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Determination of the Doubling Time of the Connective Tissue Cell of the Young Rat Periodontium

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DETERMINATION OF THE DOUBLING TIME OF THE CONNECTIVE TISSUE CELL OF THE YOUNG RAT PERIODONTIUM

by

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LIFE

Hsueh-Wan Kwan was born in Tientsin, China on November 11, 1923. She graduated from Yao Hua High School, Tientsin in 1942 and received a Doctor of Dental Surgery degree from the National Central University in Nanking, China in 1948.

From March, 1949 to October, 1951 she practiced general dentistry in Hong Kong at the Government Dental Clinic. From July, 1952 to February, 1956, Dr. Kwan worked in a school health program sponsored by ABMAC in Taiwan, China. In August, 1957, she became a teaching assistant in the School of Dentistry of National Taiwan University,

In September, 1961, Dr. Kwan began graduate studies in the department of Oral Pathology of the Loyola University School of Dentistry in Chicago for one year. In September of 1962, she returned to Taiwan where she began teaching Oral Pathology at the National Taiwan University until 1968. In September of 1968, she returned to Loyola University and continued her graduate studies in the department of Oral Pathology.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

It is a known fact that a series of tissue changes happens along with the physiologic aging process resulting in various tissue reactions to its environments. Many studies on different tissues of human and experimental animals had been done in this aspect. The rat molar provides a fair comparison to human tooth. Kindlova (1963), Schour (1949); except for the physiologic distal drift of the rat molars. Sicher and Weinmann (1944). For this reason, the rat molar has been used widely in experimental studies. This report is part of a general study evaluating the aging effects upon the doubling time of the connective tissue cell in the periodontium of different age groups of normal rat molar.

REVIEW OF THE LITERATURE

I. Aging and connective tissue

Connective tissue is the supporting, binding and packing tissue found everywhere in the body. It contains living cells, fibers and ground substance. It is the basic and unique reactive tissue in the body concerning development, growth, defense and repair. Any factor that may induce changes of this tissue will lead to significant varied tissue reactions. Aging, the physiologic process, has been demonstrated with distinguishable effects upon the connective tissue.

Klinsberg(1960) observed all the tissues appear to have uniform morphology and structure in the young rat and hamster and become less cellular and more fibrous with increasing age. Thinning of the periodontal membrane and osteoporosis are associated with aging.

Wentz's (1952) study of clinical normal human gingiva describes the connective tissue showing decline in cellular elements and increasing coarseness of fibers with advancing age.

Belting (1953) found that cellular activity at alveolar crest seemed to be arrested in the old rat, Bernick (1962) study of age changes in the blood supply of rat molars, noticed a progressive decrease of the interseptal vessels and their perforating branches with aging resulted from a gradual thickening and fusion of the bony trabeculae of the interseptal bone with the marrow space limited to its apical portion.

Everitt (1958) researched the aging process in rats found that all the physiologic activities decreased with age. Narrowing of human periodontal membrane and decrease in its cellular elements were described by Coolidge (1937) and Schour (1953).

Lavelle's study (1968) on the histologic structure of the rats incisors suggested a reduction of the rate of formative tissue proliferation with advancing age.

The metabolic activity of skin connective tissue is reduced in old animals as compared with young ones. There is progressive diminuation of hyaluronic acid and water content with increased accumulation of chondroitin sulfate B and collagen, Davidson (1963), Clausen (1966).

The amount of soluble collagen decreases and the insolluble collagen increases as a function of age, Mitchell (1964). Owing to the high concentration of hydroxyproline in fibrous protein and hexosamine, a constituent of the mucopolysaccharide of the ground substance which determine its gel-like quality, the ratio hexosamine to collagen represents the fibrillar density. A decline of this ratio was demonstrated in aging connective tissue as the hexosamine content fell, while the hydroxyproline content increased. This influences the nutrition and metabolism of the cells to a great extent since they are dependent on the properties of the gel state of the ground substance, Sobel and Marmoston (1956), Sobel and Gabay (1958).

Biophysical evidence revealed molecular collagen ages and the established crosslinks rendered it more aggregated with a tendency to increase in size with aging, Elden (1964).

The reduction in the production of sulfated mucopclysaccharides is proportional to the decrease of young cells, Toto (1967).

Autoradiographic studies by Jensen (1966) demonstrated a decreased labellin index in the periodontal membrane of aging rats, Pinzon (1967) found a similar reduction of labeled cells with age in the pulp.

Borg (1967) studying age changes of the periodontal membrane of mice stated that the ratio of labeled to non-labeled cells is significantly greater in the young mouse. A tendency for doubling to occur after twenty hours was shown in the sixty day old mice.

Lord (1964) found the doubling of labeled cells within twenty-four hours in the periodontium of the orthodontically moved rat molar.

II. Autoradiography

Radioactive isotopes have been the popular materials used in biologic studies. Prior to actual cell division, synthesis of DNA takes place in the nucleus. Tritiated thymidine is used as a precursor for DNA, Reichard and Estborn (1951), autoradiography makes it possible to locate cell nuclei which have incorporated tritiated thymidine during DNA synthesis. The uptake of tritiated thymidine by the tissue is generally completed about one hour following injection and the excess is excreted and can only lose the radioactive label upon cell death or dilution by further division, Hughes (1958), Messier (1960).

Tritium atoms undergo radioactive decay and emit low energy beta rays which can activate silver grains in a photographic emulsion and these grains will appear as black grains when viewed with a light microscope, King (1965).

MATERIAL AND METHOD

Thirty-seven sixty day old hooded male rats were injected with tritiated thymidine, specific activity 1.90 curies/m mole, intraperitoneally at a dose of 1.0 uc per gram of body weight. Each rat was sacrificed at two to four hour intervals through a hundred hours.

The maxillas were dissected and fixed in 10 percent neutral formalin. The specimens were decalcified with formic-citric acid solution. Histologic sections of 4 microns to 6 microns were cut after usual prodedures of dehydration and embedding. All the sections were cut through the first molar mesiodistally. Both autoradiographs and hematoxylin and eosin stains of each section were prepared.

A whipple disc was inserted into the eyepiece of the microscope. All the cells (labeled and non-labeled) in the periodontium of the mesial surface of the mesial root were counted except the endothelial cells and blood cells. It started from the level of alveolar crest and extended to the center of the apex. The cells in ten sections of each specimen were counted and the average was calculated. All of the labeled cells were counted in each section. The ratio of labeled to non-labeled cells and the doubling time of labeled cells were determined. The criterion for a labeled cell was one that contained at least four or more silver grains over the cell nucleus.

FINDINGS

The labeled cells in the periodontium on the mesial surface of the mesial root were mostly found in the vicinity of the apex and the alveolar crest.

The distribution of labeled cells per specimen at each time interval was shown in Figure I. The percentages of labeled cells rose rapidly to double between 2 hours and 16 hours. The rise in labeled cells continued showing even higher values at 52 hours, after tritiated thymidine injections.

The average number of all cells (labeled and non-labeled) in the connective tissue of the periodontium of the 60 day old rat as shown in Table I is 8,063.

Bone resorption was noted on mesial surface of the alveolar bone.

DISCUSSION

The mitotic potential can be studied and measured by the use of autoradiographic identification of cells that are actively engaged in synthesizing DNA prior to cell division.

The doubling time of the labeled periodontal connective tissue cells in rats is between sixteen to eighteen hours as the number of labeled cells gradually increased and doubled in this time interval. Pulp studies by Taiyong (1968 M.S. Thesis) showed that the doubling time in pulp of sixty-day old rats to be approximately twenty hours. A tendency for doubling to occur after twenty hours was shown in the sixty day old mice periodontium and twenty-two to forty-four in three-hundred day old mice, Borg (1967).

The greater cellular activity at the apex reflects the need for precursor cells to form both cementum and bone as the teeth erupt throughout life. Continuous cemental deposition occurs in the rat molar, Belting (1953). Apposition of cementum is necessary not only to keep the superficial surface of the root vital but also for functional orientation of periodontal membrane fibers, Sicher (1954). When the root is fully developed, bone apposition at the alveolar fundus like cementum formation compensates for eruption, Sicher (1942). The rat first molar comes into functional occlusion at twenty-three days and the roots are completed at thirty days, O'Brien and Bhaskar (1958). Secondary cementum formation begins at thirty-five days and continues throughout life, Schour and Massler (1949).

The bone resorption at mesial alveolar bone surface was not controversial to the distal drift of the rat molars characterized by bone apposition

along the mesial alveolar wall and bone resorption along the distal wall described by Sicher and Weinmann (1944) as it had to provide the necessary space for the axial movement of the strong and mesially spread mesial root.

The percentage of distribution of labeled cells at different times showed some irregularity. This fluctuation could be due to differences in the thickness of sections resulting in a failure of adequate radiation of the emulsion and also variability in the amount of radioactive thymidine arriving at the site of DNA synthesizing cells. Probably a greater number of cell counts could have decreased variability in this data. Fluctuation in the percentage of distribution of labeled cells might also be attributed to individual variation.

In Figure I, the second maximal level which occurred between fifty-two to fifty-six hours may represent the initially doubled labeled cells which contributed the sixteen to eighteen hour population , to have again gone through division. There is also a possiblity of reutilization of the tritiated thymidine (released by degenerated or dead labeled cells) by cells undergoing DNA synthesis at that particular time.

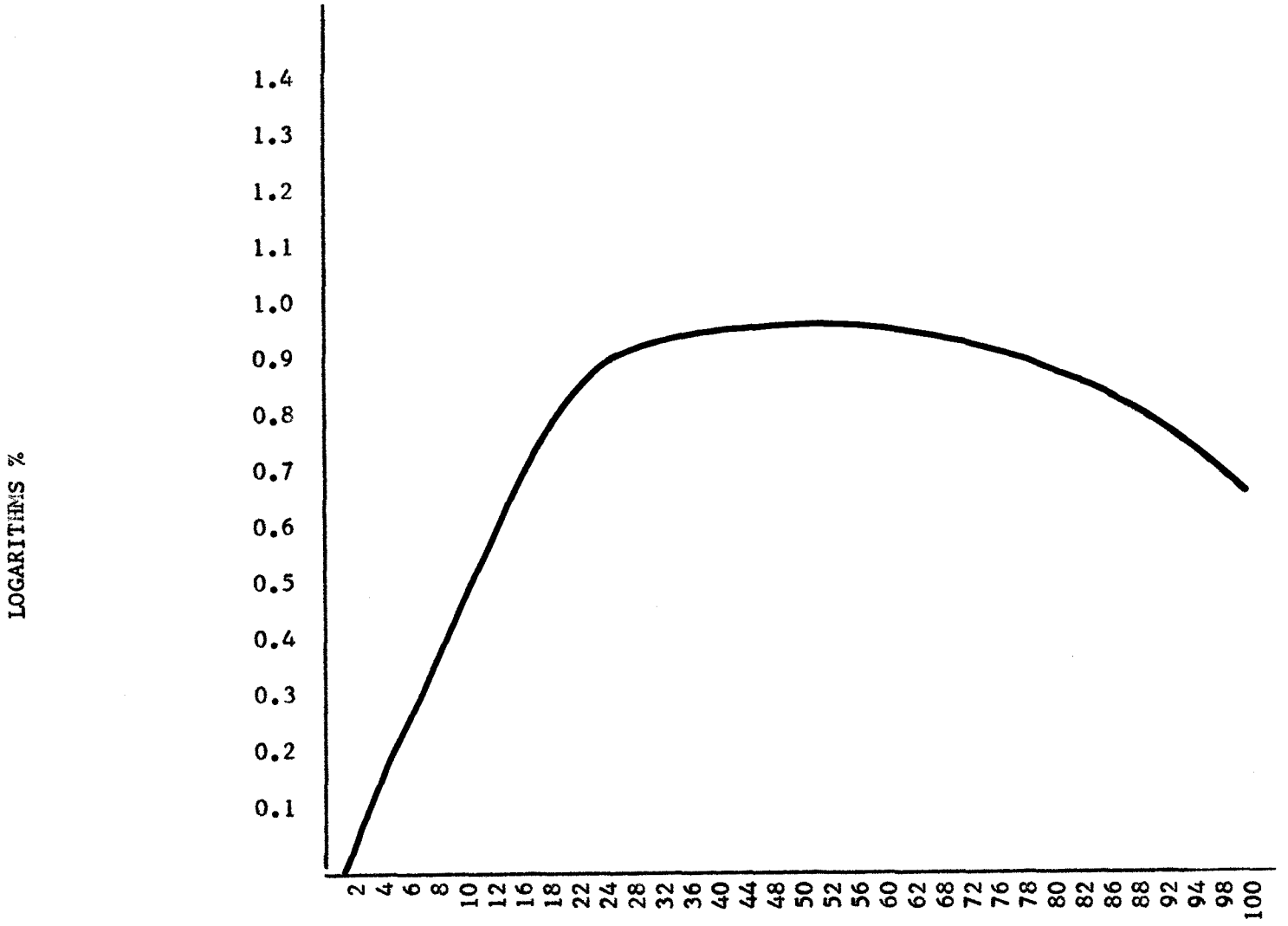
According to Jensen (1966) and Borg (1967), the labeling index was higher in young age group and the doubling time appeared to be slightly delayed in old mice. The decrease in the percentage of labeled connective tissue cell denoting the decline in sources of undifferential cells in the periodontium was steadily progressive with age. However, there are old cells available which possessed the capability of repair and growth. It was compatible with the slower metabolic rate and diminished function of all cells and

tissues in the aging periodontium that resulted in impaired homeostasis. The lack of reserve cells was interpreted as lowered resistance to infection and slower convalescence after injury. Healing in the aged is typically fibrotic, Massler (1956).

SUMMARY AND CONCLUSION

Thirty-seven sixty-day old hooded male rats were sacrificed after injection with tritiated thymidine at two to four hour intervals. The labeled and non-labeled connective tissue cells of mesial surface of mesial root were counted and the doubling time of the labeled cells was determined at sixteen to eighteen hours and again from fifty-two to fifty-six hours. It was shown that 1.24 percent of the cells were labeled after two hours. 2.48 precent were labeled after 16 hours and 4.29 percent were labeled after 52 hours.

The greater distribution of labeled cells is seen at the apex and alveolar crest.



PERCENTAGE DISTRIBUTION OF LABELED CELLS
IN THE PERIODONTIUM OF RAT

FIGURE I,

TABLE I . PERCENTAGE DISTRIBUTION OF LABELED CELLS IN THE PERIODONTIUM OF THE RAT MOLAR

TOTAL AVERAGE NUMBER OF CELLS PER SPECIMEN: 8063*

TIME INTERVAL (HOUR)	NUMBER OF LABELED CELLS	PERCENTAGE OF LABELED CELLS
2	101	1.24
4	53	0.65
6	55	0.68
8	81	1.01
10	63	0.78
12	85	1.05
16	200	2.48
18	197	2.44
22	109	1.35
24	122	1.51
28	275	3.41
32	87	1.08
36	113	1.27
40	253	3.14
44	121	1.50
48	90	1.13
50	86	1.06
52	356	4.29
56	198	2.45
60	216	2.57
62	171	2.10
68	0	
72	110	1.36
76	256	3.17
78	96	1.06
80	196	2.43
82	77	0.95
86	184	2.28
88	115	1.42
92	111	1.37
94	160	1.98
98	92	1.14
100	135	1.67

* There is an average of 8063 cells in the periodontium of the mesial surface of the mesial root of the 60 day old rat molar.

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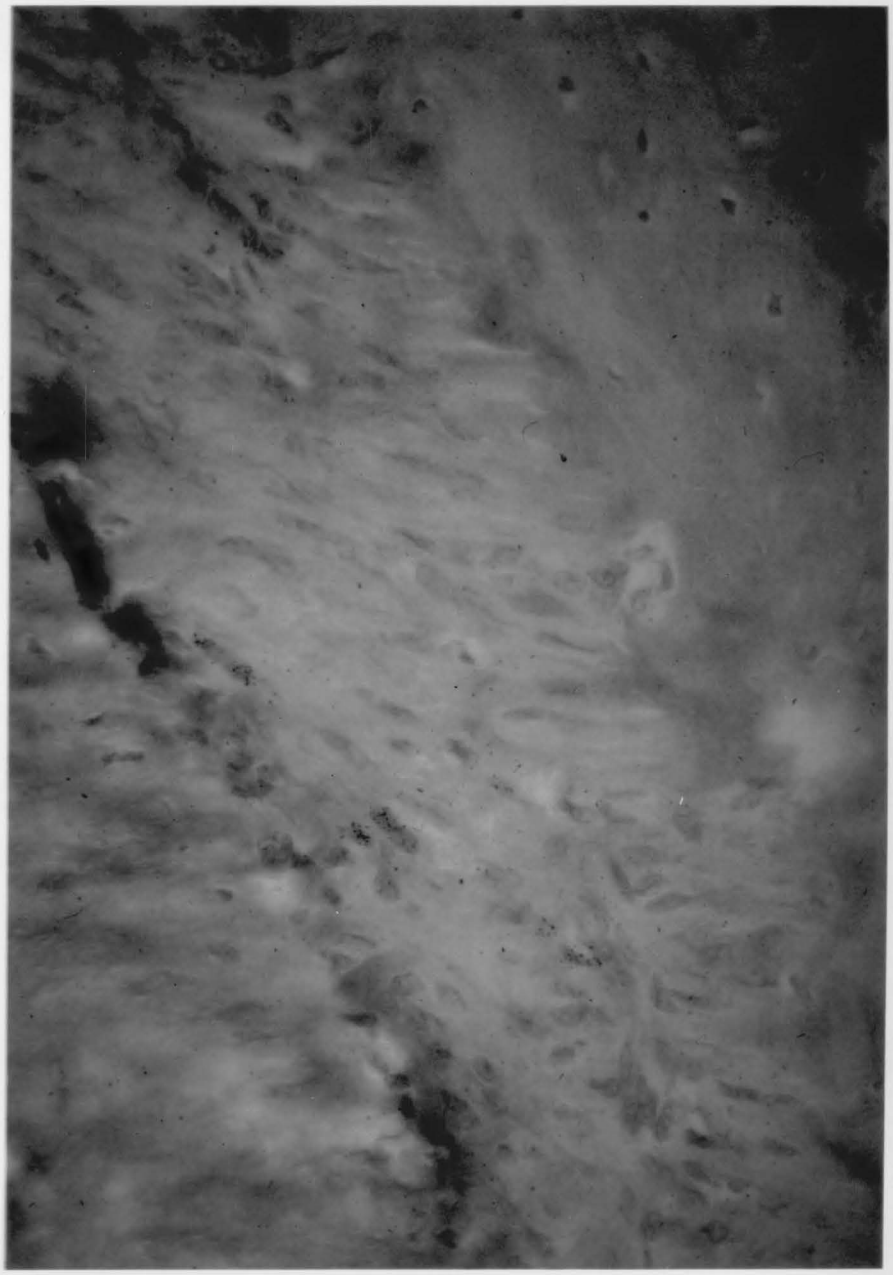

Fig 1. The labeled cells in the periodontium at two hours duration after tritiated thymidine injection.

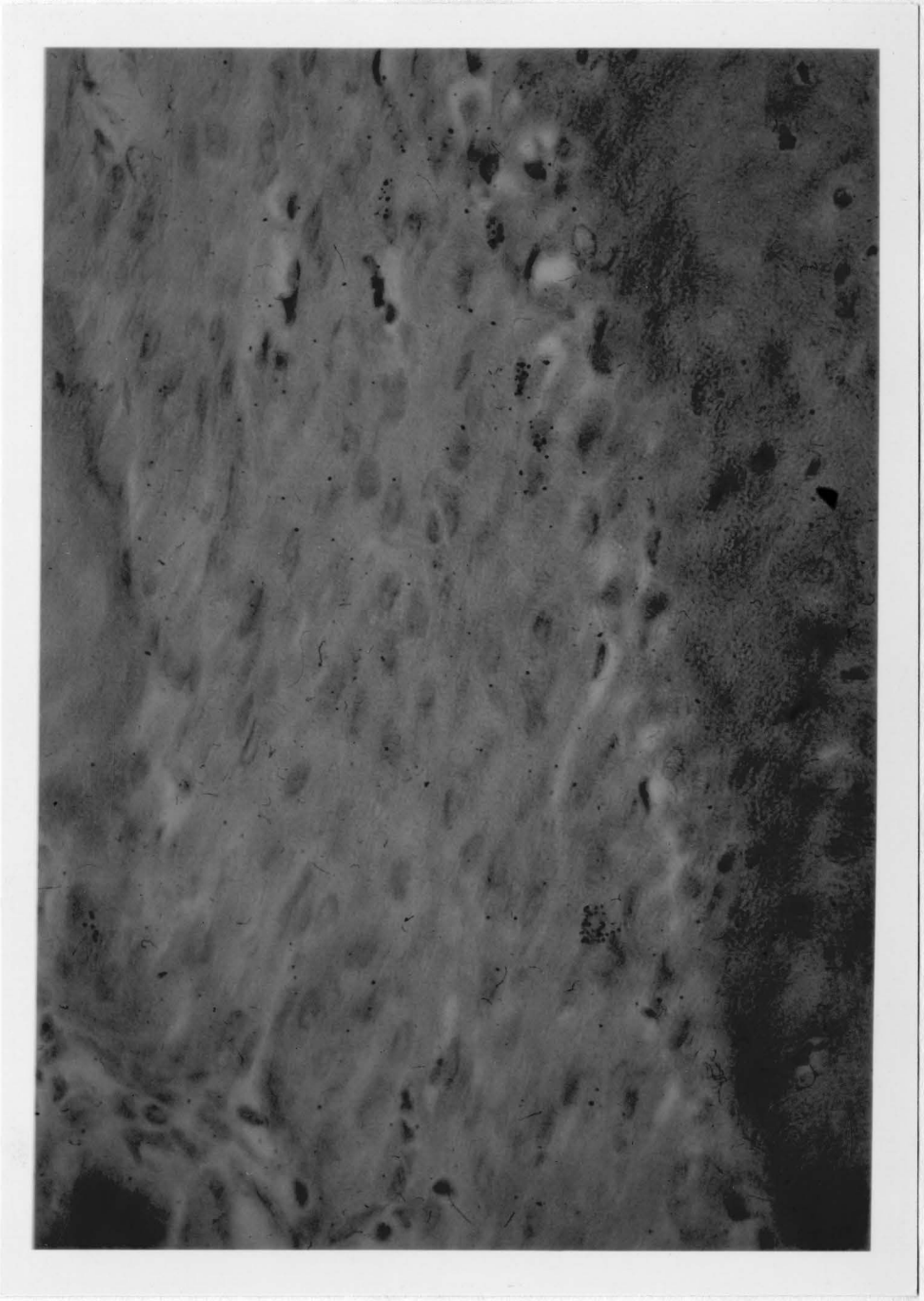

Fig 2. The labeled cells in the periodontium at sixteen hours (approximately twice in number as seen in Fig 1).

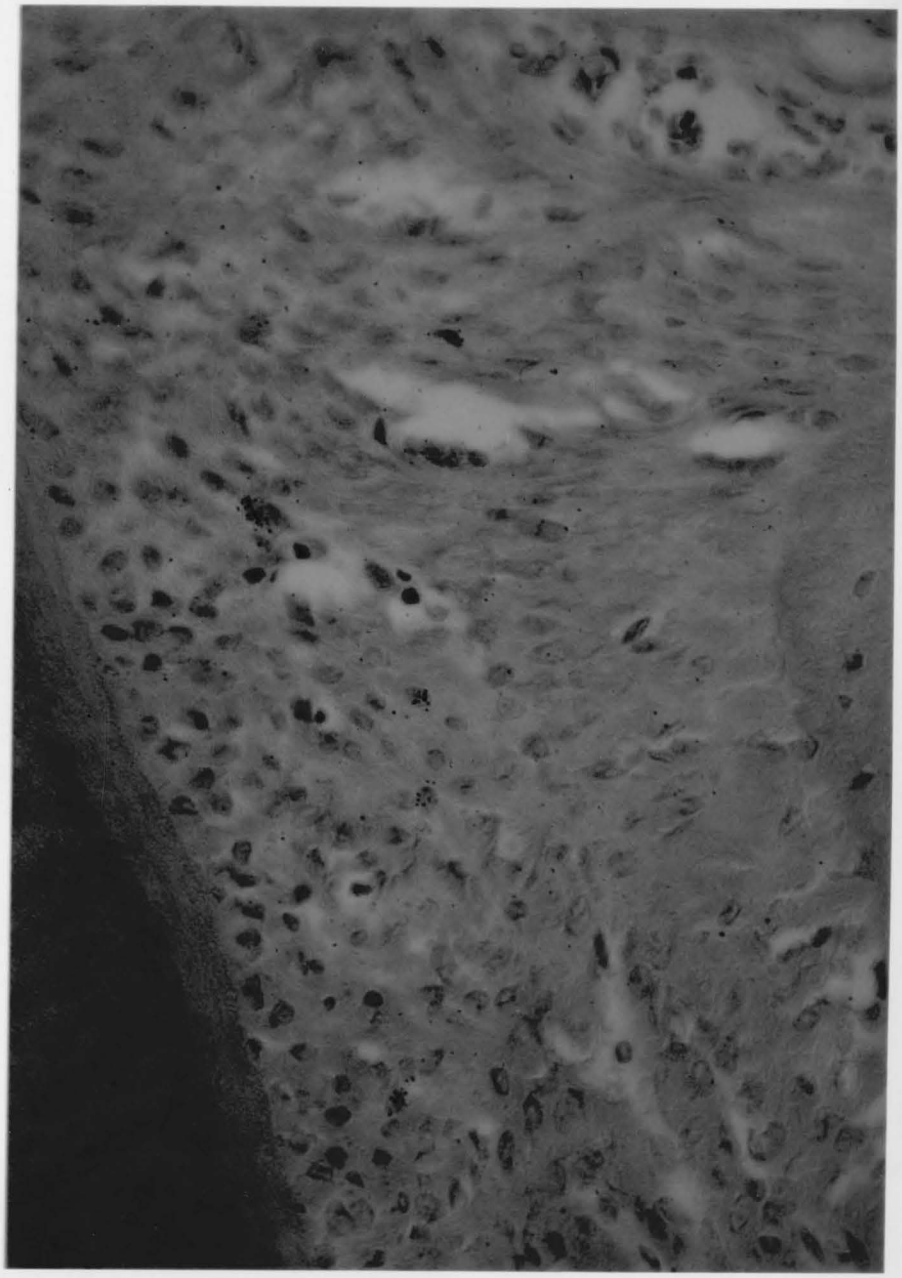

Fig 3. The labeled cells in the periodontium found at the alveolar crest.

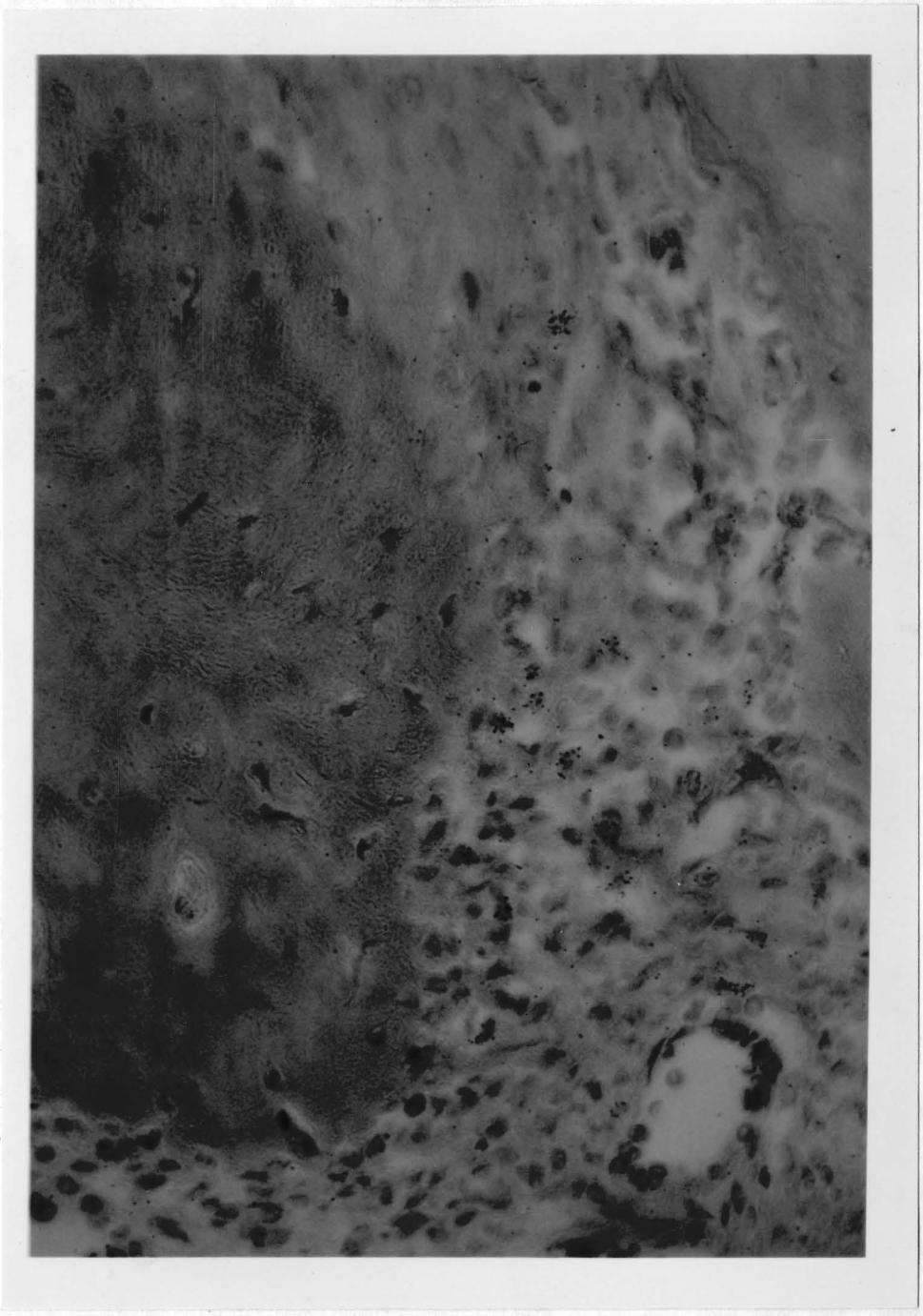

Fig 4. The labeled cells seen at the apical periodontium. (Note greater frequency as compared to that found in Fig 3.)

APPROVAL SHEET

The thesis submitted by Hseuh-Wan Kwan has been read and approved by two members of the faculty of the Graduate School.

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

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

May 22 1969
Date

Patrick D. Soto
Signature of Advisor