CONFIRMATION OF LIPIDS IN HUMAN TOOTH MOVEMENT

by

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TABLE OF CONTENTS

INTRODUCTION

There have been many histologic studies of tooth movement in experimental animals but relatively few on humans. Herzberg,[1] in this country, did one of the first studies on human tooth movement in 1932. Later, Oppenheim[2] and Stuteville[3] added to our information in this area. Since 1951, Reitan[4,5,6] has written numerous reports on this subject. In one of his studies, he described a cell-free area in the periodontal ligament after a force of 70 grams had been applied to a tooth. He called this cell-free area "hyalinization."[5] Church[7] corroborated this cell-free area after seven days of movement, and in addition after 21 days of movement, he reported on the presence of "signet ring cells" in the periodontal ligament. He described this area as being similar to tissue fatty degeneration. Due to this unforeseen development, he could not prove that there had been fat present in those cells, because he did not use any fat-soluble stains, and routine laboratory procedures destroyed all fat that may have been

present. Goya[8] further examined 21-day human tooth movement material to determine if Church's observations were in fact lipid-bearing tissue or were artifacts. Goya only demonstrated fat in one specimen out of the four examined and he felt that one possibility may have been that the tooth had simply moved into a marrow space instead of the "fatty degeneration" that Church described. Furthermore, he could not discount the possibility that Church's findings might have been tissue artifacts of some unknown nature.

Oppenheim[2] described an "angioma-like" appearance in the periodontal ligament in some of his specimens of human tooth movement. He attributed this to an abundance of large vessels and capillaries. Stuteville[3] and Reitan[4] reported vacuolar spaces in the PDL, but they termed them artifacts. The possibility exists that these may be what Church described as "fatty degeneration."

The experimental model for the following investigation is similar to Church's[7] and Goya's.[8] This model was based on Reitan's[4] work in which a 70-gram continuous tipping force was placed on a human tooth

using a fixed appliance. The tipping force had a 21-day duration.

The purpose of this experiment is to determine whether or not there is fat in the human periodontal ligament space after light orthodontic forces are applied to teeth for a 21-day time period.

REVIEW OF LITERATURE

The study of lipid as an organ and a tissue has been more difficult than the study of other organs and tissues because of its chemical nature in comparison to the routine handling of proteins. Histologic studies of fatty material are relatively recent because satisfactory fat stains have not been in use as long as other types of stains.[9] The use of dyes in histology began about 1850, but fat stains such as Sudan black B, Nile blue, oil red O, OTAN-technique and others[10] are much more recent. Daddi[11] introduced Sudan III in 1896, and for many years this was essentially the only fat stain being used. In 1926, French[12] introduced oil red O. Casselman[10] felt that the two most satisfactory fat stains are Sudan black B and oil red O.

The lipids can be described as a group of substances that are generally soluble in certain organic solvents but not water and they include the higher fatty acids, their naturally occurring compounds

and substances found in association with them.[13] Many simple classifications are used to demonstrate histochemically identifiable lipids (Table I).

The origin of the cell that makes up adipose tissue has not yet been conclusively determined. A primary difficulty in understanding the histogenesis of adipose tissue is due to the lack of clear distinction between brown fat and white fat. White fat is also called unilocular adipose tissue and is characterized morphologically by cells which contain a single large vacuole of stored lipid. Brown fat is called "multilocular adipose tissue" because the lipid is present in the cytoplasm as multiple small droplets.[14]

Toldt (1888)[15] thought that fatty tissue of mammals was a specific organ, entirely distinct from the connective tissue in which it frequently occurs. However, Fleming (1897)[16] felt that adipose tissue was merely ordinary connective tissue in which fat had been deposited. More recently, Kinsell[14] was in agreement with the earlier observations of Toldt. He said that published evidence seems to show adipose tissue

develops from special primitive connective tissue cells and that the cells of this tissue have a specific structure and function quite distinct from that of the fibroblasts of connective tissue.

Unilocular fat cells are very large due to the quantity of lipid which accumulates in them. When crowded together, they become oval or polygonal in shape. In ordinary histological sections from which the soluble fats are lost, the cells are frequently collapsed, or there is distortion of their contours. There is some degree of variation in size in individual fat cells, not only from one region of the body to another, but even in the same area.[14] Tedeschi[17] feels that the size of the adipose cell is a reflection of its level of metabolic activity and he suggests that their variation in size is an indication that the fatty tissues do not function as a unit but that each cell is provided with a certain degree of autonomic function. Reh[18] studied the size of fat cells and found that their diameter varied from 10 micra to 170 micra.

According to the traditional description of white adipose tissue,

the cytoplasm surrounding the lipid vacuole is no more than a thin film. The nucleus is flattened and displaced to one side of the cell where it occupies a thickened area of the cytoplasm.[14] This is where the term "signet ring" cell comes from. Around each individual fat cell, there is a delicate feltwork of reticular tissue which forms a coat for the plasma membrane. The cytoplasm of some cells is so thin that it cannot be seen with the conventional light microscope.

Clark and Clark[19] observed that fat frequently, but not invariably, appeared first in cells near blood vessels. Fat formation occurred in stable regions with a moderate or a slow circulation, but it failed to develop where the circulation was sluggish, and it never formed in areas of active vascular ingrowth. Fat already present diminished following periods of active circulation, especially when the nearby vessels showed evidence of inflammation.

The biologically important lipids are the neutral fats (triglycerides), the phospholipids and related compounds, and the sterols. The triglycerides are made up of three fatty acids bound to glycerol.

Naturally occurring fatty acids contain an even number of carbon atoms. They may be saturated (no double bonds) or unsaturated (dehydrogenated, with various numbers of double bounds).[20,21,22]

The lipids in cells are of two main types: structural lipids, which are an inherent part of the membranes and other parts of cells; and neutral fat, stored in the adipose cells of the fat depots. A third special type of fat is brown fat, which is found in infants but not in adult humans. It has a high metabolic rate and produces heat that aids in thermoregulation. Depot fat is mobilized during starvation, but structural lipid is preserved. The size of the fat depot varies, but in nonobese individuals they make up about 10% of the body weight.[21] They are not the inert lumps they were once thought to be but active, dynamic tissues undergoing continuous breakdown and resynthesis. Even while weight is being maintained constant, a steady turnover of fat occurs in the body. The half life of fat is about eight days.[20] In the fat depots, glucose is metabolized to fatty acids and neutral fats are synthesized. In the reverse process, neutral fat is broken

down, and free fatty acids are liberated into the circulation.[20,21,22]

Fatty acids in man usually have 16 carbon atoms. None have more than 16. There are small amounts of 12 and 14 carbon atom fatty acids. The free fatty acids are an extremely labile circulating fat component, with a half life of only a few minutes.[20] They are a major source of energy for many organs, particularly the heart.[21]

Disturbances in fat metabolism and adipose tissue are very common in disease processes. When fat occurs free in tissue spaces under pathologic conditions, it elicits an inflammatory reaction, usually of foreign body type.

Anderson[23] listed several types of pathology in which a disturbance in fat metabolism is involved. He said obesity is the condition in which there is an abnormally large amount of adipose tissue. Heredity and environmental factors have both been shown to have important roles in the development of obesity, but metabolic studies have indicated that obesity is invariably the result of an energy intake by the body greater than its total dissipation. "Adiposa dolorosa" is a term used

for certain cases of generalized obesity in which tenderness and pain of the fat deposits is an outstanding feature.[23,24]

Progressive lipodystrophy is a disease of unknown origin characterized by loss of adipose tissue in certain regions of the body with remaining parts retaining their adipose tissue or even showing an increase in fat.[23]

Fatty infiltration is the condition of excessive accumulation of fat in connective tissue fibers between parenchymatous cells of organs. The parenchymatous structures, abnormally separated by their fatty accumulation, may be subject to some interference with their function and tend to undergo atrophy.[23]

Fatty change of the liver is an excessive accumulation of fat in the parenchymatous cells of the liver. The liver is an organ that plays an important role in the normal metabolism of lipids. Excess accumulation of fat in liver cells occurs very commonly in metabolic disturbances, without primary injury or degeneration of the liver cells.[24] However, a true fatty degeneration, in which fat appears in liver cells

as a result of their injury, also occurs.[23,24]

Fatty degeneration is characterized by the appearance of visible or stainable fat in the cytoplasm of cells in which none normally can be observed. It most frequently affects the kidneys, heart, and liver but also may affect the cells of many other structures. It is usually an indication of a rather severe injury to the involved cell, although the process is usually reversible. It is often associated with disturbance in functional efficiency of the involved organ. It develops from a variety of causative factors, the most important of which are severe anemias or anoxemias, certain chemical poisons, and many toxic and infectious conditions.[23] Affected organs are flabby and may have a distinct yellowish color of either patchy or diffuse distribution. The fat appearing in parenchymatous cells in fatty degeneration has long been considered to be due to an unmasking or a change to a visible form of fat already present within the cells (fat phanerosis) rather than to an increased total lipid content as a result of accumulation from sources

outside the affected cells.[23,24]

There are very few studies of finding fat in the periodontal ligament space after applying a tipping force to the teeth.[7,8] Research on fat associated with any dental structure is very recent.

Irving[25] showed that there is sudanophil material at sites of calcification of bone and teeth. He also described his method for unmasking the sudanophil material by extracting it with hot pyridine. He felt that it was necessary to extract with hot pyridine in order to unmask intracellular sudanophil material. After the lipids are unmasked, they can then be stained with routine fat stains. Melcher[26] said that the extent and intensity of extracellular sudanophilia is greatly increased by extraction of unfixed sections in acidified chloroform: methanol, suggesting that further bound lipid is unmasked to staining by this procedure. He said that similar extraction after fixation is ineffective so that, if hard tissues are to be examined for sudanophilic-bound lipids by these methods, they must be demineralized without prior fixation. His controls showed that the cementum was strongly sudanophilic. The trabeculae of bone were also sudanophilic

with the younger trabeculae showing it more intensely. Parts of some fibers of the periodontal ligament were sudanophilic. Extraction in acidified chloroform:methanol at room temperature increased both the extent and intensity of the extracellular sudanophilia.

Itoiz, et al.[27] produced localized ischemia in the gingival tissues of monkeys by injecting microspheres into the right carotid circulation of nine squirrel monkeys. He found after 24 and 72 hours of arteriolar occlusion, that the lipid content increased. Small droplets of Sudan black B positive material were localized in the intercellular spaces of the basal and lower prickle cell layers.

Das and Harris[28] found microscopic globules of fatty materials present in tufts and spindles of enamel and in dentinal tubules. They showed that fat is present in tubules of sound dentin, that the major lipids of human teeth are triglycerides, cholesterol, cholesterol esters, phospholipids and free fatty acids, and that the peripheral dentinal extensions of odontoblasts are rich in lipids.

In another report, Das and Harris[29] extracted lipids and fatty

acids from eight specimens of fossil teeth that were reliably estimated to be 100,000 to 230,000,000 years old. The kinds and relative amounts in the extract were estimated by gas-liquid chromotography. These ancient teeth were found to be similar to modern animal teeth in lipid and fatty acid content. It is noteworthy that these lipids and fatty acids survived storage in teeth for millions of years.

Anneroth and Ivemark[30] studied the distribution of various lipids in the human dental pulp of teeth. In the odontoblastic zone, sphingomyelin and free fatty acids were observed specifically in the cytoplasm of the odontoblasts and in the pulpo dentinal membrane. The sheaths of the myelinated nerves in the pulpal tissue contain sphingomyelin, saturated and unsaturated fatty acids and free cholesterol.

Prout and Tring[31] showed that a deficiency of essential fatty acids in the diet produces histologic changes in the enamel organ of rats. This showed that lipids are involved in the calcification process of teeth for rats.

Shapiro[32] studied bovine compact bone for lipid content before

demineralization and after demineralization. Triglycerides comprised over 94% of the pre-demineralized lipid extract, and cholesterol esters were the predominant lipid of the post-demineralized extract. Of the total lipids released from the bone, 65% were removed prior to decalcification of the tissue.

Ingervall, Freden and Heyden[33] did a histological study of the lipid content of rat alveolar bone after traumatic loading of the teeth. In their introduction, they say that in a preliminary histochemical study of the occurrence and distribution of phospholipids in 60 to 70 year old patients with arthrosis of the hips, it has been found that regions with radiographically demonstrable sclerosis and cystic lesions revealed a higher lipid content than control biopsy specimens from corresponding regions in bone of normal structure. The changes have been tentatively ascribed to a damaged vascular bed, possibly due to abnormal loading.

In their experiment they used 26 white male rats. In the experimental animals, the maxillary and mandibular incisors were loaded by fastening an inclined plane on the mandibular teeth. They used six

experimental animals that were six weeks old, and 12 experimental animals that were 52 weeks old. The young experimental animals lost the inclined bite plane after 10 to 17 days. They were then sacrificed immediately. The older experimental animals were killed after 18 to 27 days. None of them had lost the bite plane.

The preparations of the right and left maxillary and mandibular incisors together with surrounding bone were immediately placed in 4°C EDTA solution. After demineralization for one week, they were sectioned on a cryostat. They were never fixed. The sections were stained with van Gieson, OTAN-technique and rhodamine B-technique.

Ingervall, et al.[35] found no difference between control and experimental young rats in the lipid content of the alveolar bone. In the older experimental rats, the amount of lipids was larger, and some osteons in the bony tissue around the loaded incisors showed fluorescence reactions. They attributed the increased occurrence of lipids to the abnormal loading of the teeth causing repeated trauma. Degenerative changes in bone can be promoted by such insults.

In 1971, Church[7] did a study on human tooth movement. At 21 days of continuous tipping movement, he reported a widened periodontal ligament space with stasis of blood vascular channels. In three of his specimens, he reported the morphologic evidence of fatty degeneration. He based this assumption on the appearance of vacuoles in the periodontal ligament which resembled the characteristic signet ring cells. He attributed the formation of these cells to fatty degeneration due to local ischemia.

In 1972, Goya did a study to corroborate Church's study. He found three possible explanations for Church's findings of vacuoles within the periodontal ligament, but he did not find nearly as many vacuoles as Church did. His first explanation was that the presence of a large number of capillaries within the reorganizing periodontal ligament gave the vacuolar appearance. The second was that the tooth had moved into a marrow space where there were fat cells, rather than fat cells being due to fatty degeneration. The third possible explanation he gave was that the vacuoles were artifacts of the

histologic staining technique.

MATERIALS AND METHODS

Eight dental patients who required the extraction of their maxillary first premolars for orthodontic therapy were utilized in this study. These patients included three males and five females who were 12 to 14 years of age. The maxillary first premolar which had the least hindrance to its movement by contacts or occlusal relationships served as the experimental tooth, and the other first premolar as an in-patient control.

An appliance, using an .045 lingual arch attached by vertical posts to cemented molar bands, was fabricated so that an .016 finger spring produced a buccal tipping force on the experimental maxillary first premolar. The appliance is similar to that used by Church[7] and Goya.[8] The spring was calibrated with an elastic tension gauge to produce a force of 75 grams over its working range. The spring was also checked in the mouth at the time of insertion with the same tension gauge to ascertain that the force was delivered within $\pm$10%

of 75 grams.

Small indentations were placed on the tip of the lingual cusps of both maxillary first premolars with a dental handpiece using a No. $\frac{1}{4}$ round bur. Bi-maxillary width measurements were taken on the 7th, 14th, and 21st days after appliance insertion using a needlepoint divider and transferring these to an index card.

On the 21st day following insertion of the appliance, the maxillary first premolars with approximately 7 mm. of buccal alveolar bone were surgically removed. A technique similar to that used by Goya,[8] and Church,[7] and by Reitan[4,5,6] was used to remove the teeth and alveolar bone. Healing following these extractions was uneventful.*

The specimens were immediately placed in a 10% neutral buffered formalin solution. After 24 hours, the crowns of these teeth were sectioned from the root and bone by a dental handpiece. The specimens were returned to the formalin solution for one week. They were then rinsed and placed in Kristensen Decalcification Solution (sodium formate

*Surgery was under the supervision of Dr. Ralph G. Merrill of U.O.D.S., whose aid is gratefully acknowledged.

and formic acid). After 14 days, x-rays (90 KP, 10 ma, 1/10 sec.) were taken of all specimens to verify decalcification. Three specimens required 16 days of decalcification and two specimens required 18 days. The specimens were then rinsed and placed in lithium carbonate for 24 hours. After 48 hours of rinsing, frozen sections were cut on a clinical microtome using carbon dioxide as the freezing agent.

The specimens were randomly divided into two groups, keeping the experimental and control specimen from each patient in the same group. Transverse sections were taken on group number one and frontal sections on the other group. Hemotoxylin-and-eosin and Mallory stains were done on representative sections of each group. The lipid specific stain Sudan IV was used on the frontal sections and Oil Red O on the transverse sections.*

All slides were coded and read by three independent observers using a Zeiss GL 4 Brightfield microscope and photomicrographs were

* The histologic procedures were performed by the University of Oregon Dental School Tissue Laboratory under the supervision of Dr. Robert Quinton-Cox whose aid is gratefully acknowledged.

made on a Zeiss photoscope.

After the slides were viewed, additional sections of the specimens, which demonstrated a positive Oil Red O fat stain in the cell-free area, were re-stained with Sudan Black B. This procedure was done as an additional cross check on the laboratory procedures to rule out that the Oil Red O staining might be an artifact.

The observed data are listed in Table II.

HISTOLOGIC OBSERVATIONS

The sample for this study consisted of 16 teeth with the attached buccal alveolar bone. Four experimental and four control teeth were sectioned frontally, and the remaining four experimental and four control teeth were sectioned transversely. The staining of these histologic sections is described in the MATERIALS AND METHODS section. All the experimental specimens were subjected to a continuous tipping force for 21 days. The histologic sections of all specimens were satisfactory for study.

The purpose of this study was to investigate through the use of fat specific stains the nature of the "signet-ring" cells described by Church[7] and Buck and Church[35] after 21 days of tooth movement. The possible interpretations for these "signet-ring" cells are fat cells or artifacts. After viewing the slides, only one frontally sectioned control specimen demonstrated morphologic evidence of fat cells in the periodontal ligament (Fig. 1). These "signet-ring" cells

were insignificant in number when compared to those reported by Church.[7] They were isolated to a small area of the periodontal ligament and did not demonstrate any histochemical Sudan IV fat specific stain. Another control specimen did show some evidence of Sudan IV histochemically stained fat cells in the periodontal ligament. However, these were not conclusive since they were few in number and were located adjacent to the alveolar bone.

Five specimens did show morphologic evidence of fat cells in the alveolar bone marrow spaces. In the specimens stained with Sudan IV, one experimental and one control specimen demonstrated histochemically stained fat cells in the alveolar bone marrow spaces. Two control and one experimental specimen in the Oil Red O group also showed a similar histochemical staining of fat cells in the marrow.

Due to the insignificant number and lack of histochemical staining up-take of fat cells in the periodontal l'gament of the specimens, we were unable to find conclusive evidence as to whether or not these cells were true signet-ring fat cells or artifacts. However, we did

demonstrate several findings common to all the specimens which may prove to be meaningful.

In five of our eight experimental specimens, there were areas of compression of the periodontal ligament characterized by the condensation of the collagen fibers, the absence of the PDL cells, and the obliteration of all blood vascular channels and nerve bundles (Fig. 2). These were highly characteristic of the area of "hyalinized" periodontal ligament described by Reitan[5] and the cell-free areas described by Church,[7] Goya,[8] and Buck and Church.[35] These areas were located in the coronal one-third of the specimen and the measured mean width of the periodontal ligament of the cell-free area was 89 microns as compared to 290 microns for the normal periodontal ligament of our control specimens (Fig. 3). The experimental teeth which developed the cell-free areas had clinically moved between .5 and 1.5 mm. as measured with dividers from the pits in their lingual cusp tips (Table III). This cell-free area was evident in one experimental tooth of the frontally sectioned group and in all four experimental teeth in the transversely sectioned group.

All specimens with cell-free areas showed a definite intense histochemical fat stain in the cell-free area. The one frontal specimen stained with Sudan IV demonstrated intense Sudanophillic staining throughout the cell-free area (Fig. 3). All four of the transverse specimens showed a definite intense Oil Red O uptake in the cell-free zone (Figs. 4,5,6). Three comparable slides of the specimen of the transversely sectioned group were restained with Sudan Black B and demonstrated an intense histochemical Sudan Black B staining reaction in the cell-free area (Figs. 7 and 8).

The staining reaction in all the cell-free areas demonstrated extra cellular lipid material. There was no evidence of "signet-ring" cells in or near the cell-free area. The nerve trunks and marrow spaces in the specimens which showed positive histochemical fat staining in the cell-free areas, also demonstrated positive fat stain uptake indicating specificity and reliability of the staining reaction.

There was associated undermining resorption adjacent to the cell-free areas similar to that reported by Goya[8] (Figs. 3,5,8). Most

specimens also showed frontal osteoclastic activity and undermining resorption within the marrow spaces as first described by Sandstedt.[34] In some areas the undermining resorption had broken through the alveolar bone into the periodontal ligament. Several of the specimens showed evidence of fibrous immature bone adjacent to the sites of active frontal resorption.

Contrary to the findings of Goya,[8] the lateral root resorption was minimal and showed no correlation to be adjacent to the cell-free areas. The periodontal ligament adjacent to the cell-free areas was widened and undergoing reorganization similar to that reported by Goya[8] (Figs. 3,5,6, and 8).

All the control specimens showed numerous and regularly spaced glomera located in the periodontal ligament similar to those reported by Provenza[36] (Fig. 9). The fibers of the periodontal ligament were well organized and circumvented these glomera. The glomera appeared to be located in the coronal one-third of the periodontal ligament. However, it was not possible to pinpoint their exact location since

the sections were taken randomly from the specimen.

DISCUSSION

The sample investigated consisted of eight dental patients who required the extraction of their maxillary first premolars for orthodontic treatment. No difficulty was encountered in fabricating the appliance which produced tooth movement, and it proved satisfactory for this study. No appliances were lost or damaged, and the patients experienced no apparent displacement of the finger springs between appointments.

The tooth movement of each experimental specimen is recorded in Table II and plotted graphically in Table III. These measurements were taken to indicate that the amount of tooth movement accomplished should be sufficient to initiate histologic responses. The variations in measured tooth movement may have been caused by several factors. The force of some finger springs may have disipated rapidly while others remained constant. The movement of some experimental premolars may have been hindered by tight contacts or occlusal interferences. This possible hinderance could be removed by disking the contacts and

relieving the occlusion of the experimental premolars. However, the individual biologic variation to tooth movement is probably the more important factor producing this movement difference. The data on the millimeters of tooth movement was not statistically analyzed owing to the small sample size. The control specimens demonstrated no abnormal mobility or evidence of deflection during the stage of active force on the experimental premolars.

The surgical removal of all specimens was successful with no apparent separating or fracturing of the buccal alveolar bone. The postoperative healing was uneventful and it appears that the surgical removal of the tooth and attached bone is a simple, repeatable, oral surgical procedure.

The histologic sections of the eight experimental and eight control premolars with attached alveolar bone were all relatively free of artifacts and were satisfactory for microscopic study. Only two specimens presented bone which had been partially separated from the periodontal ligament. However, they presented material adequate for

interpretation and were included in the study.

The insignificant number of "signet-ring" cells found in the periodontal ligament does not support the findings reported by Church[7] and Buck and Church.[35] Contrary to our expectations, the morphologic evidence of "signet-ring" cells in the periodontal ligament was found only in two control specimens. The experimental specimens demonstrated no conclusive evidence of fat cells in the periodontal ligament. There were far more cell-free areas present in the 21 day tooth movement specimens of this investigation than in those reported by Buck and Church,[35] most likely due to our increased sample size. There may not have been 21 days of continuous tooth movement due to interferences by tight contacts, occlusion, or fatigue of the finger spring. However, this is unlikely because of the magnitude and frequency of the measured tooth movement (Tables II and III).

The lack of Sudanophillic staining of the few "signet-ring" cells found in the periodontal ligament may have been caused by improper staining techniques or that they are not fat cells. There is much

disagreement on the proper procedures for staining fat as is evident in the papers of Irving,[25] Das and Harris,[29] and Ingervall, et al.[33]

There was evidence of Sudanophillic staining of nerve trunks and fat cells in marrow spaces, so it appears that the staining techniques were adequate. By combining the findings of this investigation and those of Goya[8] the presence of cellular fat as reported by Church[7] and Buck and Church[35] cannot be corroborated.

Five of our eight experimental specimens demonstrated the cell-free areas described by Reitan,[4,5,6] Church,[7] Goya,[8] and Buck and Church.[35] The major finding of this investigation was that all five of these cell-free areas demonstrated intense Sudanophillic staining reaction when stained with Sudan IV, Oil Red O and Sudan Black B. One frontal experimental specimen stained with Sudan IV demonstrated this intense Sudanophillic staining in the cell-free area. All four of the transverse experimental specimens stained with Oil Red O showed this staining in the cell-free area. After noting this, three slides of the transverse specimens were re-stained with Sudan Black B as an

additional cross check on the histologic techniques. These also demonstrated intense Sudanophillic uptake in the cell-free area. There was no evidence of fat cells in or around the cell-free areas.

The nerve trunks and marrow space fat cells of these specimens also demonstrated a positive Sudanophillic staining which indicates specificity and reliability of the staining reaction.

This Sudanophillic staining reaction demonstrates the presence of extracellular lipid in the cell-free area. However, the investigation was not designed to analyze the cause or the types of extracellular lipids demonstrated in the cell-free area. According to Pearse,[37] the extracellular lipids demonstrated by our stains are the following: Sudan IV--lipids in general, Sudan Black B--phospholipids, and Oil Red O--neutral lipids. However, there is no field of biochemistry in which there is less uniformity than the lipids.[37] To adequately analyze the lipids stained in our specimens would involve many complicated histochemical procedures which we were unable to perform.

The extracellular fat in the cell-free area may be the results of

fatty degeneration or infiltration, which is usually initiated by ischemia, anoxemia, trauma, or metabolic disturbances.[23,24] Itoiz, et al.[27] demonstrated that localized ischemia in the gingival tissue of monkeys cause an increase of lipids in the intercellular spaces. Ingervall, et al.[33] also reported increased lipids in the alveolar bone of rats whose teeth were subjected to traumatic forces. The fat appearing in fatty degeneration is considered to be a change to a visible form of the fat already present within the cells rather than an increase in lipid content as a result of accumulations from outside sources.[23,24]

However, the data of this investigation does not allow us to form any supportable conclusions as to the origin of this extracellular fat. The composition of collagen is primarily glycine, proline and hydroxyproline. The degeneration of collagen should not provide sufficient by-products to produce lipids since they are derivatives of non-cyclic hydrocarbons, steriods, or isoprene.[37] The extracellular

lipid material may have been deposited by degenerating macrophages which had migrated to the traumatized cell-free area. However, no specimens demonstrated evidence of macrophages around or in the cell-free area. One of the most likely sources of the extracellular lipids could be the degeneration of the myalin sheaths of the nerve fibers trapped in the cell-free area.

These statements support the conclusion that the extracellular fat present in the cell-free area has been released by the degeneration of structures found in the periodontal ligament. These Sudanophillic staining reactions in the cell-free area should be investigated further. The half life of fat cells is reported to be eight days and that of free fatty acids is only a few minutes.[20] Therefore, it would be interesting to investigate when this Sudanophillic staining in the cell-free area first occurs and the length of time it remains. This information may further help answer the questions as to the cause and source of this extracellular lipid, and how it is related to the histologic tooth movement response.

The frontal and undermining osteoclastic activity was in agreement with findings previously reported.[4,5,6,8,34,35]

The lateral root resorption demonstrated in our specimens was very minimal and was not consistent with the findings of Goya,[8] that there was a direct correlation between the cell-free areas and lateral root resorption.

The numerous and regularly spaced glomera found in all the control specimens was an unexpected finding. Provenza[36] felt the glomera were possibly a pathologic response to trauma or inflammation so they were not expected to appear in our controls. However, this may be caused by the fact that the premolars investigated were undergoing active eruption. It would be interesting to compare the controls of this investigation with histologic sections from adult specimens. It was impossible to locate the exact location of these glomera in the periodontal ligament since these sections were taken randomly from the specimens. However, the majority of our sections were taken from the coronal one-third of the tooth. In contrast to the findings of Goya,[8] the experimental specimens did not demonstrate an increased number of glomera in the

periodontal ligament. From our data there is no means of explaining the difference.

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate, through the use of fat specific stains, the nature of the "signet-ring" cells described by Church[7] and Buck and Church[35] after 21 days of tooth movement.

Eight dental patients who required the extraction of their maxillary first premolars for orthodontic therapy were utilized in this study. The maxillary first premolar which had the least hindrance to its movements by contacts or occlusal relationships served as the experimental tooth, and the other premolar as an in-patient control.

An appliance, using an .045 lingual arch attached by vertical posts to cemented molar bands, was fabricated so that an .016 finger spring produced a buccal tipping force on the experimental maxillary first premolar. The spring was calibrated with an elastic tension gauge to produce a force of 75 grams over its working range.

On the 21st day following insertion of the appliance, the maxillary first premolars with approximately 7 mm. of buccal alveolar bone were

surgically removed.

The specimens were fixed in 10% neutral buffered formalin solution and were decalcified in Kristensen Decalcification Solution (Sodium formate and formic acid).

The specimens were randomly divided into two groups, keeping the experimental and control specimen from each patient in the same group. Transverse sections were taken on one group and frontal sections on the other group by a liquid nitrogen frozen section sliding microtome technique. Hemotoxylin-and-eosin and Mallory stains were done on representative sections of each group. The lipid specific stain Sudan IV was used on the frontal sections and Oil Red O on the transverse sections.

Our histologic findings did not demonstrate the presence of fat cells during tooth movement in the compression zone of the periodontal ligament. In five of our eight experimental specimens, there were cell-free areas in the periodontal ligament space. All five of these specimens showed a definite intense histochemical fat stain in the

cell-free area. Furthermore, all the control specimens showed numerous and regularly spaced glomera located in the periodontal ligament similar to those reported by Provenza[36] but of no apparent significance to the study of lipid-bearing tissue.

The findings of this study will support the following conclusions:

1) Fat of unknown origin is definitely present in cell-free areas of the periodontal ligament space after light orthodontic tipping forces have been applied to human teeth for 21 days.

2) Very little is known about this fat and it should be studied further, not only in human tooth movement material but in animal tooth movement material as well.

3) In the young patient with actively erupting teeth, numerous regularly spaced glomera in the periodontal ligament space adjacent to the cervical area appear to be a normal biologic finding.

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TABLE I[10]

A CLASSIFICATION OF HISTOCHEMICALLY DEMONSTRABLE LIPIDS

SIMPLE LIPIDS

- Triglycerides
- Ester Waxes

COMPOUND LIPIDS

- Phospholipids
  - Phosphatidic Acids
  - Phosphatidyl Esters
- Glycolipids
  - Cerebrosides
  - Gangliosides

DERIVED LIPIDS

- Fatty Acids
- Fatty Aldehydes
- Alcohols

STEROLS & STEROL ESTERS

- Cholesterol
- Cholesteryl Esters

ASSOCIATED SUBSTANCES

- Carotenoids
- Fat Soluble Vitamins
- Steroids
- Lipid Pigments

Table II. Summary of Histologic Observations

Specimen Number	Exp. or Control	Histochemical Fat Stain	Cell-free Area	Amount of movement in mm.	Evidence of Glomera
21 R	C	o	o	o	yes
21 L	E	o	no	2.0 mm.	no
22 R	E	CFA Sudan IV+ M Sudan IV+	yes	.9 mm.	no
22 L	C	o	o	o	yes
23 R	C	o	o	o	yes
23 L	E	o	no	1.2 mm.	no
24 R	C	M Sudan IV+ PDL Sudan IV+	o	o	yes
24 L	E	o	o	.7 mm.	no
25 R	E	CFA Oil Red O+	yes	1.3 mm.	no
25 L	C	M Oil Red O+	o	o	yes
26 R	E	CFA Oil Red O+ M Oil Red O+	yes	.5 mm.	no
26 L	C	o	o	o	yes
27 R	C	o	o	o	yes
27 L	E	CFA Oil Red O+	yes	1.5 mm.	no
28 R	E	CFA Oil Red O+ Sudan Black B+	yes	1.1 mm.	no
28 L	C	M Oil Red O+	o	o	yes

Legend:
o - Not present or not applicable.
\+ - Positive for lipid presence.
CFA - Cell-free area.
M - Marrow
PDL - Periodontal ligament.

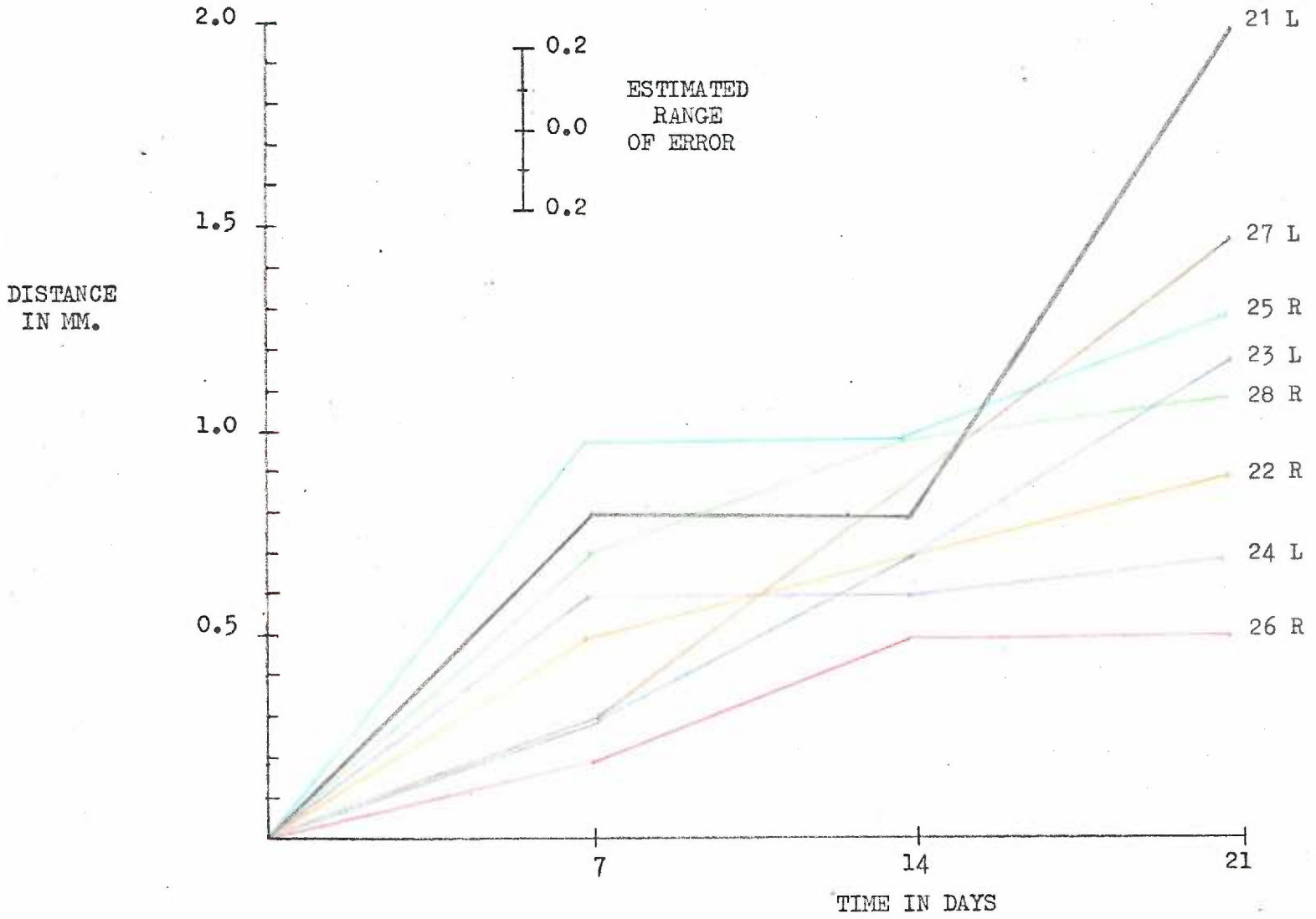

TABLE III. MOVEMENT OF MAXILLARY FIRST BICUSPIDS

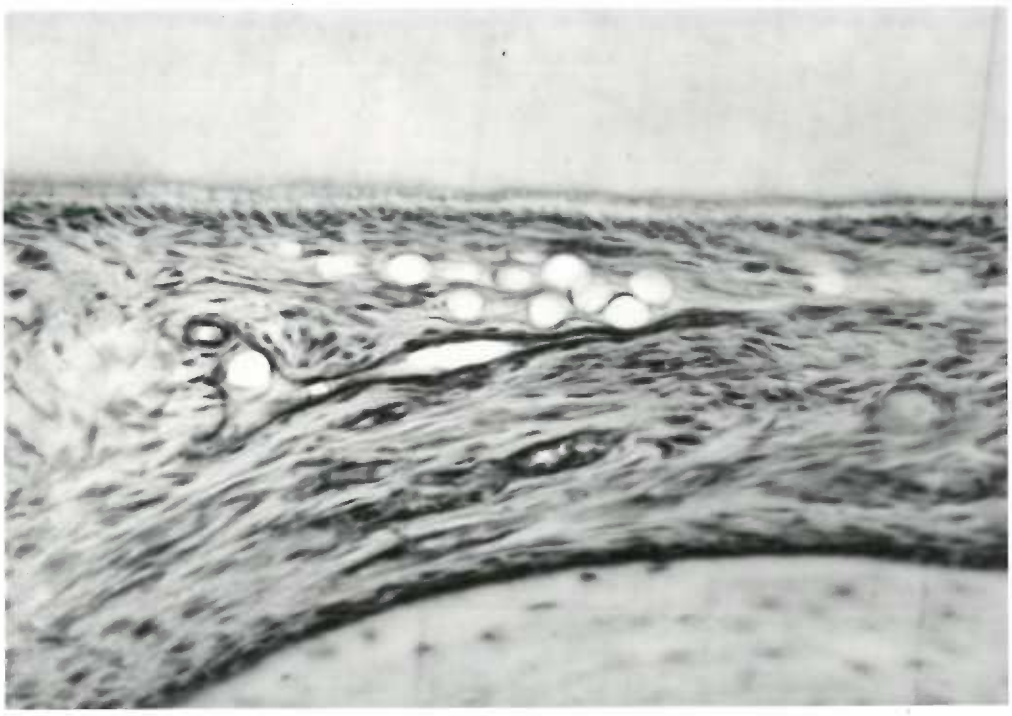

FIG. 1 PHOTOMICROGRAPH OF A FRONTAL SECTION OF PERIODONTAL LIGAMENT DEMONSTRATING CELLS SIMILAR TO FAT CELLS, BUT NOT POSITIVE TO STAIN. (SUDAN IV)

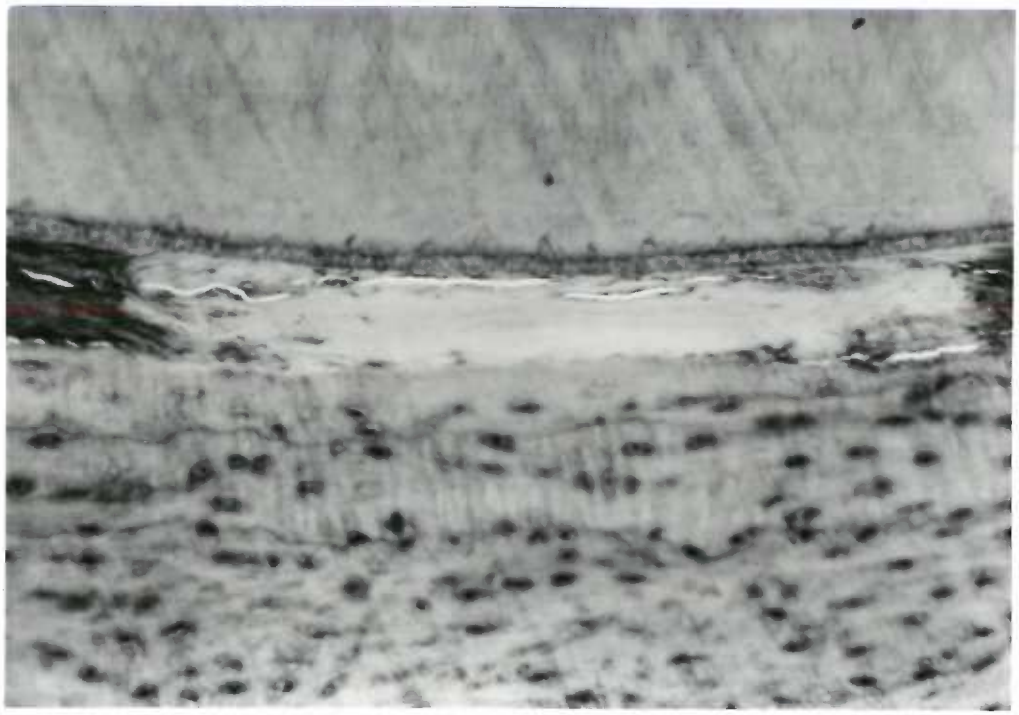

FIG. 2 FRONTAL SECTION OF AN AREA OF COMPRESSION OF THE PDL DEMONSTRATING THE CHARACTERISTIC CELL-FREE ZONE. (STAIN - HEMATOXYLIN AND EOSIN)

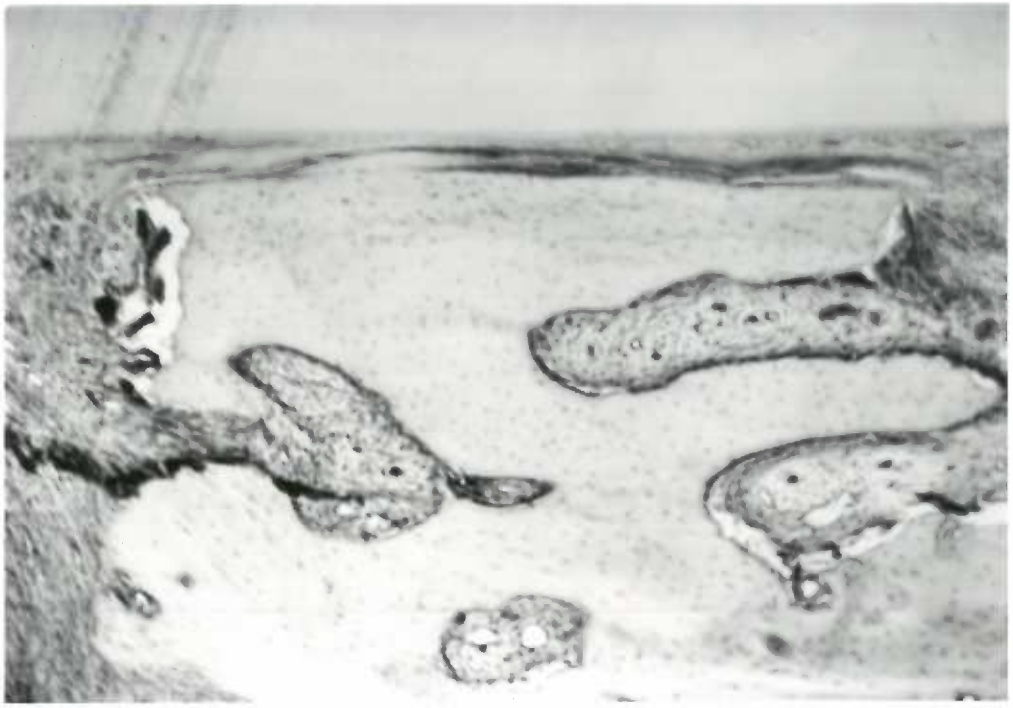

FIG. 3 A COMPRESSION AREA OF THE PDL DEMONSTRATING THE REDUCED WIDTH OF THE CELL-FREE ZONE WITH SUDAN POSITIVE STAIN IN THE COMPRESSION ZONE. (FRONTAL SECTION) (STAIN - SUDAN IV)

FIG. 4 A CELL-FREE AREA DEMONSTRATING A LIPID POSITIVE REACTION. (TRANSVERSE SECTION) (STAIN - OIL RED O)

FIG. 5 A CELL-FREE AREA DEMONSTRATING A LIPID POSITIVE REACTION AND ADJACENT REORGANIZATION. (TRANSVERSE SECTION) (STAIN - OIL RED O)

FIG. 6 A LOCALIZED CELL-FREE AREA WITH REORGANIZATION OF PDL ADJACENT TO CELL-FREE AREA. (TRANSVERSE SECTION)
(STAIN - OIL RED O)

FIG. 7 INTENSE SUDANOPHILLIC STAINING OF A CELL-FREE AREA. (TRANSVERSE SECTION) (STAIN - SUDAN BLACK B)

FIG. 8 INTENSE SUDANOPHILLIC STAINING OF A CELL-FREE AREA WITH POSITIVE STAIN OF AN UNDERMINING OSTEOCLAST. (TRANSVERSE SECTION) (STAIN - SUDAN BLACK B)

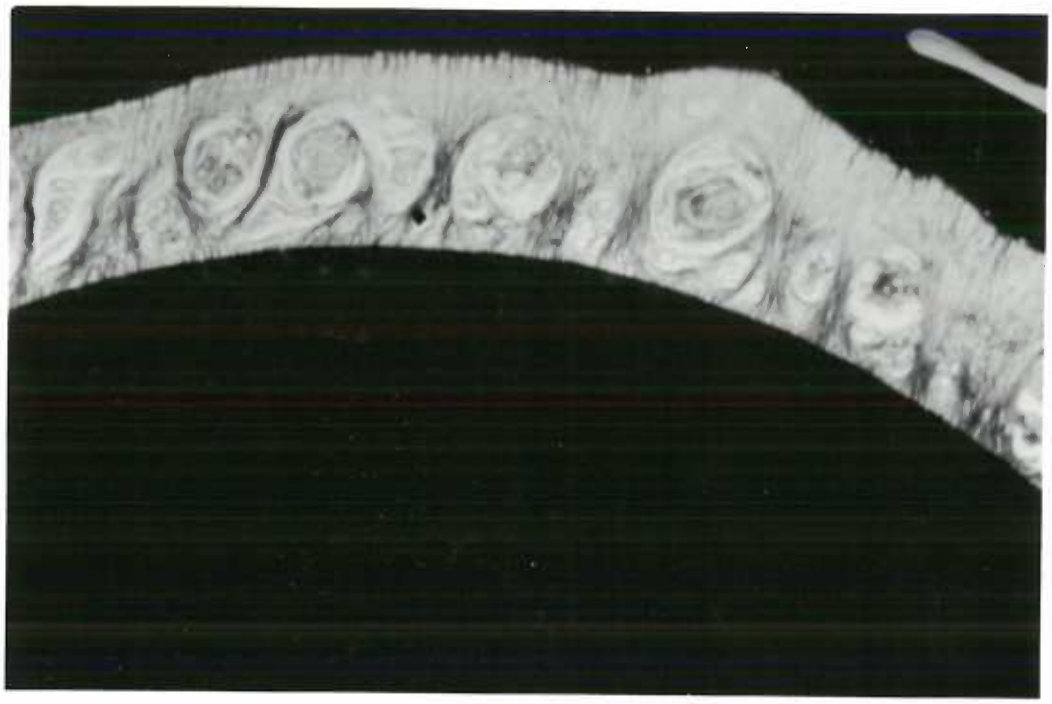

FIG. 9 TRANSVERSE SECTION OF NUMEROUS AND REGULARLY SPACED GLOMERA OCCURRING IN THE PDL OF ALL CONTROL SPECIMENS.
(STAIN - MALLORY CONNECTIVE TISSUE STAIN)