gingivitis in 70 percent of the pregnant patients examined, whereas control studies in nonpregnant women showed 15 to 18 percent incidence. They concluded that the significant change in pregnancy gingivitis is a hyperplasia of the epithelium and a loss of surface keratin, with the probability that hormones are the causative agents.

In a later study, Ziskin and Nesse (1946) found that the most prominent changes in the gingiva during pregnancy were a loss of surface keratin, hydropic degeneration of the prickle cell layer of the epithelium, hyperplasia of the germinative layer, and inflammatory changes in the lamina propria. They listed as the most prominent cause of the gingival changes to be a lack of utilization of estrogen or modification of endogenous estrogen. The explanation of the mechanisms involved as given by Ziskin and Nesse (1946) is,

> (1) The increase in serum and urinary estrogen does not have a proportional estrogen effect on the gingiva since

part of the serum estrogen is bound to the protein fraction of the blood, while the urinary estrogen is excreted as the conjugated glucuronide which is a detoxification product; (2) There may be reduced ability of the oral tissues to utilize the available estrogen because of the increased amount of progesterone which has a sparing action on estrogen, modifying its utilization; (3) The large amount of chorionic gonadotropin found during pregnancy in some way affects the gingiva, although the mechanism is unknown; (4) The increased activity of the thyroid gland and the adrenal cortex may also tend to decrease or modify the effect of the available estrogen to the gingiva; and (5) Nutritional factors may alter the amount of endogenous estrogen available for the gingiva.

Maier and Orban (1949), in an extensive study, concluded that the histopathologic features of pregnancy gingivitis consist of a proliferative inflammation characterized by numerous mitotic figures in the epithelium, endothelium, and connective tissue. Another finding of possible significance was an accumulation of lymph cells repeatedly in the biopsy specimens. The lymph cells often formed fairly well-circumscribed nodes in which mitoses were sometimes seen, and also some large cells which could have been lymphoblasts were noted by them. They considered pregnancy a conditioning factor which influences the character of the inflammatory reaction but did not consider pregnancy to be the primary etiologic factor in pregnancy gingivitis.

A more recent study involving the attached gingiva in pregnancy by Turesky, Fisher, and Glickman (1958) has shown a relative increase in epithelial glycogen, reduction in glycogen and carbohydrate-protein complexes of the connective tissue, and a thinning of the basement membrane.

Recently, Löe (1965) has attempted to correlate gingival inflammation and oral hygiene in women during pregnancy and after delivery. He found that the correlation was closer after delivery, while the quantity and character of the oral debris did not differ in the pregnant and postpartum groups. He concluded that during pregnancy some other factor is introduced which, with the bacteria, may be responsible for the accentuated inflammatory changes.

Autoradiography

Autoradiography is a method for detecting radioisotopes, based on their ability to affect the silver bromide crystals of photographic emulsions. These crystals act as detectors of radiation and are useful in localizing labeled substances in the tissues of the body. According to Gross, Bogoroch, Nadler, and Leblond (1951) an autoradiogram is obtained by placing a tissue section containing radioactive material in contact with the emulsion, allowing sufficient time for exposure, and then developing. The resulting autoradiogram consists of black silver granules overlying those areas in the tissue section which contain the radioactive material.

The autoradiographic technique has been used extensively to localize carbohydrates, fats, and proteins, and to monitor uptake of radioactive materials in such as teeth, bone and thyroid, as documented by McDonald, Cobb, and Solomon (1948), Leblond, Stevens, and Bogoroch (1948), Doniach and Pell (1949), Belanger and Leblond (1950), and others. The radioactive material is administered by injection prior to sacrifice or

biopsy in these instances. Reports of *in vitro* uptake of radioactive elements are few in number. Pelc and Spear, as reported by Gross and co-workers (1951), have shown that a cell cultured for 48 hours in a medium containing P^{32} would incorporate the isotope into organic molecules.

Antigen-antibody reactions have also been shown by autoradiography. Warren and Dixon (1948) have used plasma globulin labeled with iodine to produce anaphylaxis in guinea pigs. They found that radioactivity presumably due to the antigen-antibody reaction occurred in the edematous connective tissue of the bronchi. In another study, Pressman, Hill, and Foote (1949) have found that the kidney glomeruli were the sites of specific antigen-antibody combinations. At this time, reports of *in vitro* studies relating to antigen-antibody reactions are not evident.

CHAPTER III

MATERIALS AND METHODS

Gingival biopsies were obtained from 38 adult women who ranged in age from 18 to 40 years. The area of biopsy chosen was the buccal one-half of an interproximal papilla.

Nineteen women were pregnant, with the duration of pregnancies ranging from 8 to 37 weeks. Of these nineteen, ten were selected for gingival biopsy of clinically inflamed tissue and nine selected for gingival biopsy of clinically normal tissue (TABLE 1). The criteria for normal gingiva were: pink color, firm texture, pointed papillary contour, thin marginal contour, no supragingival or subgingival calculus, no bacteria plaque, no exudate upon probing, and a sulcus of one and one-half millimeters or less. The other nineteen women were not pregnant. Of these, ten were selected for gingival biopsy of clinically inflamed tissue and nine selected for gingival biopsy of clinically normal tissue (TABLE 2).

A medical history* was obtained from each woman to rule out any systemic illnesses, and only those women with negative histories were chosen. In the nonpregnant groups, only those women who were of child-bearing age and still menstruating regularly were chosen.

---

*Loyola University School of Dentistry medical history form.

Each area of biopsy was anesthetized with 2% lidocaine* containing epinephrine in a concentration of 1:50,000 injected into the buccal vestibule apical to the biopsy site. Each half papilla was excised with an Orban periodontal knife, rinsed in tap water, frozen immediately on dry ice, and placed in a freezer for future study.

A pure culture of Streptococcus mitis organism** was placed into one liter of trypticase-soy broth and incubated at 37° C. for 48 hours. After incubation, a portion of the bacterial suspension was centrifuged at 3800 RPM for ten minutes. The supernate was poured off, and the bacterial sediment was collected and placed in physiologic saline solution. The bacterial cells were then broken up by the process of sonification.

The frozen and dehydrated tissue sections that had been stored in the freezer were placed in 95 percent ethyl alcohol, embedded in paraffin, and sectioned at four microns on a rotary microtome. Several sections from each of the 38 specimens were stained with hematoxylin and eosin for histologic study. Other sections were kept in paraffin blocks and held for autoradiograms.

In preparation for autoradiography, the fragmented bacterial cells were labeled with the radioisotope, Iodine-131***. The prepared sample

---

*Xylocaine, Astra Pharmaceutical Products, Inc., Worcester, Mass.

**American Type Culture 903, Rockville, Md.

***Radiopharmaceutical Division, Abbott Laboratories, Abbott Park, Illinois.

consisted of 15 ml of radioactive bacterial cell components with activity of 111.1 microcuries/ml as of 12 o'clock noon, central standard time on January 7, 1969. The concentration was 0.1 ml of the original bacterial suspension/ml of prepared sample. Total millicuries in the prepared sample were 1.66, and the specific activity was 1.06 millicuries/ml of the original bacterial suspension. One drop of the prepared sample thus contained 5.5 microcuries of radioactivity. In order to protect against bacterial contamination, 1.5 ml of colorless merthiolate in a concentration of 1:10,000 were added to the prepared radioactive material.

Other tissue sections from each of the 38 specimens were deparaffinized, brought to water, and air dried. One drop of the radioactive bacterial solution was placed on a tissue section from each specimen using a sterile tuberculin syringe. Each microscopic slide containing a tissue section was placed in a sterile petri dish moistened with wet filter paper and incubated at 37° C for two hours. After this time, each section was rinsed with distilled water from an eye dropper and then run under tap water for ten minutes.

The glass slides containing the tissue sections were then dipped in a photographic emulsion* while in a dark room, sealed in light-tight boxes, and placed in a freezer for a period of 27 hours. The sections were washed and stained with indigo carmine and nuclear fast red dyes.

---

*Kodak NTB_3. Kodak Corporation, Rochester, N.Y.

Silver bromide grains were counted in the connective tissue immediately adjacent to the epithelium, and the grains in the background were counted at a considerable distance from the tissue section. The grains were counted under oil immersion at 1000 X using a Whipple micrometer disc with 100 equal squares, the total area of the squares being 0.25 mm.

As a control, other tissue sections from each of the 38 specimens were deparaffinized, brought to water, and air dried. One drop of unlabeled, sonified, S. mitis cells in saline solution, with clear merthiolate added as described above, was placed on each tissue section. The tissue sections were incubated in the same manner as the labeled sections, washed after incubation, and air dried. Immediately afterward, one drop of radioactive bacterial solution was placed on each section, incubated as before, washed, and air dried. The tissue sections were then processed for autoradiography as described above.

The technique for this in vitro autoradiographic study was worked out by trial and error. Two days after receiving the radioactive material the procedure was performed as described above, except that the incubation time was 24 hours and the sections were exposed for ten days. This proved to be unsatisfactory since the labeling was far too heavy to count. The concentration of the solution was then diluted 100 times, incubation time reduced to six hours, and the sections exposed for five days. This again turned out to be unsatisfactory for the same reason. The original solution was then diluted 1000 times, incubation time reduced to two hours, and the sections exposed for three days. This time the grains

were barely visible and could not be counted accurately. The problem had to be either an overdiluted solution or a complete loss of the radioactivity. The half-life of Iodine-131 is 8.1 days, and it was calculated that at this time there was about two percent activity remaining in the original solution. The results were ultimately obtained on February 25, 1969, using the original solution at 1.6 percent activity, incubated for two hours, and exposed for 27 hours.

CHAPTER IV

FINDINGS

The hematoxylin and eosin stained sections from the clinically normal gingiva of nine non-pregnant women and from the clinically "normal gingiva" of nine pregnant women revealed histologic features that were compatible with normality (FIGS. 1 and 2). The oral epithelium had well-defined epithelial ridges and an intact keratin layer on the surface. The stratified squamous epithelium showed three distinct cellular layers, an outer stratum corneum with flattened cells, a prickle layer of cuboidal cells, and a one-cell thick basal layer. The sulcular epithelium demonstrated these same cellular layers, but the epithelial ridges were flattened.

The connective tissue appeared dense with collegenous fibers and fibrocytes. Perivascular undifferentiated mesenchymal cells, plasma cells, lymphocytes, and histiocytes were seen only occasionally.

The hematoxylin and eosin stained sections from the clinically inflamed gingiva of ten non-pregnant women revealed abnormal histologic features in both the epithelium and the connective tissue (FIG. 3). The epithelium was characterized by varying degrees of intracellular edema. In some cases microscopic ulcerations were present in the sulcular epithelium. Disorganization of the epithelial layers was often seen, and neutrophils were found dispersed throughout the epithelium.

The connective tissue was generally edematous. Many neutrophils were present in areas adjacent to the sulcular epithelium and in some areas were seen to migrate through the epithelium onto the surface. There was evidence of perivascular connective tissue proliferation, with plasma cells seen throughout this proliferating tissue. There were few lymphocytes present. Although cell counts were not done, it was generally observed that plasma cells greatly outnumbered the lymphocytes.

The hematoxylin and eosin stained sections from the clinically inflamed gingiva of ten pregnant women showed histologic features similar to those of gingival inflammation in the non-pregnant group, with one exception (FIG. 4). Within the connective tissue, proliferation of endothelial cells and capillary dilatation in some areas were the most marked features distinguishing gingival inflammation during pregnancy from that of non-pregnancy.

Each autoradiogram of both the experimental and control groups showed silver grains randomly dispersed over the epithelium, the connective tissue, and the background.

The experimental autoradiograms from all four groups of women, PI (pregnant with inflamed gingiva), PN (pregnant with normal gingiva), NPI (non-pregnant with inflamed gingiva), and NPN (non-pregnant with normal gingiva) had higher grain counts over the connective tissue than over the background of the slides (FIGS. 5 and 6). The control autoradiograms (unlabeled fragmented S. mitis cells which were added to each tissue section before the labeled fragments) also had higher grain counts

over the connective tissue than over the background (FIGS. 7 and 8).

The mean difference of grain counts over the connective tissue and grain counts over the background in PI was recorded as 302.7 (TABLE 5). This value was found to be significant at the 0.001 level by application of the "t" test for non-paired experiments.

The mean difference in the control autoradiograms in PI was 48.6 (TABLE 5). This value was significant at the 0.01 level.

PN had a mean difference of 208.5 between the connective tissue and the background in the experimental autoradiograms, which was significant at the 0.001 level (TABLE 6). The mean difference in the control was 30.0, which was significant at the 0.01 level (TABLE 6). In the control, two autoradiograms had higher grain counts over the background than over the connective tissue.

In NPI the mean difference between the connective tissue and the background was 373.7, a value significant at the 0.001 level (TABLE 7). The autoradiograms in this group had very wide-ranging grain counts over the connective tissue. The mean difference in the control was 39.5, which was significant at the 0.01 level. In the control, one autoradiogram had a higher grain count over the background than over the connective tissue, a 100 percent effective blocking reaction.

In NPN the mean difference between the connective tissue and the background was 246.1, which was significant at the 0.001 level (TABLE 8). The mean difference in the control was 51.0 which was also significant at the 0.001 level (TABLE 8).

Since each mean difference between the connective tissue and the background in the control was significant to at least the 0.01 level, it had to be determined whether the grain counts in the experimental autoradiograms were significantly greater than the grain counts in the control autoradiograms. In each group, PI, PN, NPI, and NPN, the experimental grain counts were found to be significantly greater than the control grain counts at the 0.001 level (TABLE 9). Thus, even though the grain counts over the connective tissue were significantly greater than the grain counts over the background in the control, the control itself was not significant when compared to the experimental.

Each of the four groups was measured against the others by comparing the mean grain-count values of each group (TABLE 10). The grain counts of PI were found to be significantly greater than the grain counts of PN at the 0.01 level. NPI was also found to be significantly greater than PN at the 0.01 level. NPI was significantly greater than NPN at the 0.05 level, and NPN was significantly greater than PN at the 0.05 level. However, the grain counts of NPI and PI, and of PI and NPN, were not found to be significantly different at the 0.1 level.

No correlation could be found in the pregnant women between the grain counts and age, duration of pregnancy, number of times pregnant, number of viable births, or area biopsied. Likewise, there was no correlation found in the non-pregnant women between the grain counts and age or area biopsied.

CHAPTER V

DISCUSSION

This study has shown that antibody activity against fragmented Streptococcus mitis cells occurred in the inflamed gingiva and in clinically normal gingiva in both pregnant and non-pregnant women.

Labeled antigenic fragments of S. mitis cells were used to bind specific antibody sites within the gingival tissue. This microorganism was chosen since it is one of the most commonly found bacteria in the gingival sulcus, and because antigenic properties have been ascribed to its cell wall by Kalonaros and Bahn (1965). The bacterial cells were fragmented since it is not known what other parts of the cells might be antigenic in gingival tissue.

Clinically normal gingiva was used in order to determine if specific antigen-antibody reactions occur in the healthy gingiva as they have been reported to occur in the inflamed gingiva by Schneider and co-workers (1966), and others. The fact that plasma cells and lymphocytes are occasionally seen in clinically normal gingiva indicates that specific antibodies may always be present in response to the ever present bacteria in the gingival sulcus, a fact supported by this study. In all 18 gingival specimens classified as clinically normal in this study, few plasma cells and lymphocytes were seen in the connective tissue, and apparent antigen-antibody reactions could be detected. In the healthy gingiva

these antibodies might play a role in controling the antigens of S. mitis. Thus, host resistance of the gingiva to S. mitis antigens might be kept high if the number of the organisms was kept relatively low. The possibility of blood-borne antibodies aiding in the tissue defense against S. mitis antigens must be considered in the light of the findings of Toto and co-workers (1968), where globulins produced against streptococcal hyaluronidase were found in the gingival blood of patients with periodontitis.

The technique of autoradiography was used in an attempt to quantitate specific antigen-antibody activity in the gingiva. Autoradiograms show silver grains not only over the tissue section containing the radioisotope but also over the background of the slide. For this reason, any attempt to count the grains in an autoradiogram must take into account the number of grains found over the background.

A significant amount of bound, labeled antigen was apparently found within the connective tissue of each autoradiogram from each group of subjects (PI, PN, NPI, and NPN). This was determined from the findings that the connective tissue grain counts were significantly greater than the background grain counts in each autoradiogram studied.

Although the silver grains were counted over the connective tissue of each tissue section, the grains were not confined to this area. Random distribution of grains also were seen over the epithelium, but no attempt was made to count them and determine their significance with regard to the background grain counts. There is a possibility of antigen-antibody

reactions occurring in the epithelium since globulins have been found here. The presence of globulins in the epithelium is probably due to migration from the connective tissue since antibody-producing cells are not found in the epithelium.

The control used in this study was set up as a conventional blocking reaction. By using unlabeled S. mitis fragments on each tissue section, the antibody sites in the gingiva for S. mitis antigens should have been bound by these fragments. Thus, after incubation, rinsing, and adding the labelled S. mitis fragments, there should have been no antibody sites left for the labeled antigenic fragments to bind, and these labeled fragments should have been lost after rinsing. The blocking reaction was 100 percent effective in only five of the 38 specimens. This is indicated by the findings in five autoradiograms of the background grain counts being approximately equal to the connective tissue grain counts. In each of the four control groups (PI, PN, NPI, and NPN) considered as a whole, the blocking reaction was not 100 percent effective. The unlabeled S. mitis antigenic fragments did not react with all of the S. mitis-specific globulins in the connective tissue, and a signfficant amount of labeled S. mitis antigenic fragments reacted with the remaining S. mitis-specific globulins.

A very important fact is the significantly greater antigen-antibody activity that apparently occurred in the experimental autoradiograms than in the control autoradiograms in each group of subjects, showing that the blocking reaction was effective, even if not 100 percent effective. This

was determined from the findings that the mean grain counts in the experimental autoradiograms were significantly greater than those in the control autoradiograms.

The foregoing discussion dealt with the presence of immune reactions in both clinically normal gingiva and in the inflamed gingiva. Since these reactions were found in all of the groups studied (PI, PN, NPI, and NPN), the findings of intergroup comparisons were evaluated to determine if inflammation, or pregnancy, or both, significantly affect the antigen-antibody activity in the gingiva.

This study has shown that in all cases there was significantly greater antigen-antibody activity, using S. mitis antigenic fragments, in the inflamed gingiva than in clinically normal gingiva. This fact held true in both the pregnant and non-pregnant groups. This is to be expected as there are more plasma cells found in the inflamed gingiva than in clinically normal gingiva. It was pointed out earlier that plasma cells are the most prominent antibody-producing cells found in the inflamed gingiva.

It was also found that there was significantly greater antigen-antibody activity, using S. mitis antigenic fragments, in the clinically normal gingiva of the non-pregnant group than in the clinically normal gingiva of the pregnant group. Thus, there would seem to be a depression in the immune reactions to S. mitis antigens in the clinically normal gingiva during pregnancy.

In the light of the findings of Nelson and Hall (1964, 1965) and Nelson (1967), regarding depression of the thymolymphatic system during pregnancy, one might assume that this is the reason for the apparent decrease in the immune function that is seen in the clinically normal gingiva during pregnancy. This apparent reduction was not seen in the inflamed gingiva during pregnancy when compared to the inflamed gingiva during non-pregnancy, since the antigen-antibody activity was not significantly different in the inflamed gingiva of the pregnant and non-pregnant groups.

Nelson and Hall (1965) have further suggested that plasma cells may be derived from small lymphocytes, which are produced in the thymolymphatic system. Thus, a depressed thymolymphatic system during pregnancy could result in decreased plasma cell production and a subsequent decrease in antigen-antibody activity, if the above hypothesis is assumed. It appears unlikely though, that in the gingiva plasma cells are derived from lymphocytes. The findings of Toto (1961) strongly suggest that plasma cells are derived locally from undifferentiated mesenchymal cells. If this is the case, plasma cells would not be a product of the thymolymphatic system. Thus, even if the thymolymphatic system might be depressed during pregnancy, it is apparent that plasma cell production is not depressed in the gingiva.

As stated above, there is an apparent reduction in the antibody activity to S. mitis antigens in the clinically normal gingiva during pregnancy when compared to the antibody activity seen in clinically normal

gingiva during non-pregnancy. In the inflamed gingiva there was no significant difference in antibody activity to S. mitis antigens during pregnancy as compared to non-pregnancy.

A possible explanation is that immune functions might be depressed during pregnancy, but not reduced to the point where host resistance of the gingival tissue is lowered. Hence, a normal state could be maintained. Even if the clinically normal gingiva of the pregnant woman does not have the same level of antibody acitivity to S. mitis antigens as does the clinically normal gingiva of the non-pregnant woman, the antibodies that are present probably would be sufficient to maintain the host resistance of the gingiva.

Another possible explanation is that blood-borne antibodies might play a role in the maintenance of normal gingiva, while locally produced antibodies are active during gingival inflammation. If this hypothesis were true, a depression of the thymolymphatic system during pregnancy could account for the decreased antibody activity to S. mitis antigens seen in clinically normal gingiva of the pregnant women in this study. Also, if the thymolymphatic system does not function in the defense against gingival inflammation, as the above hypothesis states, a depression of this system during pregnancy would have no effect on the level of antibody activity against S. mitis antigens occurring in the inflamed gingiva of pregnant women. Thus, the inflamed gingiva during pregnancy and during non-pregnancy would have approximately the same level of

antibody activity to S. mitis antigens, as this study has shown.

Further research into the immunology associated with inflammatory periodontal disease could be directed toward the isolation of the particular antigen or antigens of S. mitis that are active during gingival inflammation. Other oral microorganisms could be used in the manner described in this study to determine if they also possess antigens capable of provoking an immune response in the gingiva. Finally, with regard to the possible depression in immune activity during pregnancy, the qualitative and quantitative aspects of serum globulins could be studied in an effort to determine their possible role in the defense of the gingiva to antigens during pregnancy.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Thirty-eight adult women, ranging in age from 18 to 40 years, were selected for gingival biopsy in order to evaluate the antibody activity in the gingiva to Streptococcus mitis antigens, and to determine whether this antibody activity is affected during pregnancy. Nineteen women were pregnant, with ten having inflamed gingiva and nine having clinically normal gingiva. The other 19 women were not pregnant, with ten having inflamed gingiva and nine having clinically normal gingiva. Each excised interproximal papilla was quick-frozen on dry ice and stored in a freezer. The tissue was prepared for histologic and autoradiographic study at a later time.

A pure culture of Streptococcus mitis organisms was cultured in trypticase-soy broth for 48 hours at 37° C, fragmented, and labeled with the radioisotope, I-131 (specific activity, 111.1 microcuries/ml of radioactive bacterial suspension).

Tissue sections were cut in a bucco-lingual direction at a thickness of four microns, and each sixth section was used for autoradiography. One drop of radioactive bacterial fragments, at 1.6 percent of the original activity, was placed on each tissue section, incubated for two hours at 37° C, and exposed in a photographic emulsion for 27 hours. The autoradiograms were stained with nuclear fast red and indigo carmine.

As a control, unlabeled bacterial fragments were added to other tissue sections before the addition of the labeled fragments.

Silver grains were counted over the connective tissue and over the background of each section at 1000 X. Grain counts over the connective tissue were found to be significantly greater than the counts over the background in each instance, indicating apparent antibody activity to S. mitis antigens in the connective tissue. The control autoradiograms showed these same findings. However, the control grain counts were significantly less than the experimental grain counts, indicating that the control was a partially successful blocking reaction.

Comparison of inter-group grain counts revealed significantly greater counts in the inflamed gingiva than in clinically normal gingiva in each autoradiogram, indicating an apparently greater antibody activity to S. mitis antigens in the inflamed gingiva than in clinically normal gingiva, in both pregnancy and non-pregnancy. These comparisons further showed a significantly greater number of grains in clinically normal gingiva of the non-pregnant women than in the pregnant women. This indicates apparently less antibody activity to S. mitis antigens in the clinically normal gingiva during pregnancy. However, there were no significant differences in the grain counts in the inflamed gingiva of the non-pregnant women and of the pregnant women. This indicates an apparently equal amount of antibody activity to S. mitis antigens in the inflamed gingiva during pregnancy and during non-pregnancy.

The following conclusions can be drawn from this study:

1. S. mitis cells have the ability to provoke an antibody response in the gingiva of pregnant and non-pregnant women.
2. Clinically normal gingiva showed apparent evidence of antibody activity to S. mitis antigens in both pregnant and non-pregnant women.
3. Inflamed gingiva showed significantly more antibody activity to S. mitis antigens than clinically normal gingiva in both pregnant and non-pregnant women.
4. There was apprently less antibody activity to S. mitis antigens in clinically normal gingiva of pregnant women than in that of non-pregnant women.
5. Antibody activity to S. mitis antigens was apprently equal in the inflamed gingiva of pregnant women and in the inflamed gingiva of non-pregnant women.
6. Autoradiography can be an important tool in the in vitro assessment of antigen-antibody reactions in human gingiva.

BIBLIOGRAPHY

Angervall, L., and Lundin, P. Administration of cortisone to the pregnant rat. Effects on lymphoid tissue. Acta. Endocrinology. 50:104, 1965.

Arnim, S. Microcosms of the mouth-Role in periodontal disease. Texas D.J. 82:4, 1964.

Baer, P.N., Newton, W.L., and White, C.L. Studies on periodontal disease in the mouse. VI. The older germ-free mouse and its conventional control. J. Periodont. 35:388, 1964.

Belanger, L.F., and Leblond, C.P. Mineralization of growing tooth as shown by radiophosphorus autographs. Proc. Soc. Exper. Biol. and Med. 73:390, 1950.

Bibby, B.G. Role of bacteria in periodontal disease. O.O.O. 6:318, 1953.

Billingham, R.E. Transplantation immunity and maternal-fetal relation. New England J. Med. 270:667, 1964.

Billingham, R.E., and Silvers, W.K. Sensitivity to homografts of normal tissues and cells. Annual Review of Microbiology. 17:531, 1963.

Cohn, B. Comparative studies in periodontal disease. Proc. Roy. Soc. Med. 53:275, 1960.

DePetris, S., Karlsbad, G., and Perni, B. Localization of antibodies in plasma cells by electron microscopy. J. Exp. Med. 117:849, 1963.

Desai, R.G., and Creger, W.P. Maternal-fetal passage of leukocytes and platelets in man. Blood. 21:665, 1963.

Dewar, M.R. Bacterial enzymes and periodontal disease. J. Dent. Res. 37:100, 1958.

Doe, R.P., Zinneman, H.H., Flink, E.B., and Ulstrom, R.A. Significance of concentration of nonprotein bound plasma cortisol in normal subjects, Cushing's syndrome, pregnancy, and during estrogen therapy, J. Clinical Endocrinology. 20:1484, 1960.

Doniach, I., and Pelc, S.R. Autoradiographs with radioactive iodine. Proc. Roy. Soc. Med. 42:957, 1949.

Dougherty, T.F., Berliner, M.L., Schnecbeli, G.L., and Berliner, D.L. Hormonal control of lymphatic structure and function. Annals N.Y. Acad. Science. 113:825, 1964.

Douglas, G.W., Thomas, L., Carr, M., Cullen, N.M., and Morris, R. Trophoblast in the circulating blood during pregnancy. Amer. J. Obst. & Gynec. 78:960, 1959.

Eastman, N.J., and Hellman, L.M. Williams Obstetrics, Twelfth Edition, Appleton-Century-Crofts. New York. p. 169, 1961.

Goodlin, R.C., and Herzenberg, L. Further studies on the mechanism of pregnancy-induced isoimmunization in mice. Amer. J. Obst. & Gynec. 95:133, 1966.

Gross, J., Bogoroch, R., Nadler, N.J., and Leblond, C.P. The theory and methods of the radioautographic localication of radioelements in tissues. Amer. J. Roetgenology and Radium Therapy 65:420, 1951.

Howell, A., and Jordan, H.V. Production of an extra-cellular levan by Odontomyces viscosus. Arch. Oral Biol. 12:571, 1967.

Jordan, H.V., Fitzgerald, R.J., and Stanley, H.R. Plaque formation and periodontal pathology in gnotobiotic rats infected with an oral actinomycete. Amer. J. Path. 47:1157, 1965.

Kalonaros, I.V., and Bahn, A.N. Antigenic composition of the cell wall of Streptococcus mitis. Arch. Oral Biol. 10:625, 1965.

Lanman, J.T., Dinerstein, J., and Firkig, S. Homograft immunity in pregnancy: Lack of harm to fetus from sensitization of mother. Annals N.Y. Acad. Science. 99:706, 1962.

Leblond, C.P., Stevens, C.E., and Bogoroch, R. Histological localication of newly-formed desoxyribonucleic acid. Science. 108:531, 1948.

Löe, H. Periodontal changes in pregnancy. J. Periodont. 36:209, 1965.

MacDonald, J.B., and Gibbons, R.J. Relationship of indogenous bacteria to periodontal disease. J. Dent. Res. 41:320, 1962.

Maier, A.W., and Orban, B. Gingivitis in pregnancy. O.O.O. 2:334, 1949.

McDonald, H.M., Cobb, J., and Solomon, A.K. Radioautographic technique with C^{14}. Science 107:550, 1948.

Medewar, P.B. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp. Soc. Exp. Biol. vol. 7, 1953.

Mergenhagen, S.E., and Scherp, H.W. Lysis of reconstituted collagen and catabolism of products of collagenolysis by the oral microbiota. Arch. Oral Biol. 1:333, 1960.

Monash, S. Proliferative gingivitis of pregnancy. Surg. Obst. and Gynec. 42:794, 1926.

Nelson, J.H., and Hall, J.E. Regeneration of pelvic lymph nodes after pelvic lymphadenectomy. Amer. J. Obst. & Gynec. 93:102, 1965.

_____. Studies on the thymolymphatic system in humans. I. Morphologic changes in lymph nodes in pregnant women at term. Amer. J. Obst. & Gynec. 90:482, 1964.

_____. Studies on the thymolymphatic system in humans. II. Morphologic changes in nodes in early pregnancy and during puerperium. Amer. J. Obst. & Gynec. 93:1133, 1965.

Nelson, J.H. Effect of pregnancy on the thymolymphatic system. I. Changes in the intact rat after exogenous HCG, estrogen, and progesterone administration. Amer. J. Obst. & Gynec. 98:895, 1967.

Pirofsky, B. Immunologic aspects of the maternal-fetal state. Ann. Allerg. 24:109, 1966.

Pressman, D., Hill, R.F., and Foote, F.W. Zone of localization of anti-mouse kidney serum as determined by radioautographs. Science 109:65, 1949.

Ramfjord, S. Local factors in periodontal disease. J.A.D.A. 44:647, 1952.

Rosebury, T. Nature and significance of infection in periodontal disease. Amer. J. Orth. and Oral Surg. 33:658, 1947.

Schneider, T.F., Toto, P.D., Gargiulo, A.W., and Pollock, R.J. Specific bacterial antibodies in the inflamed human gingiva. Periodontics 4:53, 1966.

Schultz-Haudt, S., Bibby, B.G., and Bruce, M.A. Tissue destructive products of gingival bacteria. J. Dent. Res. 33:625, 1954.

Schultz-Haudt, S., and Stig, G. Bacteria and Gingivitis. Norske Tandl. Tidskr. 66:203, 1956.

Simmons, R.L. The immunologic problem of pregnancy. II. Ultrastructure of isogenic and allogenic trophoblastic transplants. Amer. J. Obst. & Gynec. 97:218, 1967.

Simmons, R.L., and Russell, P.S. Antigenicity of mouse trophoblast. Annals N.Y. Acad. Science. 99:717, 1962.

Toto, P.D. Plasma cells in inflamed oral mucosa. Dental Progress. 1:61, 1961.

Toto, P.D., Wittwer, J.W., and Dickler, E.H. Anti-streptococcal hyaluronidase and periodontitis. J. Dent. Res. 47:174, 1968.

_____. unpublished material. 1968.

Turesky, S., Fisher, B., and Glickman, I. A histological study of the attached gingiva in pregnancy. J. Dent. Res. 37:1115, 1958.

Waerhaug, J. and Stein, E. The presence or absence of bacteria in gingival pockets and the reaction in healthy pockets to certain pure cultures. Odont. Tidskr. 60:1, 1952.

Warren, S., and Dixon, F.J. Antigen tracer studies and histologic observations in anaphylatic shock in guinea pigs. Am. F. M. Sc. 216:136, 1948.

Wentz, F.M. Bacteria in the primary role and a concept of therapy. Dent. Clin. N.A. p. 693, Nov., 1960.

Zarow, D.M., Lichtman, H.C., and Hellman, L.M. The transmission of chromium^{51} tagged maternal erythocytes from mother to fetus. Amer. J. Obst. & Gynec. 88:565, 1964.

Zipursky, A., Pollack, B., Chown, B., and Israels, L.G. Transplacental fetal hemorrhage after placental injury during delivery or amino-centesis. Lancet 2:493, 1963.

Ziskin, D.E., Blackberg, S.N., and Stout, A.P. The gingiva during pregnancy. Surg. Obst. and Gynec. 57:719, 1933.

Ziskin, D.E., and Nesse, G.J. Pregnancy gingivitis: history, classification, etiology. Amer. J. Orth. and Oral Surg. 32:390, 1946.

	Age	Gravida* Para**		Duration of Pregnancy	Clinical Evaluation and Areas Biopsied	
PI# 1	24	G,IV	P,III	21 weeks	4,3	inflamed
PI 2	25	G,III	P,I	20 weeks	6,5	inflamed
PI 3	27	G,VI	P,V	31 weeks	6,5	inflamed
PI 4	18	G,I	P,0	35 weeks	7,6	inflamed
PI 5	22	G,I	P,0	17 weeks	2,1	inflamed
PI 6	26	G,IV	P,III	17 weeks	4,5	inflamed
PI 7	18	G,I	P,0	37 weeks	5,6	inflamed
PI 8	19	G,II	P,I	21 weeks	7,8	inflamed
PI 9	20	G,III	P,II	35 weeks	6,5	inflamed
PI 10	26	G,II	P,I	13 weeks	7,6	inflamed

TABLE 1

PREGNANT WOMEN SELECTED FOR GINGIVAL BIOPSY

*a pregnant woman. The number following "G" refers to all previous and present pregnancies.

**The number following "P" refers to all past pregnancies which have produced an infant which has been viable, whether or not the infant is dead or alive at birth.

#pregnant, with inflamed gingiva.

	Age	Gravida Para	Duration of Pregnancy	Clinical Evaluation and Areas Biopsied	
PN* 1	25	G,I P,0	33 weeks	5,4 |	normal
PN 2	23	G,II P,I	35 weeks	| 5,6	normal
PN 3	26	G,I P,0	25 weeks	| 3,4	normal
PN 4	27	G,III P,II	31 weeks	| 4,5	normal
PN 5	23	G,I P,0	10 weeks	| 4,5	normal
PN 6	24	G,I P,0	13 weeks	| 3,4	normal
PN 7	28	G,II P,0	31 weeks	4,3 |	normal
PN 8	29	G,II P,I	35 weeks	| 4,5	normal
PN 9	27	G,II P,I	33 weeks	| 4,5	normal

TABLE 2

PREGNANT WOMEN SELECTED FOR GINGIVAL BIOPSY

*pregnant, with clinically normal gingiva.

	Age	Interproximal Area Biopsied	Clinical Evaluation of Biopsy Site
NPI* 1	40	⌈7,8⌉	inflamed
NPI 2	34	⌈7,8⌉	inflamed
NPI 3	38	⌊5,6⌋	inflamed
NPI 4	40	⌈6,7⌉	inflamed
NPI 5	20	5,4	inflamed
NPI 6	29	⌊7,8⌋	inflamed
NPI 7	27	⌊4,3⌋	inflamed
NPI 8	22	⌊7,6⌋	inflamed
NPI 9	34	5,4⌋	inflamed
NPI 10	24	5,4⌋	inflamed

TABLE 3

NON-PREGNANT WOMEN SELECTED FOR GINGIVAL BIOPSY

*non-pregnant, with inflamed gingiva.

	Age	Interproximal Area Biopsied	Clinical Evaluation of Biopsy Site
NPN* 1	39	$\overline{5,4}\|$	normal
NPN 2	32	$\overline{5,4}\|$	normal
NPN 3	29	$\overline{3,2}\|$	normal
NPN 4	27	$\overline{5,4}\|$	normal
NPN 5	24	$\overline{6,5}\|$	normal
NPN 6	18	$\overline{5,4}\|$	normal
NPN 7	37	$\overline{5,4}\|$	normal
NPN 8	40	$\overline{6,5}\|$	normal
NPN 9	21	$\overline{5,4}\|$	normal

TABLE 4

NON-PREGNANT WOMEN SELECTED FOR GINGIVAL BIOPSY

*non-pregnant, with clinically normal gingiva.

		EXPERIMENTAL		CONTROL	
		connective tissue	background	connective tissue	background
PI*	1	366	87	145	78
PI	2	395	116	152	122
PI	3	403	109	119	75
PI	4	387	81	130	82
PI	5	325	109	168	97
PI	6	378	133	207	201
PI	7	379	100	122	73
PI	8	537	92	127	68
PI	9	341	56	133	82
PI	10	526	127	148	87
		4037	1010	1451	965
	mean =	403.7	101.0	mean = 145.1	96.5
	mean difference =		302.7	mean difference =	48.6
	t = 12.76	P > 0.001		t = 3.21	P > 0.01

TABLE 5

GRAIN COUNTS OVER CONNECTIVE TISSUE AND BACKGROUND IN EXPERIMENTAL AND CONTROL AUTORADIOGRAMS

*pregnant, with inflamed gingiva.

		EXPERIMENTAL		CONTROL	
		connective tissue	background	connective tissue	background
PN*	1	298	119	71	75
PN	2	244	80	104	107
PN	3	201	69	109	69
PN	4	273	74	93	59
PN	5	359	57	84	81
PN	6	395	89	109	58
PN	7	306	107	110	63
PN	8	215	107	123	77
PN	9	396	109	128	73
		2687	811	931	662
mean =		298.6	90.1	mean = 103.5	73.5
mean difference =			208.5	mean difference =	30.0
t = 7.42		P > 0.001		t = 3.85	P > 0.01

TABLE 6

GRAIN COUNTS OVER CONNECTIVE TISSUE AND BACKGROUND IN EXPERIMENTAL AND CONTROL AUTORADIOGRAMS

*pregnant, with clinically normal gingiva.

	EXPERIMENTAL		CONTROL	
	connective tissue	background	connective tissue	background
NPI* 1	408	149	150	121
NPI 2	330	137	100	74
NPI 3	505	77	97	79
NPI 4	418	73	85	91
NPI 5	421	69	151	87
NPI 6	597	126	185	111
NPI 7	398	105	166	118
NPI 8	605	184	148	101
NPI 9	837	156	170	104
NPI 10	375	81	108	79
	4894	1157	1360	965
mean =	489.4	115.7	mean = 136.0	96.5
mean difference =		373.7	mean difference =	39.5
t = 7.49	P > 0.001		t = 3.19	P>0.01

TABLE 7

GRAIN COUNTS OVER CONNECTIVE TISSUE AND BACKGROUND IN EXPERIMENTAL AND CONTROL AUTORADIOGRAMS

*non-pregnant, with inflamed gingiva

	EXPERIMENTAL		CONTROL	
	connective tissue	background	connective tissue	background
NPN* 1	349	129	121	80
NPN 2	367	177	151	86
NPN 3	441	224	140	71
NPN 4	386	131	117	70
NPN 5	314	82	171	119
NPN 6	430	124	139	77
NPN 7	405	90	103	74
NPN 8	364	122	114	83
NPN 9	348	110	155	91
	3404	1189	1211	751
mean =	378.2	132.1	mean = 134.5	83.5
mean difference =		246.1	mean difference =	51.0
t = 12.26	P > 0.001		t = 4.34	P > 0.001

TABLE 8

GRAIN COUNTS OVER CONNECTIVE TISSUE AND BACKGROUND IN EXPERIMENTAL AND CONTROL AUTORADIOGRAMS

*non-pregnant, with clinically normal gingiva.

	EXPERIMENTAL vs CONTROL	
	"t" value	probability
PI*	11.25	P > 0.001
PN**	6.91	P > 0.001
NPI***	7.58	P > 0.001
NPN#	13.45	P > 0.001

TABLE 9

SIGNIFICANCE OF EXPERIMENTAL GRAIN COUNTS
IN EACH GROUP OF SUBJECTS

*pregnant, with inflamed gingiva

**pregnant, with clinically normal gingiva

***non-pregnant, with inflamed gingiva

#non-pregnant, with clinically normal gingiva

	"t" value	probability
NPI* vs NPN**	2.114	$P > 0.05$
NPI vs PI***	1.610	$P < 0.1$
NPN vs PN#	2.848	$P > 0.05$
NPI vs PN	3.411	$P > 0.01$
NPN vs PI	0.938	$P < 0.1$
PN vs PI	3.170	$P > 0.01$

TABLE 10

SIGNIFICANCE OF INTER-GROUP GRAIN COUNTS

*non-pregnant, with inflamed gingiva

**non-pregnant, with clinically normal gingiva

***pregnant, with inflamed gingiva

#pregnant, with clinically normal gingiva

FIGURE 1. Area of clinically normal gingiva from non-pregnant woman, stained with hematoxylin and eosin. Original magnification 250 X.

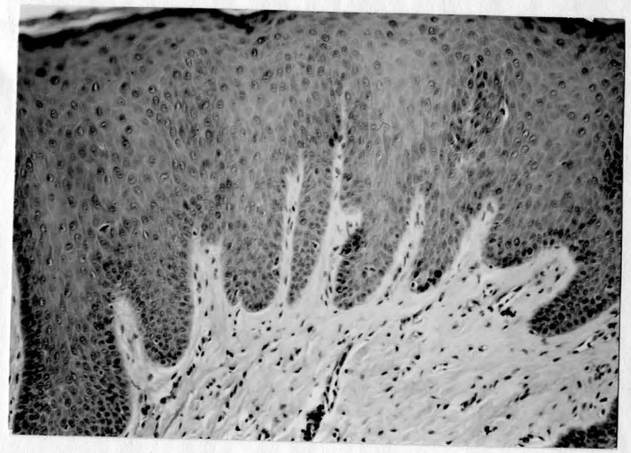


FIGURE 2. Area of Clinically normal gingiva from pregnant woman, stained with hematoxylin and eosin. Original magnification 100 X.

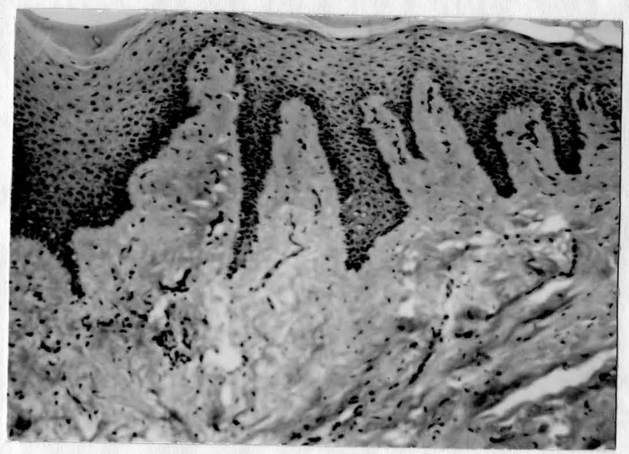


FIGURE 3. Area of inflamed gingiva from non-pregnant woman, stained with hematoxylin and eosin. Original magnification 250 X.

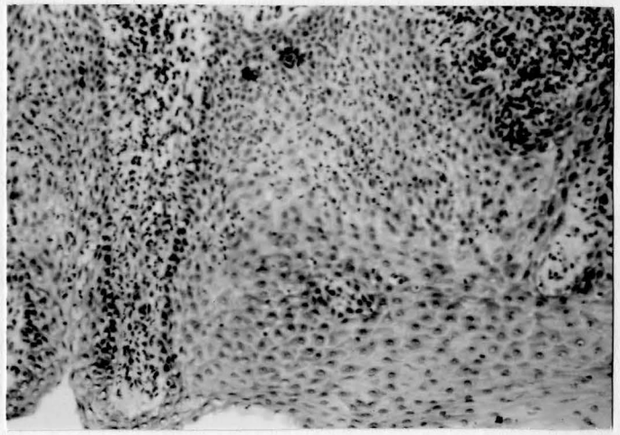


FIGURE 4. Area of inflamed gingiva from pregnant woman, stained with hematoxylin and eosin. Original magnification 250 X.

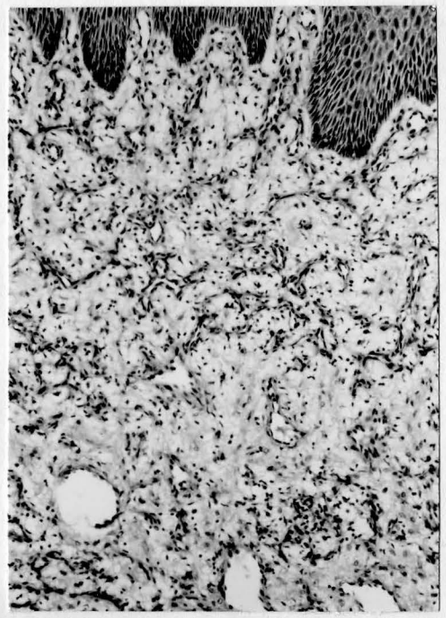
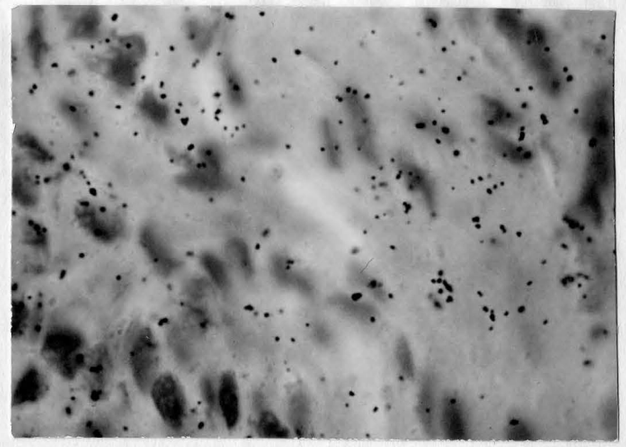


FIGURE 5. Experimental autoradiogram showing silver grains over the connective tissue. Original magnification 1000 X.

FIGURE 6. Experimental autoradiogram showing silver grains over the background. Original magnification 1000X.



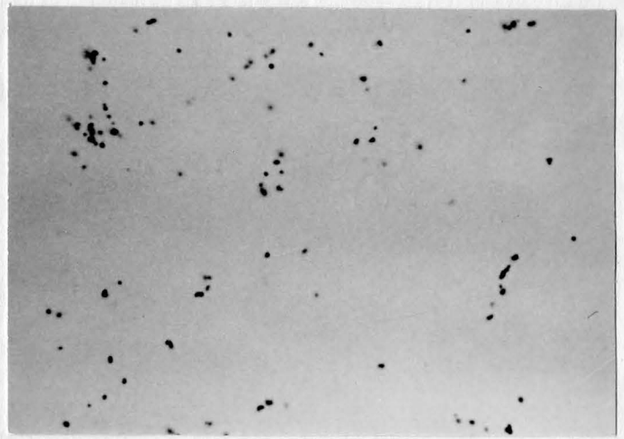
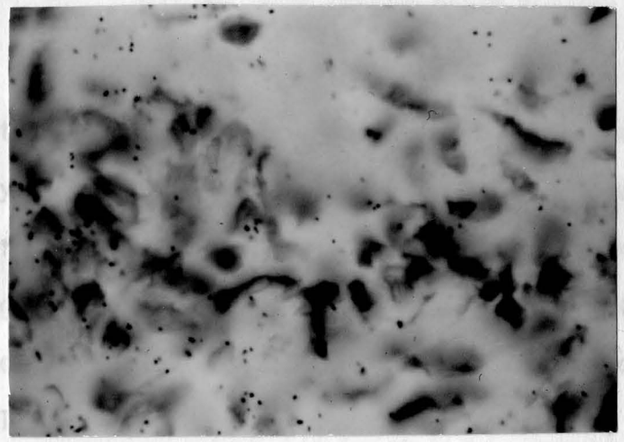
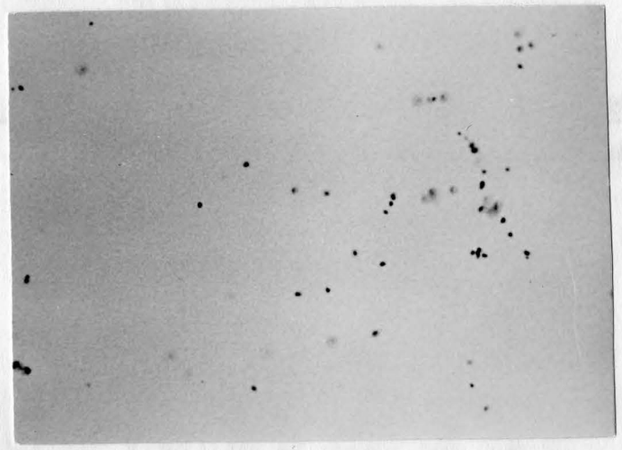


FIGURE 7. Control autoradiogram showing silver grains over the connective tissue. Original magnification 1000X.

FIGURE 8. Control autoradiogram showing silver grains over the background. Original magnification 1000X.





APPROVAL SHEET

The thesis submitted by Dr. Ronald C. Hartzer 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 requirement for the Degree of Master of Science.

May 21 1969
Date

Patrick D Tate
Signature of Advisor