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The Effect of Cortisone on Mitotic Activity in the Maxillary Incisor of the Young Female Albino Rat

Michael Lawrence Kiely
Loyola University Chicago

THE EFFECT OF CORTISONE ON MITOTIC ACTIVITY IN THE

MAXILLARY INCISOR OF THE YOUNG FEMALE

ALBINO RAT

STRITCH SCHOOL
LOYOLA
UNIVERSITY
OF MEDICINE

by

Michael Lawrence Kiely

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LIFE

Michael Lawrence Kiely was born on June 17, 1938, in Springfield, Illinois.

He was graduated from Cathedral High School, Springfield, June, 1956, and from Lewis College, Lockport, Illinois, June, 1960 with the Degree of Bachelor of Science.

In September, 1960, he began his graduate studies in the Department of Anatomy, Stritch School of Medicine, Loyola University, and was awarded a United States Public Health Service Predoctoral Fellowship in April, 1962.

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INTRODUCTION

Numerous investigations have been carried out which indicate that cortisone plays a role in the eruptive process of the rat incisor. These studies have shown that this hormone accelerates the rate of eruption of the incisor. Conversely, the removal of the adrenals was found to result in a reduced rate of eruption. Despite these and other similar observations, the actual mechanism underlying the eruptive process has never been fully explained. Nevertheless, histological and physiological studies have given rise to several theories as possible explanations for tooth eruption, and one of these, the so called "Cell Proliferation Theory", has gained considerable acceptance. This hypothesis assumes that the dividing cells, in dental and peridental tissues, are the principal cause of tooth eruption. The present studies are concerned with an effort to determine the role of dividing cells in the eruptive process.

The effect of cortisone on cell division in various tissues and organs has been studied by a number of investigators. Although results to the contrary have been reported, many workers have found that cortisone exhibits an anti-mitotic effect on tissues. However, this problem needs further investigation since many specialized tissues have not yet been studied. To our knowledge comparatively few, if any, studies have been made relating cortisone with mitotic activity in dental tissues.

It is known that certain body tissues exhibit cyclic cell division; i.e. variation in mitotic activity over a 24 hour period. The epidermis, esophagus, epididymis and thyroid gland as well as regenerating liver and proliferating

corneal epithelium are among the tissues that have been shown to demonstrate
this phenomenon; (Bullough, 1948; Muhlemann et al, 1956; Jaffe, 1954; and
Vasama and Vasama, 1958) but no reports concerning mitotic periodicity in the
incisor were found. It is however likely, that a study on the tissues of the
incisor will also reveal such a diurnal variation in mitotic activity.
Should this be true then a correlation between this periodicity and the
effects of cortisone on cell division in the incisor could yield specific and
vital information.

REVIEW OF LITERATURE

A. Cortisone and Eruption Rate

Parmer, Katonah and Angrist (1951) observed that cortisone inhibited body growth but stimulated the eruption rate of the incisors in newborn rats. The incisors of cortisone treated rats erupted 2.5 days sooner than those of control animals. Domm and Marzano (1954) found that cortisone greatly accelerated the growth rate of both upper and lower incisors in normal adult male and female rats. They noted that hypophysectomized rats revealed a decrease in the growth rate of the incisor while the administration of cortisone in such animals greatly increased this rate. These investigators also observed a precocious eruption of the incisors in newborn rats following cortisone injection. Leroy and Domm (1955) reported precocious eruption of incisors in young postnatal rats where cortisone had been administered to the pregnant mother, to the fetus in utero or to newborn rats. Similarly, Goldsmith and Ross (1956) observed a precocious development of the lower incisors in 18 and 20 day fetal rats following the administration of varied doses of cortisone to the pregnant mothers. These investigators also noted that eruption of the incisors in cortisone treated neo-natal rats occurred 2.9 days earlier than in controls.

Garren (1955) and Garren and Greep (1960) reported a reduction in the eruption rate of the incisors in hypophysectomized rats. Cortisone was found to restore the eruption rate to normal in these animals. These investigators also reported that normal adult male rats receiving varied doses of cortisone

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showed an accelerated incisor eruption rate. Domm and Wellband (1960) observed a decrease in the eruption rate following adrenalectomy. This rate was increased when cortisone was administered. These workers (1961) also reported a reduction in the eruption rate following thyroidectomy and thyro-adrenalectomy. Here also cortisone therapy brought about an acceleration in the reduced eruption rates.

B. Theories on Eruption Rate

Many investigators have conducted studies concerned with the basic causes of tooth eruption and several theories have been formulated as a result of these investigations. However, despite this fact there is still a lack of agreement with respect to the factors responsible for the eruptive process. Massler and Schour (1941) after reviewing the prevailing theories on tooth eruption concluded that "The eruptive force, or the force responsible for tooth movement during eruption, may be related to the vascularity of the tissue which surrounds the tooth". They, however, conceded that factors other than vascularity may play a role in the eruptive process. Similarly, Bryer (1957) concluded that the rate of eruption of the rat incisor was directly proportional to the vascularity of the pulp and periodontal membrane. He further concluded that eruption is caused by tissue tension within the pulp and periodontal tissues and that this tension is dependent upon blood pressure.

Taylor and Butcher (1951), on the other hand, found that the eruption rate in rats was not responsive to considerable changes in blood flow. Sturman (1957) after injecting vaso-dilator and vaso-constrictor drugs into

the pulp cavity of the rat incisor, concluded that vascularity merely serves as a local regulatory mechanism and that the actual eruptive force is probably due to proliferating odontogenic epithelium and pulpal cells. Similarly, Baume et al (1954 a,b,c), while noting a reduction in the vascularity of the rat incisor following hypophysectomy and thyroidectomy, concluded that eruption is controlled by a growth and differentiation process of the dental and periodontal tissues.

C. Cortisone and Cell Division

The effect of cortisone on cell division in various tissues and organs has been the object of a number of investigations. Bullough (1952) observed that the administration of cortisone as well as of adrenaline in adult male mice resulted in a noticeable decrease in the incidence of mitosis in the epidermis. These results were observed regardless of whether the hormones were administered in vivo or in vitro. He also found that the effects of stress, brought about by overcrowding adult mice, resulted in an 80% increase in the size of the adrenal medulla and a 30% increase in the size of the adrenal cortex. Epidermal mitotic activity was observed to decrease by 60% in such mice. The author concluded that the anti-mitotic effect of stress observed in these experiments was due to a high rate of secretion of adrenaline and cortisone and that these hormones act through some interference with carbohydrate metabolism. He suggested that the anti-mitotic action of these hormones may be related to an inhibition of hexakinase. Studer and Frey (1952) found that where cortisone was administered parenterally in rats there was a strong

inhibition in the mitotic activity of both resting and proliferating epithelium which led to atrophy of the epidermis. Proliferation of the epidermis, normally stimulated by the administration of vitamin A orally, or testosterone propionate parenterally, was observed to be inhibited when cortisone was administered simultaneously. Colchicine was injected prior to sacrifice in order to arrest mitosis and thus facilitate the counting of dividing cells. Studer (1952), in addition to the above, noted that cell division, in resting and proliferating entodermal-epithelial tissue (small intestine and thiouracil inhibited thyroid), was not affected by cortisone. The effects of single and of multiple injections of cortisone on mitotic activity in young rats were studied by Tier and Isotalo (1953). They detected a definite anti-mitotic effect in the epidermis of these animals following injection of single doses of cortisone. The rate of mitosis was often inversely proportional to the dose administered. Single doses were observed to have little effect on the mitotic activity of the orbital gland. Prolonged administration of cortisone had no effect on cell proliferation in the skin or orbital gland. These observations led the authors to conclude that cell division is controlled by a superior humoral system, composed of several internal secretory organs, as well as a peripheral control system in the tissues. Ghadially and Green (1957) noted a severe depression in the mitosis of the ear epidermis of rats after adrenaline as well as cortisone treatment. Adrenalectomized rats showed some increase in mitotic activity 48 hours after the operation while sham-operated controls exhibited a marked depression in cell division. By 18 days after the operation, both the adrenalectomized and the sham-operated

animals revealed a normal mitotic cycle. Exercise had no effect on mitotic activity in the adrenalectomized animals, but it did result in a noticeable depression in the sham-operated controls.

Unlike the above investigators, Babick and Gatz (1952) reported an increase in mitotic activity in the epidermis of cortisone treated rats when compared with controls. A general loss in body weight was noted in the experimental animals in direct proportion to the dosage administered. Cortisone treated animals revealed a reduced stratum corneum and spinosum, an almost complete disappearance of the stratum granulosum and a decrease in the nuclear size of epidermal cells.

A profound decrease in lamellar osteoblastic activity in the human clavicle and rib was detected by Frost and Villanueva (1961) following cortisone administration. Similarly, Storey (1958) observed a rapid resorption of bone and a partial inhibition of new bone formation in rabbits beginning at 4 days after cortisone administration. By 11 days following treatment histological observations revealed only inhibition of bone growth. Incisors forced laterally by mechanical means showed normal bone formation on the medial side; however, after such rats received injections of cortisone the incisors exhibited resorption and inhibition of bone formation on this side. On the other hand, Goldsmith and Stahl (1953) and Goldsmith and Ross (1956) noted an increase in the growth of alveolar bone as well as a disorganization of the periodontal connective tissue in rats following cortisone therapy.

Hypophysectomized and adrenalectomized rats were observed by Tier and Carpen (1959) to exhibit a distinct increase in mitotic activity in the epider-

mis and fore-stomach. The adrenalectomized rats showed a more significant increase than the hypophysectomized ones. These workers observed no change in the mitotic activity of the liver or the orbital gland of these animals when compared with controls. Räsänen and Tier (1961) noted an increase in mitosis of the epidermal and gastric mucosa in rats as well as in the rectal mucosa of humans after ACTH administration. Adrenalectomized rats exhibited this increase to a lesser degree. However, when such rats received ACTH no increase in mitotic activity of the epidermis or gastric mucosa was observed.

D. Diurnal Mitotic Activity

Other investigators have observed a mitotic rhythm associated with various tissues and organs. Blumenfeld (1939) was one of the first to investigate periodic mitotic activity. He studied the epidermis of male albino rats. The animals were killed at 2 hour intervals over a 24 hour period. The mitotic activity in the epidermis of these animals was observed to be almost four times as great between 8 and 10 am as between 8 and 10 pm. This activity was significantly greater between 8 am and 2 pm and significantly lower between 8 pm and 2 am than during the rest of the 24 hour period. Blumenfeld (1942), in addition to confirming the results of the above study, reported a mitotic rhythm in the renal cortex and submaxillary glands of the rat. There was little correlation in the mitotic activity of these organs as the 24 hour curves were seen to differ for each organ. The epidermis showed a peak mitotic activity between 8 and 10 am and the renal cortex between 2 and 4 pm while the submaxillary gland had no significant peak. Low mitotic activity was noted between 6 and 12 pm for the epidermis, between 10 and 12 pm for the renal cortex and

between 2 and 4 am for the submaxillary gland. This investigator concluded that mitosis is stimulated by factors acting on or residing within the organ as a unit. A periodic mitotic activity in the epidermis of the rat was also observed by Babick and Gatz (1952). They noted that control rats exhibited a peak mitotic rate at 9:15 am and a low at 10:30 am as well as 11 am. Cortisone treated animals showed a peak in mitotic activity at 9:15 and at 10:15 am.

Scheving (1959) observed a cyclic cell division in the human epidermis. However, the high and low periods were found to differ from those reported by other investigators. This worker found the peak in mitotic activity to be between 12 midnight and 4 am. During these hours, the rate was approximately 100% greater than the daily mean. A "minor" peak was detected between 2 and 4 pm and low periods between 7 and 11 am and between 7 to 11 pm. He placed the dividing cells into three categories: "pre-chromosomal" or very early prophase, "chromosomal" or actual dividing cells and "reconstruction" or daughter cells. From these categories, the approximate time necessary for a cell to divide was determined to be from 4 to 5 hours. Cell division was observed to occur mostly in "nests" which were generally surrounded by inactive areas.

Scheving and Pauly (1960) studied the ear epidermis of the rat and reported a peak in mitotic activity between 12 midnight and 4 am with a high at 3 am. They also noted a nocturnal high in spontaneous activity and rectal temperature in their animals. This study does not support the observations of those workers who noted that an inverse relationship exists between body activity, body temperature, light conditions and mitotic activity.

Bullough (1948) studied the diurnal mitotic cycle and its relation to bodily activity in the adult male mouse. The maximum mitotic activity was noted in the ear epidermis at 6 am and at 2 pm and the minimum at 10 am and 8 pm. The same mitotic cycles were also seen in the esophagus, epididymis and duodenal mucosa. A considerable degree of individual variation was observed in these animals. The author correlated the epidermal mitotic activity with spontaneous bodily activity and observed an inverse relationship; i.e., an increase in bodily activity was accompanied by a decrease in mitotic activity and vice versa. From this he concluded that individual variation in bodily activity may account for variation in individual mitotic activity. Halberg and workers (1954) reported a morning "high" (sacrifice between 6:35-8-48 am) and a night "low" (sacrifice between 9:21-11:56 pm) in the mitotic activity of the epidermis of the ear lobe, the retro-molar epithelium and the periodontal membrane of rats. A significant day-night difference was not seen in the interdental papillae. A morning high and a night low were also noted for eosinophil counts in tail blood while the converse (morning low and night high) was recorded for rectal temperatures in these animals. Similarly, Chaudhry and coworkers (1958) observed a daily high at 12:30 pm in the mitotic activity of both the pinna and pouch epithelium of hamsters which roughly corresponded with the daily low in the rectal temperature of these animals. These investigators also reported a daily low at 8:30 pm in the mitotic activity of the pinna and pouch epithelium of these animals which was accompanied by a daily high in the rectal temperature.

The diurnal mitotic activity of the corneal epithelium in mice was investigated by Vasama and Vasama (1958) who found cell division to be signi-

ficantly greater in the hours after midnight and in the morning than in the afternoon or evening. They observed a high rate of mitotic activity from 2 to 10 am with the peak at 8 am. Animals that engaged in spontaneous activity showed a decrease in mitosis of the corneal epithelium. Scheving and Chiakulas (1962) also studied the 24 hour mitotic rhythm in the corneal epithelium of urodele larvae. They reported a high mitotic activity between 12:30 and 2:30 pm, and at 10:30 pm and 2:30 am while low activity was seen at 8:30 am, 6:30 pm and at 12:30 am.

Jaffe (1954) studied partially hepatectomized animals and reported that more mitotic activity was seen in the regenerating liver between 6 and 10 am than between 6 and 10 pm in each 12 hour interval after the operation. A gradual diminution in mitosis was observed beginning at 3 days after the operation. On the basis of experimental results, the author linked carbohydrate metabolism with mitotic activity. Muhlemann et al (1956) investigated mitotic periodicity in the oral epithelium, the thyroid gland, the adrenal cortex and the duodenal epithelium of rats. The animals were sacrificed at two periods: "day rats" between 7 and 7:30 am and "night rats" between 9:30 and 10 pm. The day rats showed more cell division in the oral epithelium and in the thyroid gland than the night rats, while the night rats revealed more mitotic activity in the adrenal cortex than the day rats. No significant difference was seen in the crypts of the duodenal mucosal membrane between the two groups. Simmons (1962) studied mitotic activity in the epiphyseal cartilage of both the femur and tibia of young mice. "Day mice" were sacrificed between 2 and 10 am and "night mice" between 3 and 11 pm. The day mice revealed more mitotic activity in the epiphyseal cartilage of both the femur and the tibia than did the night

mice. The average growth rate of this cartilage was calculated to be approximately 25% greater during the day than at night.

Since cortisone has been shown to accelerate the rate of eruption of the incisor in the albino rat and since this hormone is also known to influence the rate of cell division, a study to determine its effect on the mitotic activity of the rat incisor as a possible factor in the eruptive process would appear to be significant. Moreover, we found no information in the literature with respect to the problem of mitotic periodicity in the tissues of the incisor.

MATERIALS AND METHODS

Young, female, albino rats[1] of the Sprague-Dawley strain were employed throughout this investigation. All animals were kept under standard laboratory conditions and maintained on a diet of Purina Dog Chow Pellets and tap water _ad libitum_. The animal quarters were lighted from 7 am to 7 pm and darkened from 7 pm to 7 am throughout the experiments.

Cortisone (Cortone acetate)[2], concentration 25 mg per cc, was the hormone used in this investigation. Colchicine (Colchicum autumnale[3] dissolved in 55% alcohol at a concentration of 4 mg per cc was also employed in this study. This drug is known to arrest dividing cells in the metaphase stage and thus facilitates the counting of these cells (Eigsti and Dustin, 1955). Pilot experiments were performed in order: 1) to determine the dosage of colchicine required to sufficiently arrest mitosis and 2) to establish an optimal time lapse between colchicine injection and sacrifice. Following these pilot studies, an additional preliminary experiment was undertaken with colchicine in an effort to determine the occurrence of a cyclic mitotic activity in the tissues of the rat incisor.

[1]Purchased from the Hormone Assay Laboratories, Incorporated, Chicago, Ill.

[2]Generously supplied to Dr. L. V. Domm by Sharp and Dohme, Division of Merck and Company, Inc., Philadelphia, Pa.

[3]A product of Eli Lilly and Company, Indianapolis, Ind.

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The animals (30 to 40 days old) were randomly divided into three experimental groups and each of these further divided into four sub-groups. Thus, beginning at 7 am, 1 pm, 7 pm, and 1 am the animals of these sub-groups of the three experimental groups were injected subcutaneously with cortisone. The animals of experimental Group I received 0.5 mg. This injection was repeated every 12 hours for a period of two days. Thus each animal received 2 mg in a total of four injections. The corresponding control animals simultaneously received injections of an equal volume of a normal saline solution. At 12 hours after the final injection, both treated and control animals were given a single subcutaneous injection of colchicine (1 mg/kg body wgt). All animals were sacrificed exactly three hours after this injection. Body weights were recorded prior to all injections and at the time of sacrifice.

The same procedure was followed for Groups II and III with the following exceptions: 1) Group II animals received 1 mg of cortisone per injection or a total of 4 mg in 4 injections and 2) Group III animals were given 2 mg of cortisone in a single injection which in this group was followed 6 hours instead of 12 hours later with colchicine administration. Table I gives a summary of the experimental procedures.

Sacrifice was by decapitation preceded by ether anesthesia. Both right and left maxillary incisors were immediately recovered and fixed for histological study. Samples of the duodenum were also taken in order to confirm the effectiveness of colchicine. All tissues were fixed for 48 hours in a neutral 10% formalin solution containing 1% calcium chloride. The incisors were decalcified in a sodium citrate-formic acid solution or by means of an ionic

bone decalcifier containing an 8% hydrochloric 10% formic acid solution. They were then dehydrated, imbedded in paraffin in vacuo, sectioned sagittally at 6 micra and stained with Harris' alum hematoxylin and eosin. Weigert's iron hematoxylin with Van Gieson's counter stain was employed with a few preparations.

Mitotic counts were made on four cell layers of the labial (basal) loop of the incisor (Figure 5). These cell layers were: the stratum intermedium, the ameloblast layer, the odontoblast layer, and the adjacent pulp cells. Successive circumscribed fields were counted beginning at the base of the loop and up to the point of dentin formation (Figure 6). The field was defined by means of an 8 x 8 mm square etched on an ocular micrometer disc. The area of each oil immersion field was 6400 u^2. Care was taken so that overlapping areas were not counted twice (Figure 6).

Counts were made using a Leitz binocular microscope with an oil immersion lens (100X). At least four sagittal sections were counted for each tooth. Adjacent sections were not counted in order to avoid duplication. All data were statistically analyzed employing the t-test or the Analysis of Variance Test (Batson, 1956). Differences were considered significant at the 5% level of probability.

The Analysis of Variance Test was used to statistically evaluate cyclic mitotic activity in both experimental and control animals. This test enabled us to simultaneously determine the significance of the difference between the means of the four periods (7 am, 1 pm, 7 pm, 1 am) under observation for each cell layer and in addition to make determinations of the significance of the difference between the means of particular periods; i.e. 7am and 1 pm vs 7 pm

and 1 am. These differences were calculated in the following way:

$$Sx^1 = 7am + 1pm + 7pm + 1am$$

$$N^2 = 7am + 1pm + 7pm + 1am$$

$$CF^3 = \frac{Sx^2}{N}$$

$$\text{Total } SS^4 = 7am^2 + 1pm^2 + 7pm^2 + 1am^2 - CF$$

$$\text{Between Groups } SS = \frac{Sx^2}{N} + \frac{Sx^2}{N} + \frac{Sx^2}{N} + \frac{Sx^2}{N} - CF$$
$$\text{(7am)} \quad \text{(1pm)} \quad \text{(7pm)} \quad \text{(1am)}$$

$$\text{Within Groups } SS = \text{Total } SS - \text{Between Groups } SS$$

The Between Groups SS and the Within Groups SS values were then entered in an analysis of variance table, as may be observed in Tables V-X, and the variance ratio was calculated from this table in the following manner:

$$\frac{\text{Between Groups } SS}{\text{Between Groups } DF^5} = \text{Between Groups } MS^6$$

$$\frac{\text{Within Groups } SS}{\text{Within Groups } DF} = \text{Within Groups } MS$$

$$\frac{\text{Between Groups } MS}{\text{Within Groups } MS} = \text{Variance Ratio}$$

[1]Sx = Sum of the Total Observations

[2]N = Number of the Observations

[3]CF = Correction Factor

[4]SS = Sum of the Observations Squared

[5]DF = Degrees of Freedom = N-1

[6]MS = Mean Squares

The variance ratio or value was then entered into a significance table and the appropriate P value determined. A significant P value was interpreted to indicate that diurnal mitotic activity was present for the cell layer under analysis.

However, no conclusion could be drawn as to whether a significant difference existed between the mean mitotic activity of individual time periods, (i.e. if 7 am differed significantly from 7 pm, etc.). Consequently a further analysis was carried out in order to answer this question. Inspection of the mean mitotic activity indicated, in most cases, more mitotic activity at 7 am and 1 pm than at 7 pm and 1 am. Thus as shown in Tables V to X inclusive calculations were carried out to determine if this difference in mitotic activity was statistically significant. This difference was determined as follows:

$$SS = \frac{(Sx + Sx)^2}{\underset{(7am)(1pm)}{N + N}} + \frac{(Sx + Sx)^2}{\underset{(7pm)(1am)}{N + N}} - CF$$

$$\frac{SS}{DF} = MS$$

$$\frac{MS}{Within\ Groups\ MS} = Variance\ Ratio$$

The P value was determined in the same way as previously stated. A significant P value was interpreted to indicate that mean mitotic activity was significantly greater at 7 am and 1 pm than at 7 pm and 1 am.

EXPERIMENTAL RESULTS

A. Preliminary Observations

As a result of pilot studies the dosage of colchicine required to suf-
ficiently arrest mitosis in metaphase in our rats was determined to be 1 mg
per kg of body weight. Fleischmann (1939), Leblond et al (1955) and Hooper
(1961) employed this dosage in their experiments on rats and reported consist-
ent results. Our observations revealed three hours to be the optimal time of
action of colchicine. We observed very little pycnosis in the tissues of the
incisors or the duodenum of rats sacrificed three hours after injection, thus
confirming the observations of Hooper (1961). Employing this dosage of colchi-
cine and action time we found only a minimal number of cells in prophase, ana-
phase, or telophase thus indicating sufficient arrest of mitosis and little
if any escape from this arrest. A portion of the labial loop from an incisor
of a colchicine treated and a normal rat are shown in Figures 7 and 8.

The chromosomal pattern in colchicine metaphase arrest is seen to be quite
different from the normal metaphasic orientation. This deviation is believed
to be due to a partial or total destruction of the spindle fibers which thus
become disengaged from the chromosomes (Eigsti and Dustin, 1955). In an exten-
sive review these investigators noted the following atypical metaphase patterns
in studies on animals some of which we observed in our study. The so-called
star metaphase (Figures 9 and 10) presents chromosomes which radiate from a
central point in a star-like fashion. A distorted star-like pattern was

sometimes observed in which the chromosomes were somewhat disarranged. A second type the so-called "exploded" metaphase (Figures 11 and 12) shows the chromosomes scattered throughout the cell. The 3rd type known as the "ball-type" metaphase (Figures 13 and 14) reveals chromosomes clumped tightly together in the center of the cell. Finally, typical metaphase patterns were observed in which the chromosomes were oriented in an equatorial plane (Figures 15 and 16).

B. Histological Observations

The present study was concerned with an investigation of the mitotic activity in the labial loop of the incisor in the area beginning at the basal end of this loop and proceeding apically to the point where dentin formation begins. The cell layers involved were the stratum intermedium, the ameloblast layer, the odontoblast layer and the adjacent pulp. In order to facilitate description we arbitrarily divided this area into three regions as follows: the basal, middle and terminal regions as shown in Figures 17, 18 and 19.

In the basal region of the labial loop the stratum intermedium is composed of two or three layers of loosely arranged flattened cells while the undifferentiated ameloblasts are short columnar in type. The undifferentiated odontoblasts make up an ill-defined single layer of cells adjacent to the pulp. Mitotic activity in this region was observed to be moderate and occurred chiefly in the ameloblasts (Figure 17).

In the middle region, the stratum intermedium becomes much more compact and the cuboidal odontoblasts begin to differentiate and become arranged into

a distinct cell layer. In this region, mitotic activity was the most preval-
ent. Dividing ameloblasts were observed to be chiefly lined up along the
border of the stratum intermedium. Proliferating ameloblasts in rare cases
were seen adjacent to the odontoblasts. Mitoses were generally scattered
throughout the other cell layers with some clustering in the reticular network
of the pulp (Figure 18).

In the terminal region, mitotic activity was noticeably reduced and it
was completely absent in the area of dentin formation. In this area, the
differentiating columnar ameloblasts had elongated considerably and the
differentiated odontoblasts were also columnar (Figure 19).

C. Observations on Mitotic Activity

1. Group I

Eight treated and eight control animals were employed in this group.
Beginning at 7 am, 1 pm, 7 pm and 1 am, two animals for each period were
given 0.5 mg of cortisone twice daily, at 12 hour intervals, for a 2 day
period. Control animals received an equal volume of normal saline solution
or the diluent of cortisone. At 12 hours after the final injection (4th) all
animals were injected with 1 mg of colchicine per kg of body weight and sacri-
ficed three hours later (Table I). The t-test was employed to determine the
significance of the difference between the mean mitotic activity, per compar-
able area (field), of experimental and control animals (Figure 6).

The 7 AM Period

The mitotic activity in the incisors of the cortisone treated animals at

this time showed a significant increase in all cell layers, with the exception of the odontoblasts. The mean mitotic activity of the odontoblasts of treated animals was 1.06 ± 0.11[1] and that of the controls, (1.11 ± 0.11). This difference was found not to be significant and was interpreted to indicate no depression in the mitotic activity of the odontoblasts following cortisone administration. The mean number of mitoses in the stratum intermedium of cortisone treated animals was 2.74 ± 0.15, which proved to be a highly significant increase ($P = .001$) when compared with the average of the controls, (2.05 ± 0.13). The ameloblasts of treated animals showed an average mitotic activity of 5.30 ± 0.23 while the average for these cells in the controls was 4.53 ± 0.20. This difference was very significant ($P = .010$). Similarly a significant difference ($P = .050$) was observed between the mitotic mean of the pulpal cells of treated (3.17 ± 0.17) and control (2.60 ± 0.19) animals. These results are summarized in Table II and shown graphically in Figure 1.

The 1 PM Period

In each of the cell layers at this period the cortisone treated animals revealed an increase in mitotic activity, which was found to be significant, with the exception, again, of the odontoblasts. The stratum intermedium of treated animals showed an average of 3.10 ± 0.18 and the controls an average of 2.49 ± 0.15, a very significant difference ($P = .010$). Similarly the administration of cortisone resulted in a mean mitotic activity in the ameloblasts of 5.56 ± 0.25 which was as above the average (4.62 ± 0.23) of the controls and very significant ($P = .010$). The pulpal cells of the treated rats showed an average of 4.02 ± 0.25 while the average (2.24 ± 0.18) of the

[1] Standard Error

controls was significantly less (P = .001). Table II and Figure 1 summarize
these observations.

The 7 PM and 1 AM Periods

At these periods, the mitotic activity of control animals in all cases,
was found to be greater than that of cortisone treated rats. The difference
in the mean mitotic activity of the stratum intermedium between treated and
control animals at both 7 pm and 1 am was not significant. This finding was
interpreted to indicate no inhibition of mitosis following cortisone adminis-
tration. However, proliferation of the ameloblasts was found to be greater in
the controls at both 7 pm and 1 am than in the cortisone treated rats (Table
II). This difference proved to be significant (P = .001) at 7 pm and also
at 1 am (P = .010). The difference between the odontoblasts of control and
treated animals was not significant at 7 pm but the controls showed signifi-
cantly more mitotic activity than the treated (P = .050) at 1 am (Table II).
The reverse of this was observed in the pulp cells where treated animals at
7 pm showed an average of 2.16 $\pm$ 0.14 and controls (2.79 $\pm$ 0.18). This
difference was significant (P = .010). However, at 1 am no statistically
significant difference was found between treated and control animals in the
mitotic activity of these cells (Table II; Figure 1).

The Average of the Combined Cell Layers

At 7 am and 1 pm, the mean mitotic activity of the combined cell layers
of the incisors of cortisone treated animals showed an increase which, statis-
tically, proved to be highly significant. However, at 7 pm and 1 am, the

mitotic activity was observed to be significantly greater in controls than in cortisone treated rats (Table II; Figure 4).

2. Group II

The experimental design for this group of rats was the same as that of Group I with the exception that 1.0 mg instead of 0.5 mg of cortisone per injection was administered (Table I).

The 7 AM Period

The average number of mitoses in the cortisone treated animals was observed to increase in all four cell layers when compared with controls. However, the increase in the stratum intermedium did not prove to be significant (Table III), whereas the mean of the ameloblasts in treated rats (5.65 ± 0.35) showed a significant increase (P = .001) over the mean (3.86 ± 0.28) of the controls. Different from Group I, the dividing odontoblasts of cortisone treated animals averaged 1.78 ± 0.19 as opposed to the lower mean (0.88 ± 0.11) of the controls. This increase proved to be significant (P = .001). Similarly, the mitotic activity of the pulp cells of treated animals showed a highly significant increase (Table III; Figure 2).

The 1 PM Period

The mitotic activity of the stratum intermedium of treated rats at this time revealed an average of 2.67 ± 0.15 while the average of control animals was 2.36 ± 0.19. This difference was found not to be significant. The mitotic mean (5.60 ± 0.24) of ameloblasts in treated rats increased significantly (P = .050) over the average (4.73 ± 0.34) of controls. The difference

between the mitotic means of the odontoblasts of experimental and control animals was not significant although the treated rats showed a slight increase. The mitotic activity of the pulp cells revealed a different result from that recorded above. Here the average of controls was 0.34 greater than that of the treated. This difference was not statistically significant (Table III).

The 7 PM Period

For this period the controls, in every instance, exhibited a higher mean mitotic activity than did the cortisone treated animals. These findings are in accord with those observed in the 7 pm Group I animals. In the ameloblasts the control average was 4.90 ± 0.21 as opposed to the lower mean of 3.83 ± 0.19 of the treated rats. This was a significant difference ($P = .001$). The stratum intermedium, the odontoblasts and the pulp cells showed no significant difference in the mean mitotic activity between treated and control animals (Table III and Figure 2).

The 1 AM Period

Differing from the 1 am animals of Group I, the rats of this group at this period showed an accelerated cell proliferation after cortisone administration. While this increase was found not to be significant in the stratum intermedium, it proved to be highly so ($P = .001$) in the ameloblasts, the odontoblasts and the pulp cells (Table III). This difference in results between Group I and II animals may be due to the larger dosage of cortisone administered to Group II animals (Figure 2).

The Average of the Combined Cell Layers

While the higher mean mitotic activity of the 7 am cortisone treated rats proved to be significant (P = .001), when compared with that of controls, no such difference was observed in the 1 pm group. The non-stimulatory effect of cortisone on the pulp of the 1 pm group may in part account for this lack of significance. The higher mean for the combined cell layers (9.43 ± 0.35) observed in the 1 am treated animals was significant (p = .001) when compared with the mean (7.13 ± 0.34) of control rats. Conversely, for the 7 pm period the mitotic average of the combined cell layers of controls was significantly higher than that of the treated rats (Table III and Figure 4).

3. Group III

The animals of this group were given a single injection of 2 mg of cortisone followed 6 hours later with an injection of colchicine. In all other respects the experimental procedure was the same as that followed in Groups I and II (Table I).

The 7 AM Period

The mean mitotic activity of the stratum intermedium, the ameloblasts and of the pulp of controls was observed to be higher than that of cortisone treated animals. However, a statistically significant difference (P = .001) was found only in the pulp where the mitotic average for the controls was 3.48 ± 0.24 and that of treated 2.22 ± 0.25. Virtually no difference in the mean mitotic activity of the odontoblasts was observed between treated and control rats (Table IV).

The 1 PM Period

All four cell layers in the incisors of controls at this period exhibited a greater mitotic activity than those of the corresponding treated animals. The mean mitotic activity (3.14 ± 0.16) of the stratum intermedium of controls was higher than that (2.70 ± 0.13) of treated animals. This difference was found to be statistically significant ($P = .050$). However, no significant difference between the average mitotic activity of control and treated animals was found in the ameloblasts, the odontoblasts, or the pulp (Table IV; Figure 3).

The 7 PM Period

In the animals examined at this time the stratum intermedium of cortisone treated rats revealed a mitotic mean of 2.25 ± 0.14 which was significantly greater ($P = .050$) than the mean (1.64 ± 0.20) of these cells in controls. Conversely, the average mitotic activity of the ameloblasts, the odontoblasts and of the pulp cells of controls was found to be higher, though not significantly so, than the average of treated animals (Table IV).

The 1 AM Period

The mitotic activity of the ameloblasts, the odontoblasts and of the pulp of controls was found to be higher than the corresponding activity of these cells in treated rats. However, these differences were not statistically significant (Table IV). The stratum intermedium of control rats showed an average mitotic index of 2.38 ± 0.17 which was significantly higher ($P = .050$) than the average (1.95 ± 0.13) of the cortisone treated animals (Figure 3).

The Average of the Combined Cell Layers

For every period in this group, as a result of combining the average mitotic indices of the various cell layers studied, we found that control animals exhibited more dividing cells than treated animals. At the 7 am and 1 am periods this difference proved to be statistically significant ($P = .050$ and $.010$, respectively) whereas at the 7 pm and 1 pm periods the control and treated animals revealed no significant difference in average mitotic activity (Table IV; Figure 4).

D. Observations on Diurnal Mitotic Activity

A careful examination of the mean mitotic activity in both treated and control groups generally indicated the presence of a cyclic mitotic activity. Thus the Analysis of Variance Test was utilized to determine whether this cyclic activity was statistically significant. In each experimental group, evaluations were made of the variance between the average mitotic activity of the four periods under observation (7am, 1pm, 7pm, 1am) for each cell layer in both treated and control animals. Thus the analysis was designed to determine the presence or absence of a normal mitotic as well as a cortisone mitotic cycle. In cases where a significant variance was established between the means of the four time periods of any individual cell layer, the pooled sum of the squares of the 7 am and 1 pm groups were compared with the pooled sum of the squares of the 7 pm and 1 am groups to determine if a significant variance of means existed between these two groups. This statistical procedure was also employed with the combined cell layer averages.

1. Group I

As may be observed in Table V, all the cell layers of the cortisone treated animals studied showed a highly significant (P = .001) difference between the average mitotic activity of the four periods. This finding is interpreted to indicate that a cyclic cell division occurred in each of the cell layers of the treated animals. Further analysis of the combined 7 am and 1 pm groups compared with the total 7 pm and 1 am groups also showed a significant (P = .001) difference in every cell layer. Since the average mitotic index of the combined morning and afternoon groups was, in every case, higher than this average for the total evening and early morning groups, this variance indicates significantly more mitotic activity during the former two than during the latter two periods of the day. This finding is represented graphically in Figures 1 and 4.

The control animals revealed a somewhat different situation. No significant variance was found between the average mitotic index of the four periods in the odontoblasts or pulp cells. However, a significant variance was observed for the stratum intermedium, the ameloblasts and the combined cell layers. When the data of the pooled periods for these three groups were compared no significant difference was found. Thus the interpretation of these findings would indicate that there was a diurnal mitotic cycle in the control animals but that this cycle was not the same as that observed in the experimental animals. These results are given in Table VI and graphically presented in Figures 1 and 4.

2. Group II

As in Group I, the treated animals of Group II revealed a significant

(P = .001) variation in the average mitotic activity of the four periods for every cell layer. A highly significant difference was also found between the pooled 7 am and 1 pm periods and the combined 7 pm and 1 am periods for all cell layers indicating, once more, a significantly higher mitotic activity during the former than the latter periods (Table VII and Figures 2 and 4).

The control animals of this group also showed results which differed from those of the treated rats. The difference in the mitotic average between each of the four periods for the stratum intermedium was not significant while the average variance of the ameloblasts for these periods was found to be significant. However, an analysis of the difference between the ameloblast averages for the combined periods (7am and 1pm vs 7 pm and 1am) proved not to be significant. These findings indicate that although there is a diurnal variation in the mitotic activity of the ameloblasts of controls, this variation is different from the cyclic activity observed in the ameloblasts of treated animals. The odontoblasts, the pulp cells, and the combined cell layers showed an average mitotic activity which was significantly different between each of the four periods. In addition the pooled periods, were observed to be significantly different in their average mitotic activity. Thus the cyclic proliferation of the odontoblasts, the pulp cells and of the combined cell layers of the controls seems to be of the same character as that observed in the treated animals (Table VIII; Figures 2 and 4).

3. Group III

As in the other two groups the treated animals of this group exhibited a highly significant variance in the average mitotic activity of every cell

layer between each of the four periods. Such a difference was again observed when the averages of the pooled periods were compared, once more showing a high cell proliferation at 7 am and at 1 pm and significantly less cell division at 7 pm and at 1 am (Table IX; Figures 3 and 4).

The odontoblasts of controls revealed no significant difference in averages between each of the four periods. However, the stratum intermedium, the ameloblasts, the pulp cells and the combined cell layers showed an average mitotic index which not only proved to be significant between the four periods in each of these cell layers but also between the pooled 7 am and 1 pm periods as compared with the combined 7 pm and 1 am periods. This finding indicates that the cyclic mitotic activity observed in treated and control rats is similar in each of these cell layers (Table X and Figures 3 and 4).

DISCUSSION

The results of this study indicate the presence of a normal and a cortisone mitotic cycle in the labial loop of the rat incisor. The mitotic activity of every cell layer in all cortisone treated animals was observed to be statistically, significantly, higher at 7 am and 1 pm than at 7 pm and 1 am. The mitotic activity of the combined cell layers, as well as that of the majority of the individual cell layers, of control animals in Group II and III was also observed to be statistically, significantly, higher at 7 am and at 1 pm than at 7 pm and 1 am. The mitotic rhythm of Group I controls was found to vary, within statistically significant limits, from the rhythm observed in the other control and treated groups. However, examination of the average mitotic activity of the cell layers studied in Group I controls shows a consistently higher activity at 7 am and 1 pm than at 7 pm and 1 am. Thus, with the exception of some deviation in Group I control rats, treated and control animals were found to adhere to the same general mitotic cycle.

Bullough (1948) and others have postulated that body activity is inversely proportional to mitotic activity. Thus, according to this theory, nocturnal animals should show an increase in cell division during the day, the period of rest and tissue repair, while animals that sleep during the night would exhibit high mitotic activity at this time. Since the rat is a nocturnal animal and we observed an increase in mitotic activity during the day time our results would appear to be in accord with this theory. A review of the literature indicates substantial confirmation of this hypothesis.

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Blumenfeld (1939) found mitotic activity in the rat epidermis to be highest between 8 and 10 am and lowest between 8 and 10 pm. Babick and Gatz (1952) reported similar observations in the epidermis of cortisone treated rats. Bullough (1948) observed maximum mitotic activity at 6 am and 2 pm and minimum activity at 10 am and 8 pm in the epidermis, esophagus, epididymis and duodenal mucosa of male mice. Halberg and workers (1954) noted that the mitotic activity of the epidermis, the retromolar epithelium and the periodontal membrane of rats was high between 6:35 and 8:48 am and low between 9:21 and 11:56 pm. Muhlemann et al (1956) observed more mitotic activity in the oral epithelium and the thyroid gland of rats between 7 and 7:30 am than between 9:30 and 10 pm. Scheving (1959) in a study on human epidermis reported a high period in mitotic activity between 12 midnight and 4 am and a low period at 7 to 11 am.

We observed that cortisone exerted a consistent and significant stimulatory effect on the mitotic activity of the rat incisor at 7 am and 1 pm where a dosage of 0.5 mg and 1.0 mg per injection had been administered. These findings are in agreement with those of Babick and Gatz (1952) who reported an increase in cell division in the epidermis of rats treated with cortisone and with Goldsmith and Stahl (1953) and Goldsmith and Ross (1956) who observed an increase in the growth of alveolar bone in rats following cortisone administration. However, other investigators have reported findings which are not in agreement with these results. Studer and Frey (1952) noted a decrease in the mitotic activity of the back epidermis in rats following cortisone administration while Bullough (1952) and Ghadially and Green (1957) ob-

served a similar decrease in the ear epidermis of mice and rats, respectively, after such treatment. Storey (1958) reported an inhibition of new bone formation in rabbits and Frost and Villanueva (1961) noted a decrease in the osteoblastic activity of human clavicle and rib following cortisone treatment.

The apparent anti-mitotic effect of cortisone reported by these investigators may conceivably be related to the period of day in which the experimental animals were treated since we observed no effect, and sometimes an inhibition of mitotic activity, in the incisors of our cortisone treated animals depending upon the period of treatment. At 7 pm, in rats receiving 0.5 mg and 1.0 mg of cortisone per injection, and at 1 am in those receiving 0.5 mg per injection, the incisors exhibited less mitotic activity than those of controls. Although some cell layers showed no significant difference between treated and control, the average of the combined cell layers of the above treated groups revealed a significant decrease. This finding would seem to indicate some inhibition of mitotic activity at these periods. However, our findings did reveal a stimulatory effect on cell division in the incisor at 7 am and at 1 pm where cortisone had been administered.

The dosage of cortisone and the time of administration appear to be factors influencing the stimulatory effect of this hormone. Rats given 1.0 mg of cortisone per injection at 1 am showed a significant increase in mitotic activity where as in those receiving 0.5 mg per injection at this time there was a decrease. Thus stimulation in this experiment would appear to be due to the higher dosage administered. However, such a stimulation was not observed

where 1.0 mg had been administered at 7 pm indicating that at some periods of the day an increase in the dosage will not bring about an increase in mitotic activity.

The duration of cortisone administration was also found to influence the effect of this hormone on the mitotic activity of the incisor. Rats given 2 mg in a single injection showed no stimulation and little if any inhibition in mitotic activity during the periods under observations. However, rats receiving the same daily dosage but in 2 injections per day and over a 2 day period revealed a significant increase in cell division at all periods studied except the 7 pm period. This finding would seem to indicate that mitotic activity in the incisor, within the limits of our experiment, while influenced little if at all by a single injection, is significantly stimulated following several injections per day over a 2 day period.

From our results it would appear that the effect of cortisone on the mitotic activity of the incisor is determined by three factors, namely: the period of administration, the dosage administered and the duration of the injections. In other words, the receptivity of the tissues of the incisor to cortisone may be cyclic in nature and may be influenced or altered by the dosage as well as the duration of administration. Additional studies will be required to establish the importance of each of these factors and their presumed interrelationship.

Most of the published reports in this field have dealt with the effects of cortisone on the epidermis and on bone. Many of these studies have revealed an anti-mitotic effect. However, Studer (1952) noted that cell divisi-

on in the small intestine and in the thyroid of the rat following thiouracil administration was not affected by cortisone. Tier and Isotalo (1953) also found that single and prolonged injections of cortisone had no effect on the mitotic activity of the orbital gland in the rat. It is conceivable that the tissues of the body vary in their response or receptivity to cortisone. Certain tissues, including the incisor, may be highly responsive to cortisone resulting in an increase in mitotic activity. In our study, this responsiveness appeared to be somewhat greater in some cell layers than in others. The ameloblasts of normal and cortisone treated rats exhibited a higher rate of mitotic activity than did the other three cell layers of the incisor. Moreover, this activity was shown to be somewhat lower in the odontoblasts than in the stratum intermedium, the ameloblasts, or the pulp cells. However, one must remember that the comparative width and density of these cell layers are quite different. The odontoblast layer, for example, is much thinner and more loosely arranged than the ameloblast layer. Thus, since the mitotic activity of all cell layers was observed to increase with cortisone administration during some periods of the day, the degree of receptivity of the various cell layers to this hormone is difficult to determine on a comparative basis.

Many investigators have come to the conclusion that proliferation and differentiation of the dental and periodontal tissues are the basic factors in tooth eruption. Schour and Van Dyke (1932a) reported a progressive retardation in the rate of eruption of the incisors in hypophysectomized rats evident as early as one week following the operation. Their histological findings revealed degeneration of the enamel epithelium, absence of enamel in the

basal zone, overgrowth of dentin and a reduction in the blood supply of the
pulp, periodontal membrane and the labial alveolar periostium. Sturman (1957),
after studying the effects of injecting vaso-dilator and vaso-constrictor
drugs into the pulp cavity of the rat incisor, came to the conclusion that
eruption of the incisor is controlled locally by vascularity while the actual
eruptive force is probably due to proliferating odontogenic epithelium and
pulp cells. Baume et al (1954 a,b,c) studied the effect of thyroidectomy and
hypophysectomy on the rate of eruption of the rat incisor. Following these
operations they observed a reduced eruption rate, reduction in differentiation,
and atrophy of the odontogenic and inner enamel epithelium. The administration
of growth hormone in hypophysectomized rats resulted in some renewal of dental
tissues but they observed no stimulation in the eruption rate. Thyroxin, on
the other hand, accelerated the eruption rate and resulted in improved amel-
ogenesis. When both hormones were administered simultaneously they noted an
optimal increase in the rate of eruption and a maximum restoration of the e-
namel organ. As a consequence these investigators concluded that tooth erup-
tion is due to, "a basic process of proliferation of the dental structures"...
.."which becomes effective only through a differential growth of the dental
and peridental tissues induced by the proper interaction of the epithelial
sheath". The results of our study would appear to support this conclusion.

In normal rats we observed a high degree of mitotic activity in the bas-
al loop of the incisor, primarily in the ameloblasts, but also in the stratum
intermedium and the pulp cells. Following cortisone administration at 7 am
and at 1 pm, the mitotic activity of these cell layers was observed to be
significantly stimulated. Even during the period of day when cortisone

was found to inhibit, or have no effect on mitotic activity (7 pm and 1 am), mitotic activity remained at a relatively high level. Cortisone is known to accelerate the eruption rate of the rat incisor, (Domm and Marzano, 1954; Domm and LeRoy, 1955; Goldsmith and Ross, 1956 and others) however, thus far no one has found a completely satisfactory explanation for this phenomenon. Our study has shown for the first time that mitotic activity in the tissues of the incisor is cyclic in nature and that cortisone stimulates the mitotic activity of the incisor. The results, therefore, support the concept that cell proliferation may be one of the factors responsible for tooth eruption. Additional studies are needed to establish this concept.

SUMMARY AND CONCLUSIONS

1. The effect of cortisone on the mitotic activity of the maxillary incisor was studied at 7 am, 1 pm, 7 pm, and 1 am in rats having received 0.5 and 1.0 mg per injection twice daily, at 12 hour intervals, for a two day period, and in rats given 2.0 mg in a single injection. Colchicine was administered to all rats 6 or 12 hours following the final injection and the animals sacrificed exactly three hours after treatment.

2. Mitotic counts were made on the stratum intermedium, the ameloblasts and the odontoblasts of the labial loop and the adjacent pulp of the incisor. All data were statistically analyzed.

3. In the cortisone treated animals mitotic activity was observed to be significantly higher at 7 am and 1 pm than at 7 pm and 1 am.

4. The controls likewise showed a cyclic mitotic activity which, with some exceptions, also was significantly higher at 7 am and 1 pm than at 7 pm and 1 am.

5. A single injection of 2.0 mg of cortisone produced little change in mitotic activity in any of the periods studied.

6. The mitotic activity of rats that had received 0.5 and 1.0 mg of cortisone per injection, twice daily, over a two day period, revealed either no affect or some inhibition at 7 pm. This was also the case at 1 am in those rats that had received the 0.5 mg dosage.

7. The mitotic activity of rats having received 0.5 and 1.0 mg of cortisone per injection was consistently and significantly higher at 7 am and 1 pm.

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This was also true at 1 am for rats that had received 1.0 mg per injection.

8. The dosage, as well as the time, and duration of cortisone administration were found to influence the mitotic activity of the incisor.

9. It is concluded from the results of this study, that cell proliferation is an important factor in the mechanism controlling the eruption of the rat incisor.

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TABLE I

SUMMARY OF EXPERIMENTAL GROUPINGS

Groups	No. Rats T^1 C^2	Average Weight in gm	Age in Days	Cortisone Treatment Dose in mg per inject.	Time Schedule	Colchicine Treatment	Termination
I	8 8	94.3	33	0.5	Beginning 7AM; 1PM; 7PM; 1AM; injection every 12 hours for 2 days. Total: 4 injections	1 mg/kg body wt. 12 hrs. after cortisone treatment	3 hours after colchicine adminstr.
II	8 8	90.1	32	1.0	Same as above	Same as above	Same as above
III	9 8	102.5	36	2.0	Beginning 7AM; 1PM; 7PM; 1AM; Single injection	1 mg/kg body wt. 6 hrs after cortisone treatment	Same as above

[1]Treated   [2]Control

TABLE II

TEST OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEAN MITOTIC ACTIVITY OF THE LABIAL LOOP OF GROUP I FEMALE RATS

Time Colch. Admin.		No. Rats	No. of Obs.	Stratum Intermedium			Ameloblasts			Odontoblasts			Pulp			Combined Layers		
				Mean per Field	S.E.[1]	P[2]	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P
7 AM	T[3]	2	115	2.74	.15		5.30	.23		1.06	.11		3.17	.17		12.26	.39	
	C[4]	2	114	2.05	.13	.001	4.53	.20	.010	1.11	.11	.100[5]	2.60	.19	.050	10.28	.38	.001
1 PM	T	2	87	3.10	.18		5.56	.25		1.49	.17		4.02	.25		14.18	.55	
	C	2	84	2.49	.15	.010	4.62	.23	.010	1.20	.13	.100[5]	2.24	.18	.001	10.55	.41	.001
7 PM	T	2	116	1.88	.13		3.46	.17		0.92	.09		2.16	.14		8.41	.28	
	C	2	112	2.26	.16	.070[5]	4.72	.23	.001	0.93	.10	.100[5]	2.79	.18	.010	10.70	.44	.001
1 AM	T	2	98	1.65	.13		2.90	.18		0.62	.09		2.15	.14		7.33	.32	
	C	2	118	1.96	.13	.100[5]	3.64	.21	.010	0.94	.11	.050	2.34	.16	.100[5]	8.87	.41	.010

[1] Standard Error
[2] Probability
[3] Treated
[4] Control
[5] Not Significant

TABLE III

TEST OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEAN MITOTIC ACTIVITY OF THE LABIAL LOOP OF GROUP II FEMALE RATS

Time Colch. Admin.		No. Rats	No. of Obs.	Stratum Intermedium			Ameloblasts			Odontoblasts			Pulp			Combined Layers		
				Mean per Field	S.E.[1]	P[2]	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P
7 AM	T[3]	2	102	2.43	.17		5.65	.35		1.78	.19		5.82	.41		15.68	.86	
	C[4]	2	99	2.20	.20	.100[5]	3.86	.28	.001	0.88	.11	.001	3.57	.26	.001	10.51	.60	.001
1 PM	T	2	129	2.67	.15		5.60	.24		0.78	.08		3.71	.22		12.77	.46	
	C	2	77	2.36	.19	.100[5]	4.73	.34	.050	0.74	.11	.100[5]	4.05	.25	.100[5]	11.88	.57	.100[5]
7 PM	T	2	141	1.87	.12		3.83	.19		0.60	.06		3.04	.18		9.33	.34	
	C	2	117	2.09	.13	.100[5]	4.90	.21	.001	0.61	.07	.100[5]	3.45	.18	.100[5]	11.04	.38	.001
1 AM	T	2	131	1.86	.10		4.24	.20		0.90	.08		2.42	.14		9.43	.35	
	C	2	92	1.84	.14	.100[5]	3.15	.19	.001	0.48	.05	.001	1.66	.15	.001	7.13	.34	.001

[1] Standard Error

[2] Probability

[3] Treated

[4] Control

[5] Not Significant

TABLE IV

TEST OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN THE MEAN MITOTIC ACTIVITY OF THE LABIAL LOOP OF GROUP III FEMALE RATS

Time Colch. Admin.		No. Rats	No. of Obs.	Stratum Intermedium			Ameloblasts			Odontoblasts			Pulp			Combined Layers		
				Mean per Field	S.E.[1]	P[2]	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P	Mean per Field	S.E.	P
7 AM	T[3]	2	58	2.29	.21		5.67	.32		1.21	.16		2.22	.25		11.40	.54	
	C[4]	2	112	2.60	.15	.100[5]	5.72	.27	.100[5]	1.21	.14	.100[5]	3.48	.24	.001	13.01	.52	.050
1 PM	T	3	168	2.70	.13		5.63	.21		1.30	.10		3.04	.17		12.67	.38	
	C	2	115	3.14	.16	.050	5.99	.22	.100[5]	1.35	.13	.100[5]	3.38	.21	.100[5]	13.86	.47	.060[5]
7 PM	T	2	113	2.25	.14		4.86	.23		1.20	.12		2.21	.18		10.52	.39	
	C	2	28	1.64	.20	.050	5.25	.34	.100[5]	1.25	.21	.100[5]	3.00	.42	.100[5]	11.14	.55	.100[5]
1 AM	T	2	112	1.95	.13		4.45	.23		0.86	.11		1.74	.16		8.99	.38	
	C	2	112	2.38	.17	.050	5.03	.25	.100[5]	1.11	.13	.100[5]	2.09	.18	.100[5]	10.60	.45	.010

[1] Standard Error
[2] Probability
[3] Treated
[4] Control
[5] Not Significant

TABLE V

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF GROUP I TREATED FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum	Between Groups	3	139.73	46.58	20.70	.001
Inter-medium	Within Groups(Error)	412	926.75	2.25		
	Totals	415	1066.48			
	7 AM & 1 PM vs 7 PM & 1 AM	1	130.43	130.43	57.97	.001
Amelo-blasts	Between Groups	3	527.65	175.88	39.08	.001
	Within Groups(Error)	412	1853.11	4.50		
	Totals	415	2380.76			
	7 AM & 1 PM vs 7 PM & 1 AM	1	507.50	507.50	112.78	.001
Odonto-blasts	Between Groups	3	36.31	12.10	9.31	.001
	Within GRoups(Error)	412	535.65	1.30		
	Totals	415	571.96			
	7 AM & 1 PM vs 7 PM & 1 AM	1	22.23	22.23	17.10	.001
Pulp	Between Groups	3	234.47	78.16	24.73	.001
	Within Groups(Error)	412	1301.72	3.16		
	Totals	415	1536.19			
	7 AM & 1 PM vs 7 PM & 1 AM	1	198.02	198.02	62.67	.001
Combined Layers	Between Groups	3	3026.92	1008.97	64.84	.001
	Within Groups(Error)	412	6415.92	15.57		
	Totals	415	9442.84			
	7 AM & 1 PM vs 7 PM & 1 AM	1	2780.95	2780.95	178.61	.001

[1]Degrees of Freedom   [2]Probability   [3]Not Significant

TABLE VI

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF GROUP L CONTROL FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum	Between Groups	3	16.27	5.76	2.61	.050
Inter-medium	Within Groups(Error)	424	934.95	2.21		
	Totals	427	951.22			
	7 AM & 1 PM vs 7 PM & 1 AM	1	1.88	1.88	0.85	N.S.[3]
Amelo-blasts	Between Groups	3	85.45	28.48	5.54	.001
	Within Groups(Error)	424	2177.98	5.14		
	Totals	427	2263.43			
	7 AM & 1 PM vs 7 PM & 1 AM	1	17.06	17.06	3.32	N.S.
Odonto-blasts	Between Groups	3	5.23	1.74	1.30	N.S.
	Within Groups(Error)	424	568.31	1.34		
	Totals	427	573.54			
	7 AM & 1 PM vs 7 PM & 1 AM	1	4.77	4.77	3.56	N.S.
Pulp	Between Groups	3	19.01	6.34	1.88	N.S.
	Within Groups(Error)	424	1427.98	3.37		
	Totals	427	1446.99			
	7 AM & 1 PM vs 7 PM & 1 AM	1	1.34	1.34	0.40	N.S.
Combined Layers	Between Groups	3	237.16	79.05	4.32	.010
	Within Groups(Error)	424	7756.60	18.29		
	Totals	427	7993.76			
	7 AM & 1 PM vs 7 PM & L AM	1	42.64	42.64	2.33	N.S.

[1]Degrees of Freedom   [2]Probability   [3]Not Significant

TABLE VII

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF GROUP II TREATED FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum	Between Groups	3	64.48	21.49	9.34	.001
Inter-	Within Groups(Error)	499	1146.58	2.30		
medium	Totals	502	1211.06			
	7 AM & 1 PM vs 7 PM & 1 AM	1	61.12	61.12	26.57	.001
Amelo-	Between Groups	3	327.47	109.16	15.16	.001
blasts	Within Groups(Error)	499	3594.42	7.20		
	Totals	502	3921.89			
	7 AM & 1 PM vs 7 PM & 1 AM	1	315.65	315.65	43.84	.001
Odonto-	Between Groups	3	90.88	30.29	23.30	.001
blasts	Within Groups(Error)	499	649.40	1.30		
	Totals	502	740.28			
	7 AM & 1 PM vs 7 PM & 1 AM	1	28.56	28.56	21.97	.001
Pulp	Between Groups	3	733.24	244.41	34.92	.001
	Within Groups(Error)	499	3492.94	7.00		
	Totals	502	4226.18			
	7 AM & 1 PM vs 7 PM & 1 AM	1	453.82	453.82	64.83	.001
Combined	Between Groups	3	3210.71	1070.24	33.98	.001
Layers	Within Groups(Error)	499	15718.73	31.50		
	Totals	502	18929.44			
	7 AM & 1 PM vs 7 PM & 1 AM	1	2728.06	2728.06	86.61	.001

[1]Degrees of Freedom   [2]Probability

TABLE VIII

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF GROUP II CONTROL FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum	Between Groups	3	12.73	4.24	1.63	N.S.[3]
Inter-medium	Within Groups(Error)	381	995.47	2.61		
	Totals	384	1008.20			
-	7 AM & 1 PM vs 7 PM & 1 AM	1	8.41	8.41	3.22	N.S.
Amelo-blasts	Between Groups	3	190.70	63.59	10.24	.001
	Within Groups(Error)	381	2365.93	6.21		
	Totals	384	2556.63			
	7 AM & 1 PM vs 7 PM & 1 AM	1	1.14	1.14	0.18	N.S.
Odonto-blasts	Between Groups	3	31.51	10.50	14.79	.001
	Within Groups(Error)	381	269.25	0.71		
	Totals	384	300.76			
	7 AM & 1 PM vs 7 PM & 1 AM	1	6.86	6.86	9.66	.010
Pulp	Between Groups	3	293.69	97.90	21.80	.001
	Within Groups(Error)	381	1711.65	4.49		
	Totals	384	2005.34			
	7 AM & 1 PM vs 7 PM & 1 AM	1	118.43	118.43	26.38	.001
Combined Layers	Between Groups	3	1175.78	391.93	17.90	.001
	Within Groups(Error)	381	8341.92	21.90		
	Totals	384	9517.70			
	7 AM & 1 PM vs 7 PM & 1 AM	1	305.23	305.23	13.94	.001

[1]Degrees of Freedom   [2]Probability   [3]Not Significant

TABLE IX

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF
GROUP III TREATED FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum Inter-medium	Between Groups	3	40.47	13.49	5.55	.001
	Within Groups(Error)	447	1085.88	2.43		
	Totals	450	1126.35			
	7 AM & 1 PM vs 7 PM & 1 AM	1	28.14	28.14	11.58	.001
Amelo-blasts	Between Groups	3	119.74	39.91	6.10	.001
	Within Groups(Error)	447	2923.31	6.54		
	Totals	450	3043.05			
	7 AM & 1 PM vs 7 PM & 1 AM	1	110.12	110.12	16.84	.001
Odonto-blasts	Between Groups	3	18.60	6.20	3.85	.010
	Within Groups(Error)	447	717.84	1.61		
	Totals	450	736.44			
	7 AM & 1 PM vs 7 PM & 1 AM	1	6.67	6.67	4.14	.050
Pulp	Between Groups	3	122.28	40.76	10.32	.001
	Within Groups(Error)	447	1764.27	3.95		
	Totals	450	1886.55			
	7 AM & 1 PM vs 7 PM & 1 AM	1	81.39	81.39	20.61	.001
Combined Layers	Between Groups	3	952.32	317.44	16.31	.001
	Within Groups(Error)	447	8698.40	19.46		
	Totals	450	9650.72			
	7 AM & 1 PM vs 7 PM & 1 AM	1	750.92	750.92	38.59	.001

[1]Degrees of Freedom   [2]Probability

TABLE X

THE ANALYSIS OF VARIANCE TEST ON THE DIURNAL MITOTIC ACTIVITY OF GROUP III CONTROL FEMALE RATS

Cell Layer	Source of Variance	DF[1]	Sum of Squares	Mean Squares	Variance Ratio	P[2]
Stratum	Between Groups	3	64.49	21.50	7.85	.001
Inter-medium	Within Groups(Error)	363	995.37	2.74		
	Totals	366	1059.86			
	7 AM & 1 PM vs 7 PM & 1 AM	1	35.88	35.88	13.10	.001
Amelo-blasts	Between Groups	3	58.91	19.64	2.96	.050
	Within Groups(Error)	363	2407.58	6.63		
	Totals	366	2466.49			
	7 AM & 1 PM vs 7 PM & 1 AM	1	53.71	53.71	8.10	.010
Odonto-blasts	Between Groups	3	3.35	1.12	0.59	N.S.[3]
	Within Groups(Error)	363	694.33	1.91		
	Totals	366	697.68			
	7 AM & 1 PM vs 7 PM & 1 AM	1	1.75	1.75	0.92	N.S.
Pulp	Between Groups	3	135.73	45.24	8.89	.001
	Within Groups(Error)	363	1846.23	5.09		
	Totals	366	1981.96			
	7 AM & 1 PM vs 7 PM & 1 AM	1	116.58	116.58	22.90	.001
Combined Layers	Between Groups	3	694.81	231.60	9.29	.001
	Within Groups(Error)	363	9045.11	24.92		
	Totals	366	9739.92			
	7 AM & 1 PM vs 7 PM & 1 AM	1	646.98	646.98	25.96	.001

[1]Degrees of Freedom   [2]Probability   [3]Not Significant

Figure 1. Graph showing the average number of mitotic figures in individual
cell layers of the labial loop of maxillary incisors in young
female rats having received 0.5 mg of cortisone per injection,
twice daily, for a two day period. The controls received an equal
volume of normal saline. The time of colchicine administration is
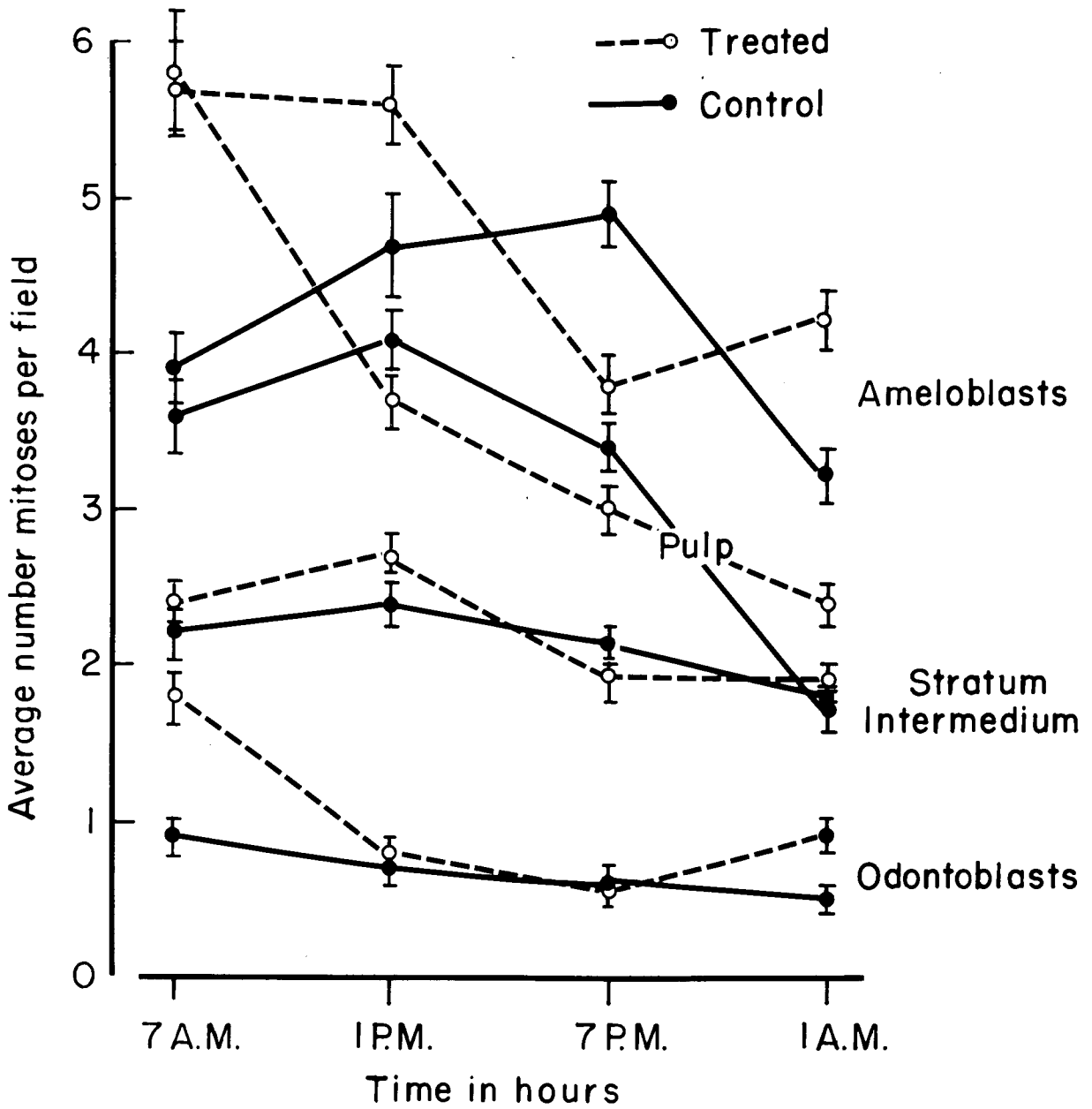indicated on the abscissa. The vertical bars indicate the standard
error.
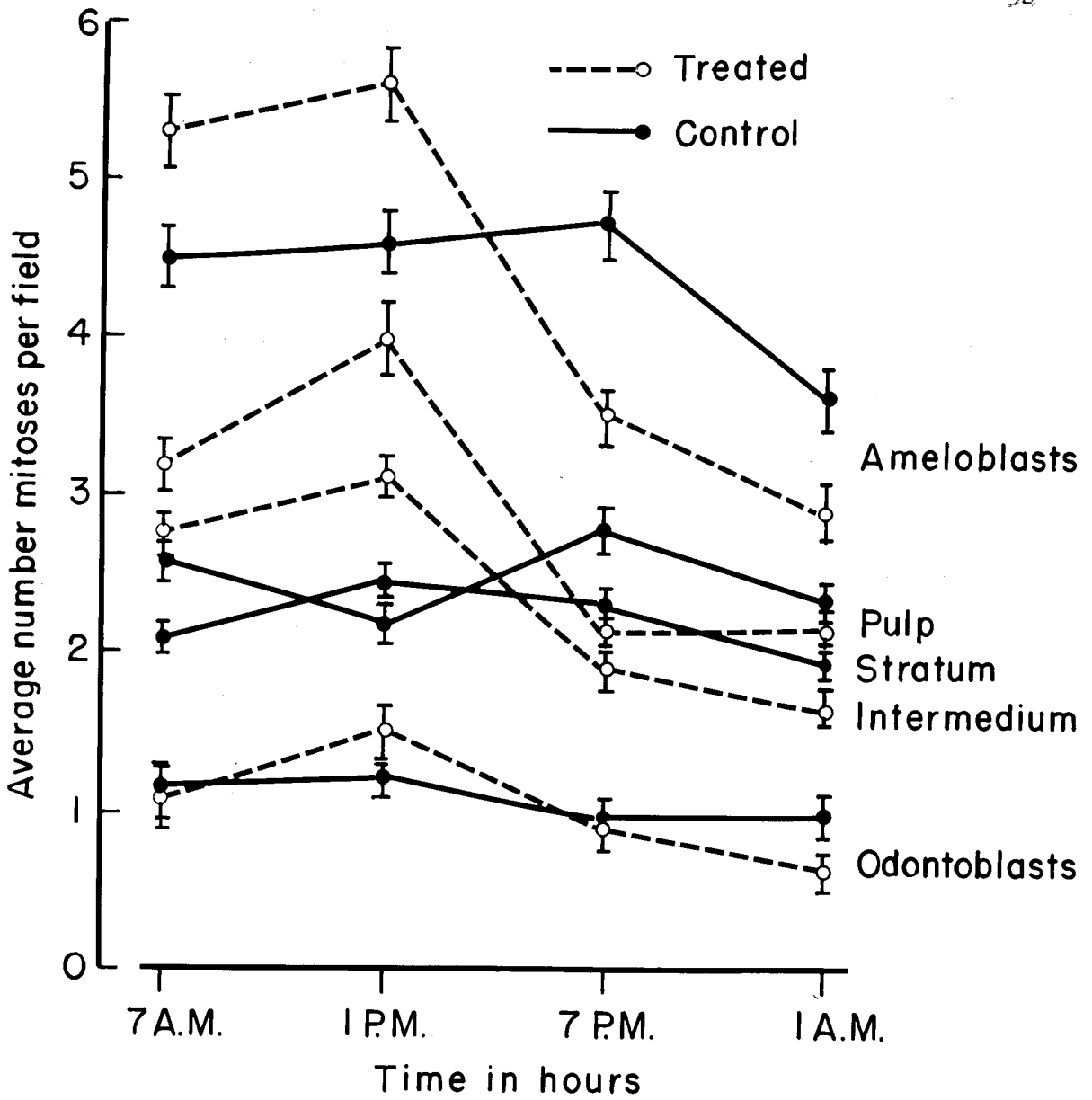
FIGURE 1

Figure 2.   Graph showing the average number of mitotic figures in individual
            cell layers of the labial loop of maxillary incisors in young
            female rats having received 1.0 mg of cortisone per injection,
            twice daily, for a two day period. The controls received an equal
            volume of normal saline. The time of colchicine administration is
            indicated on the abscissa. The vertical bars indicate the standard
            error.

FIGURE 2

Figure 3. Graph showing the average number of mitotic figures in individual
cell layers of the labial loop of maxillary incisors in young
female rats having received 2.0 mg of cortisone in a single
injection. The controls received an equal volume of normal saline.
The time of colchicine administration is indicated on the abscissa.
The vertical bars show the standard error.
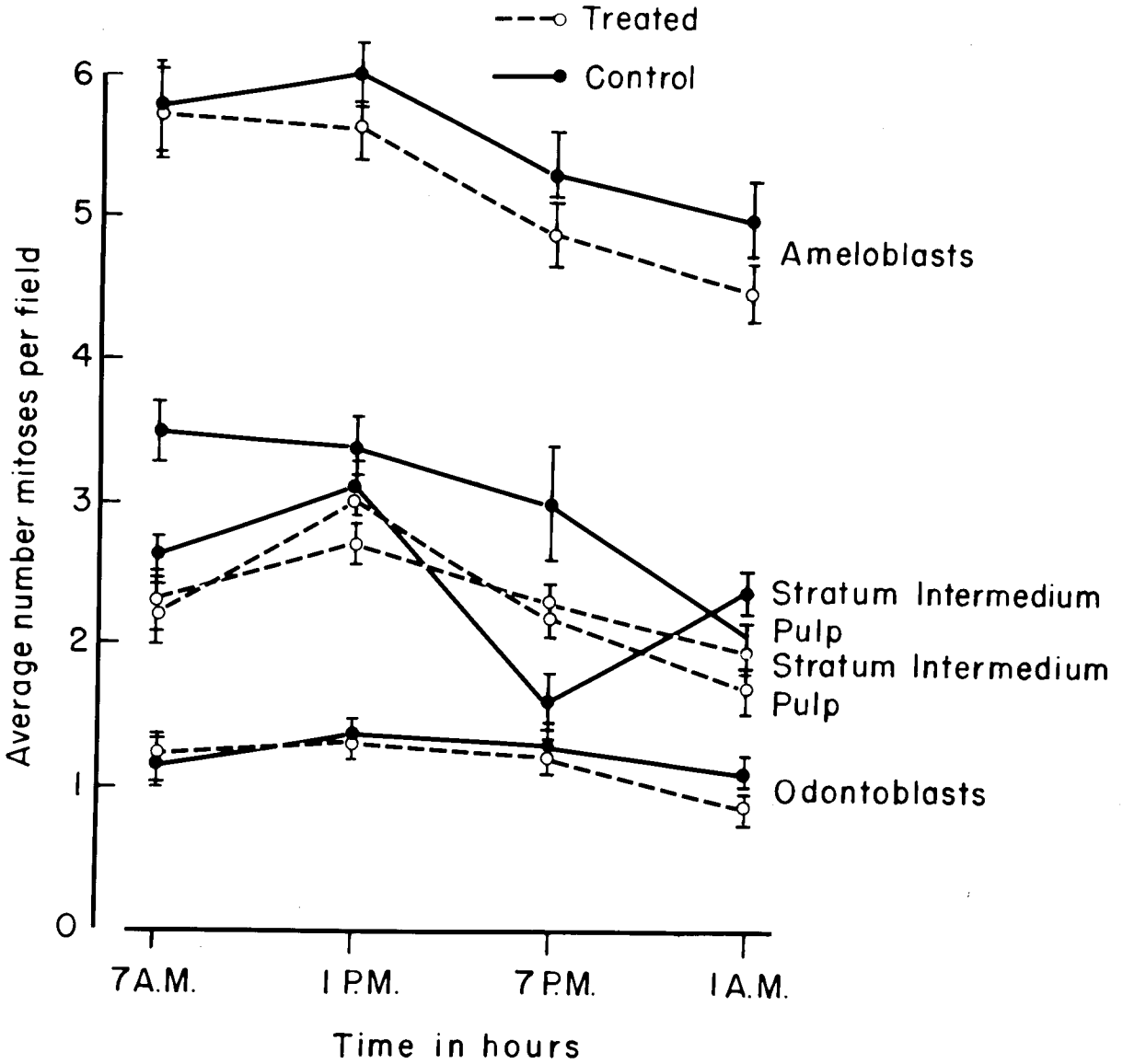
FIGURE 3

PLATE I

Figure 5. A low power view of a sagittal section of a maxillary incisor
showing the basal labial loop in a young female rat. (x30)
The circumscribed area at the basal end is shown under higher
magnification in Figure 6.

Figure 6. The labial loop of a maxillary incisor showing the areas or fields
(1-7) in which mitotic counts were made. (x200)

Abbreviations: SI - Stratum Intermedium
Am - Ameloblasts
Od - Odontoblasts
 P - Pulp
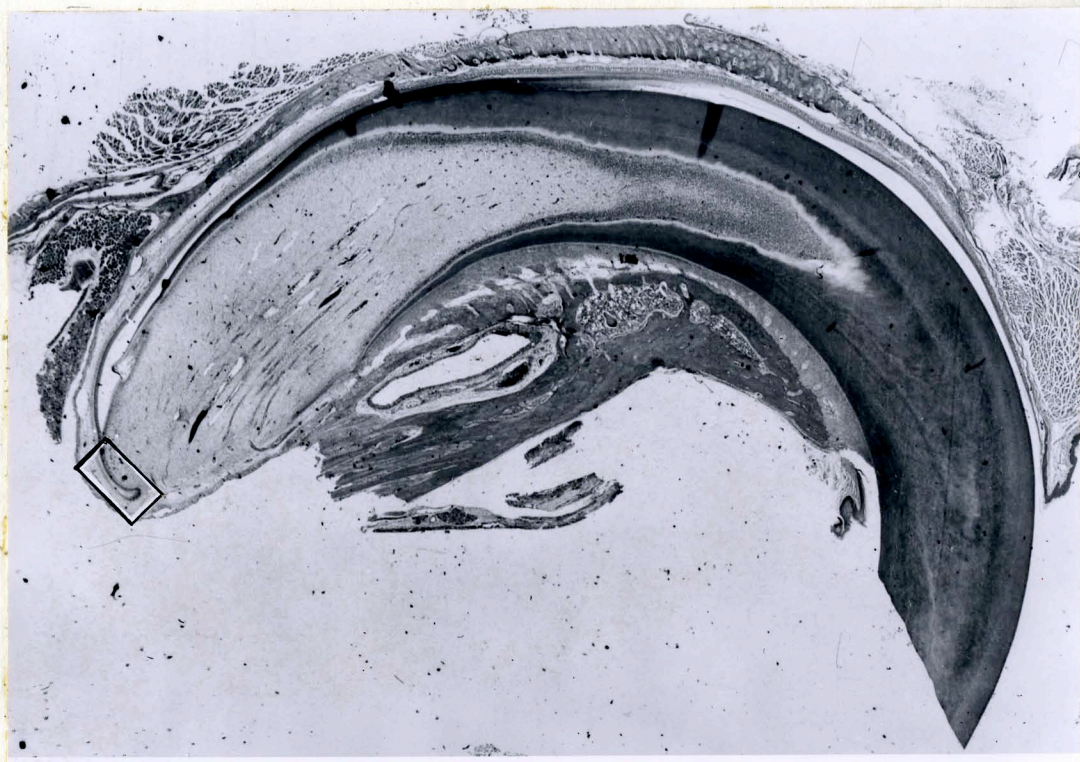
FIGURE 5
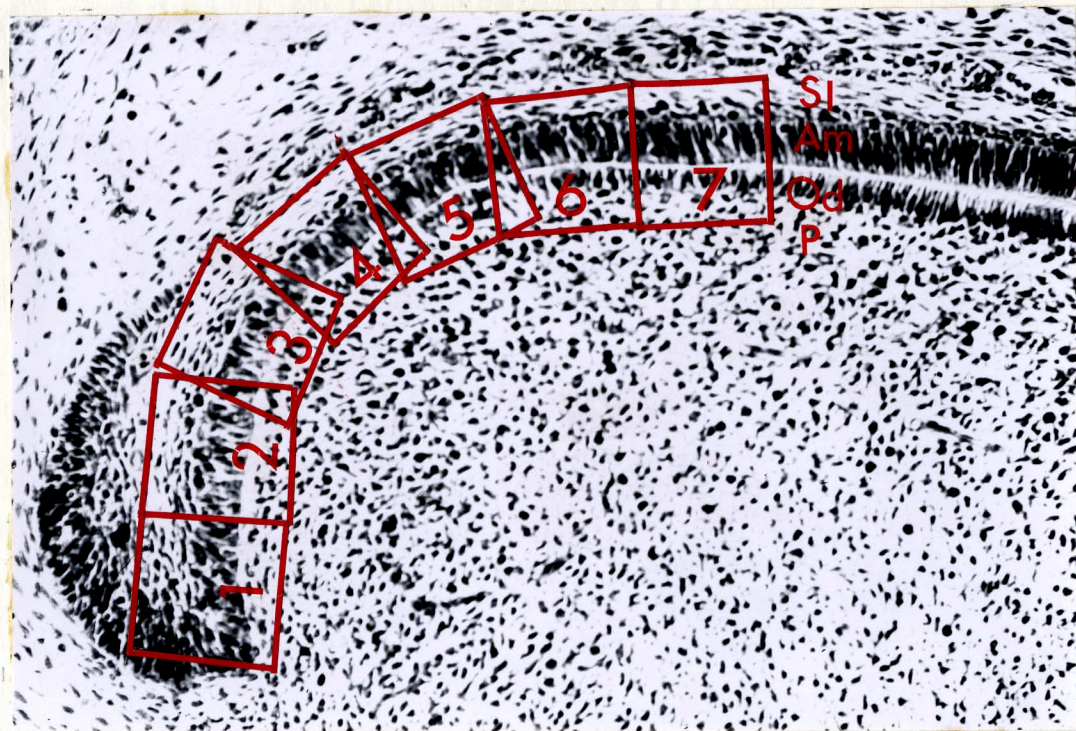
FIGURE 6

PLATE II

Figure 7. A portion of the labial loop from a maxillary incisor of a normal
young female rat. Note the scarcity of dividing cells when com-
pared with Figure 8. (x400)

        Abbreviations: SI - Stratum Intermedium
                         Am - Ameloblasts
                         Od - Odontoblasts
                          P - Pulp

Figure 8. A portion of the labial loop from a maxillary incisor of a colchi-
cine treated young female rat. Note the large number of mitotic
figures as indicated by the arrows. (x400)

        Abbreviations: SI - Stratum Intermedium
                         Am - Ameloblasts
                         Od - Odontoblasts
                          P - Pulp
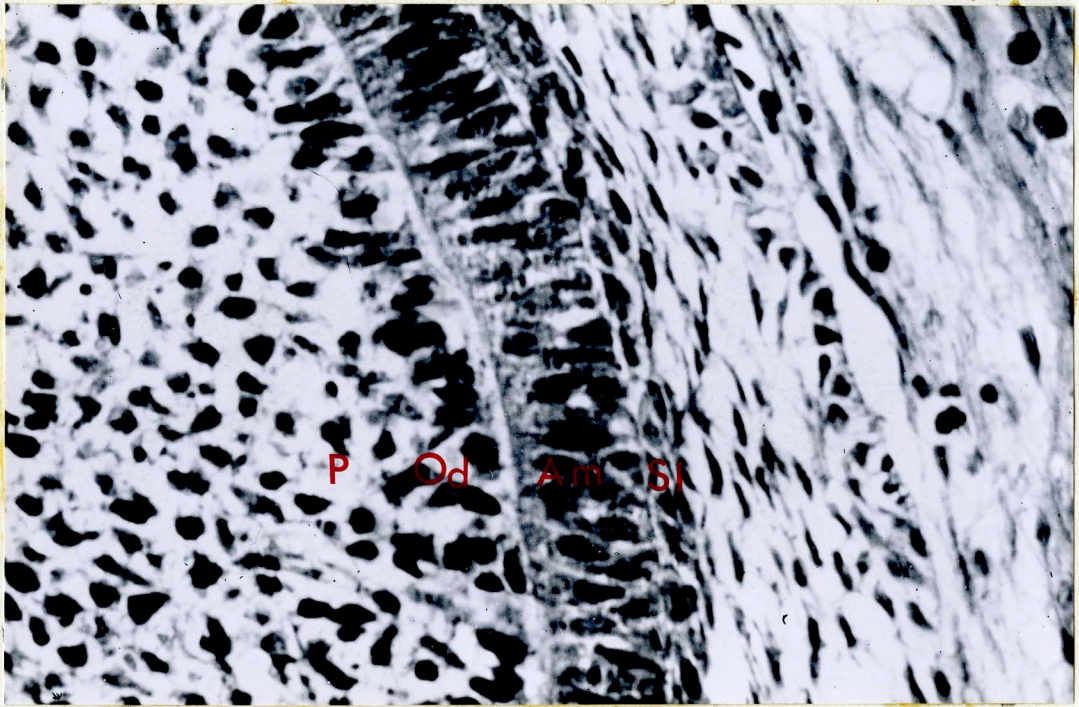
FIGURE 7
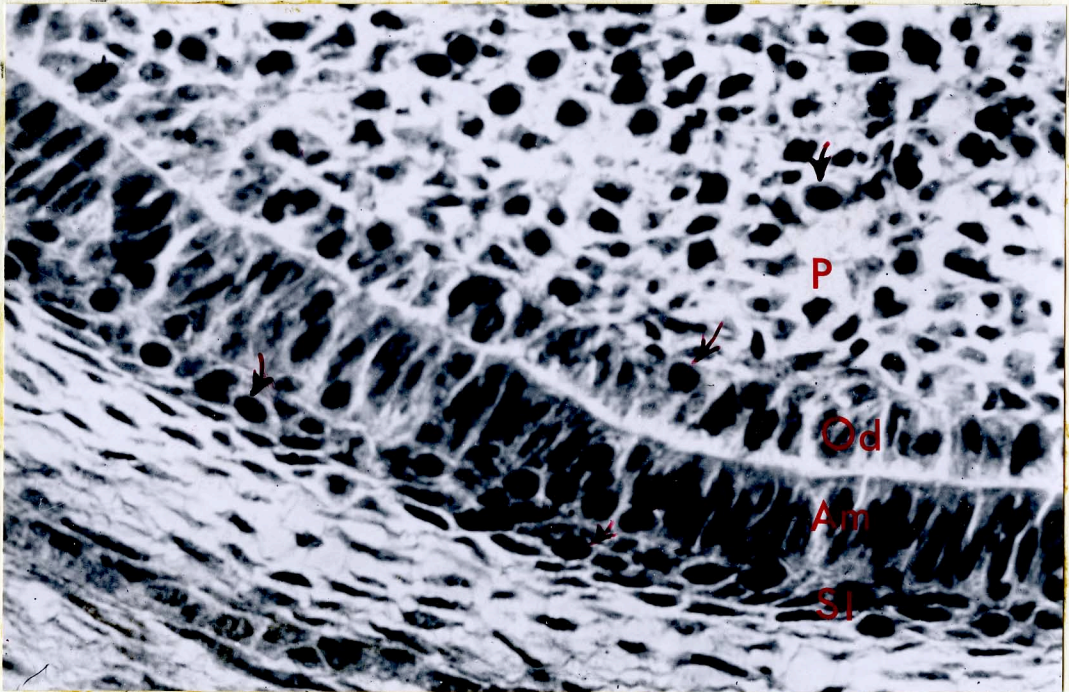
FIGURE 8

PLATE III

Figure 9. Colchicine arrested cell in metaphase of the star type. (x1000)

Figure 10. Colchicine arrested cell in metaphase of the star type in the ameloblast layer. (x1000)

Figure 11. Colchicine arrested cell in metaphase of the exploded type. Odontoblast layer. (x1000)

Figure 12. Colchicine arrested cell in metaphase of the exploded type. Odontoblast layer. (x1000)
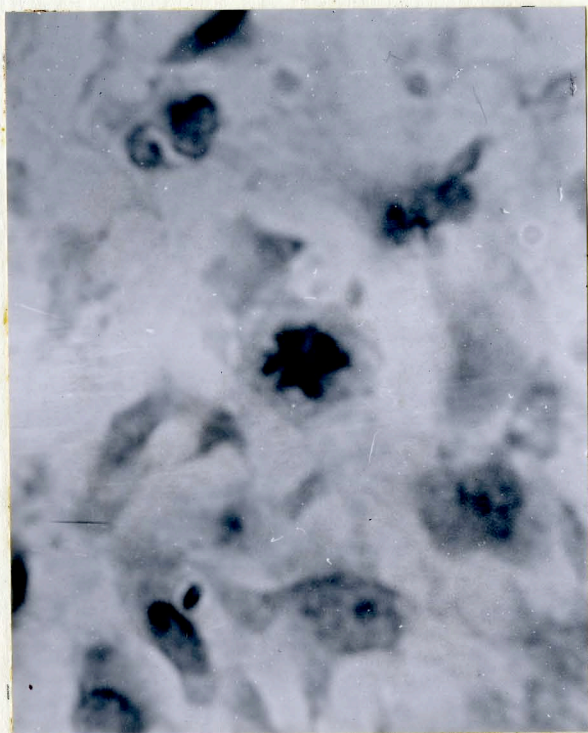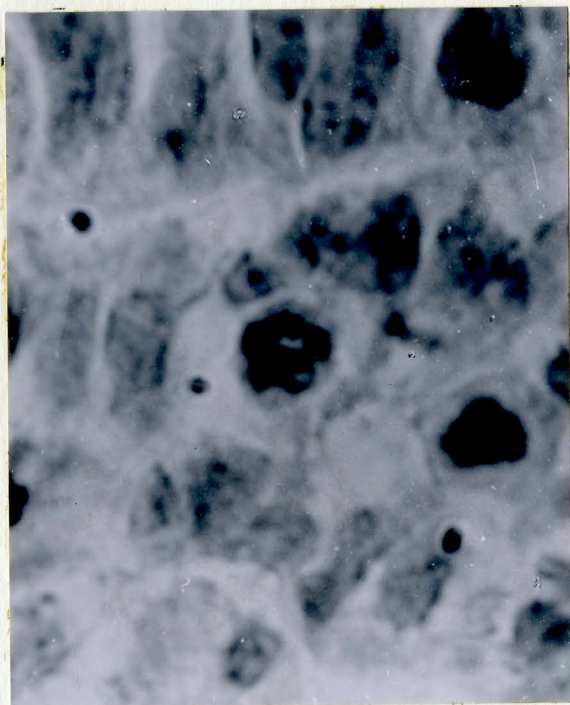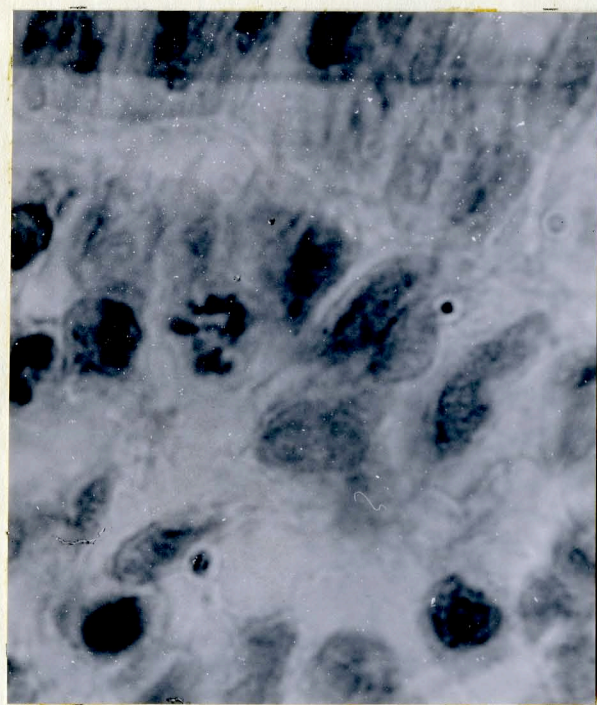
FIGURE 9
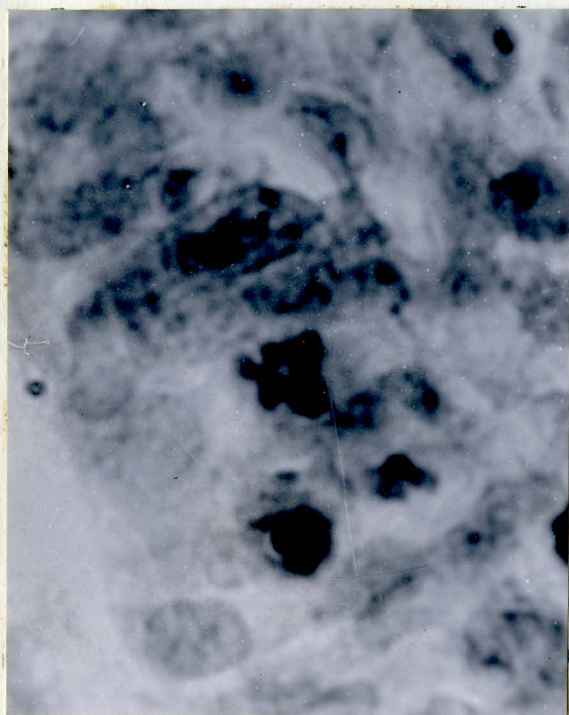
FIGURE 10

FIGURE 11

FIGURE 12

PLATE IV

Figure 13. Colchicine arrested cell in metaphase in the pulp. The ball
            type. (x1000)

Figure 14. Colchicine arrested cell in metaphase in the ameloblast layer.
            The ball type. (x1000)

Figure 15. Colchicine arrested cell in metaphase similar to the normal type,
            in the ameloblast layer. Note the equatorial plane orientation
            of the chromosomes. (x1000)

Figure 16. Colchicine arrested cells in metaphase approximating the normal
            type, in the pulp area. Note the equatorial plane arrangement
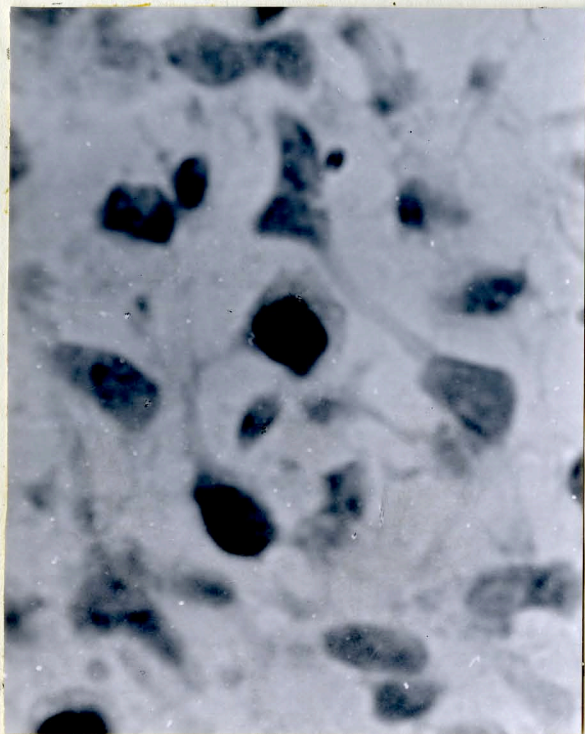   of the chromosomes. (x1000)
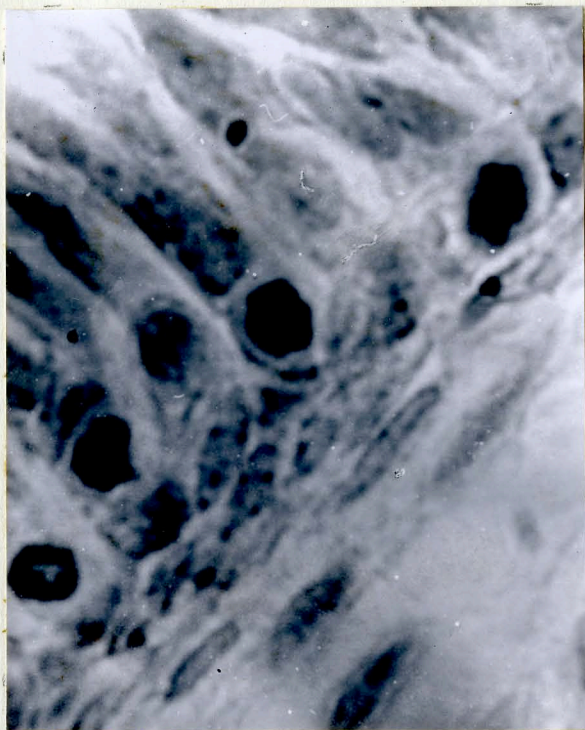
FIGURE 13
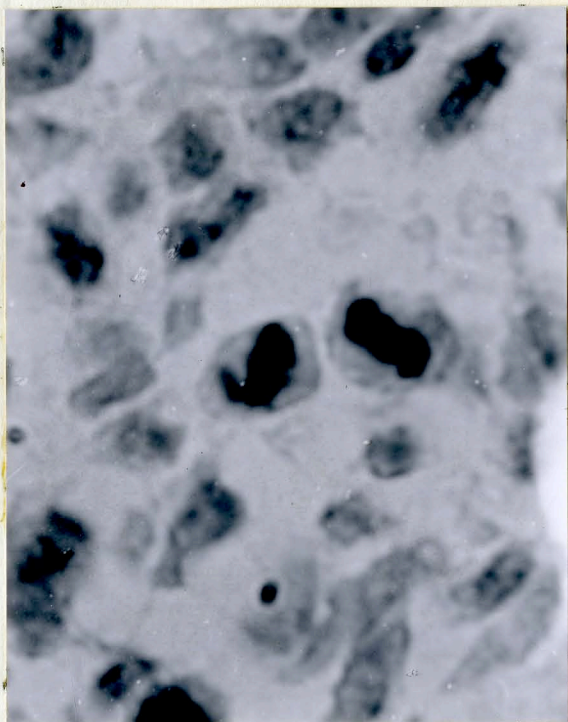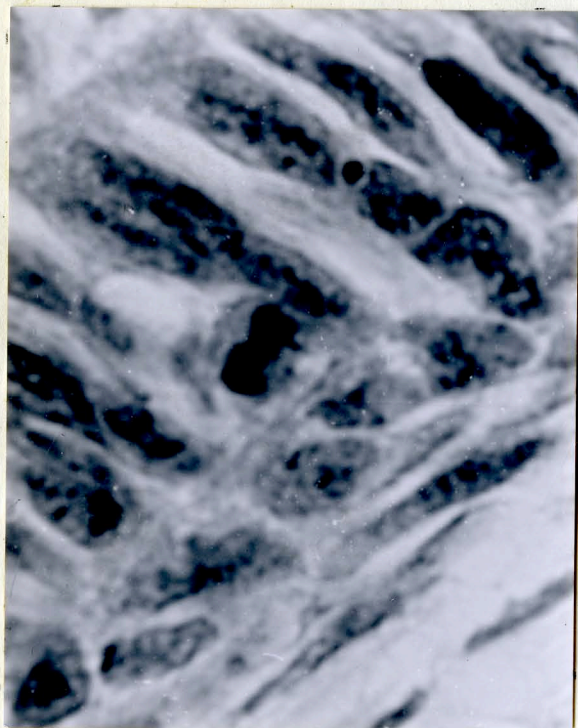

FIGURE 14

FIGURE 15

FIGURE 16

PLATE V

Figure 17.   The basal region of the labial loop of a maxillary incisor from
             a young female rat. Note the loosely arranged stratum intermedium
             and the sparse odontoblast layer. (x400)

             Abbreviations: SI - Stratum Intermedium
                             Am - Ameloblasts
                             Od - Odontoblasts
                              P - Pulp

Figure 18.   The middle region of the labial loop from a maxillary incisor
             of a young female rat. Note the large number of dividing cells
             as indicated by the arrows. (x400)

             Abbreviations: SI - Stratum Intermedium
                             Am - Ameloblasts
                             Od - Odontoblasts
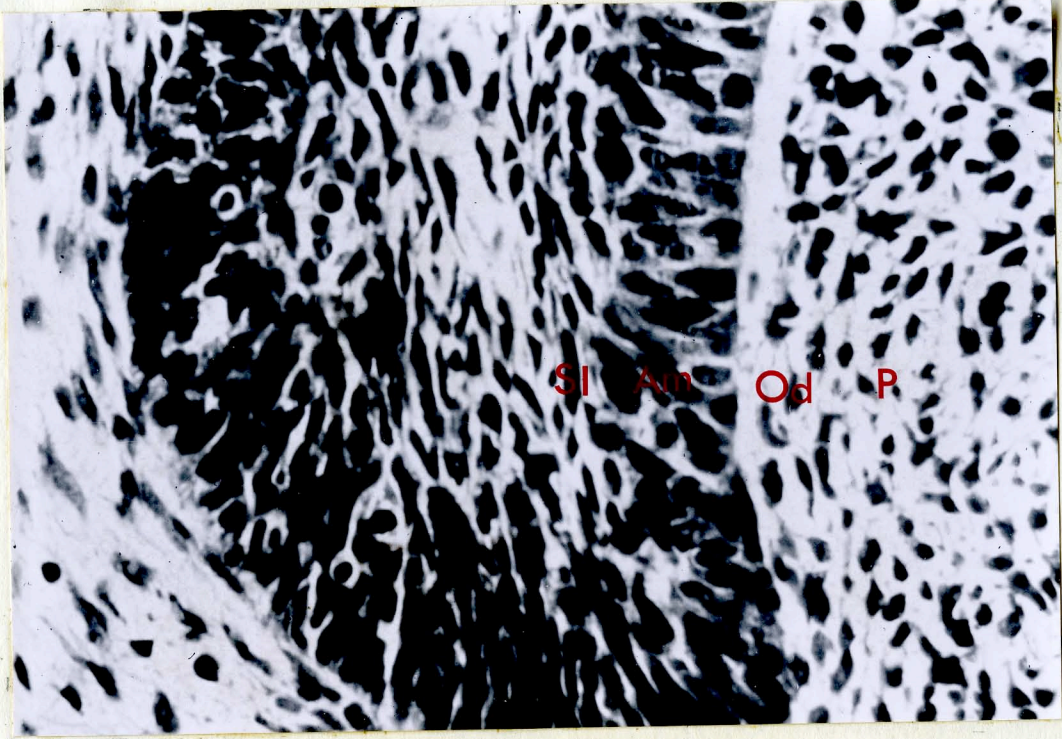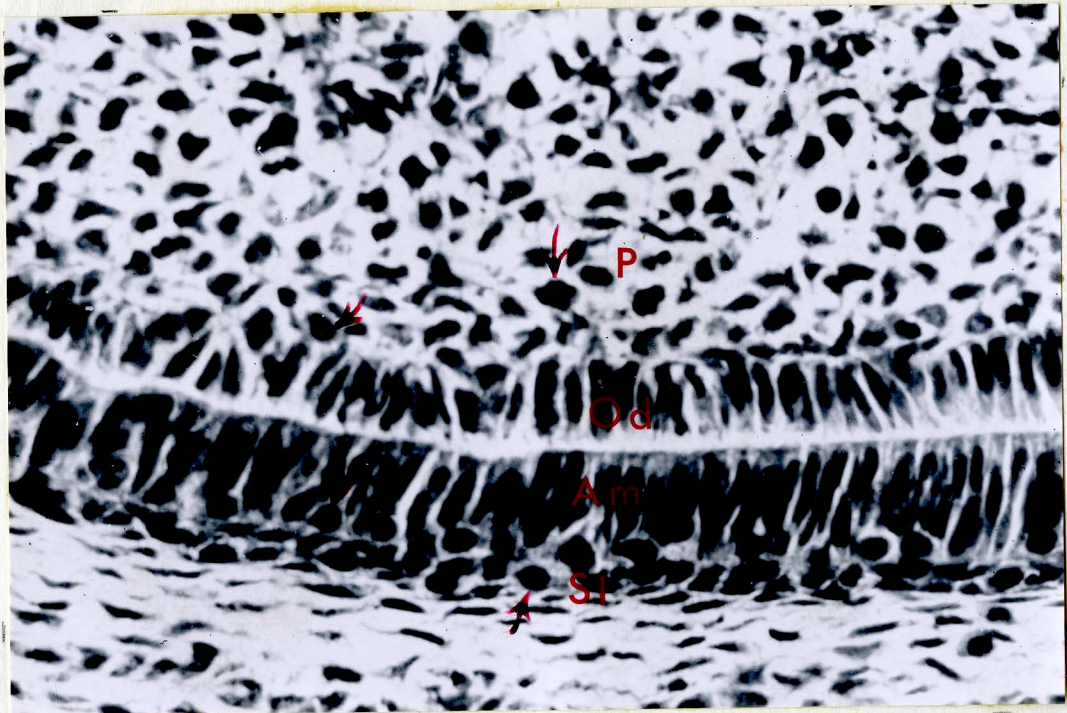   P - Pulp

FIGURE 17

FIGURE 18

PLATE VI

Figure 19. The terminal region of the labial loop from a maxillary incisor
            of a young female rat. Note the formation of dentin and the
            relative absence of mitoses when compared with Figure 18.   (x400)

            Abbreviations: SI - Stratum Intermedium
                             Am - Ameloblasts
                             Od - Odontoblasts
                              P - Pulp
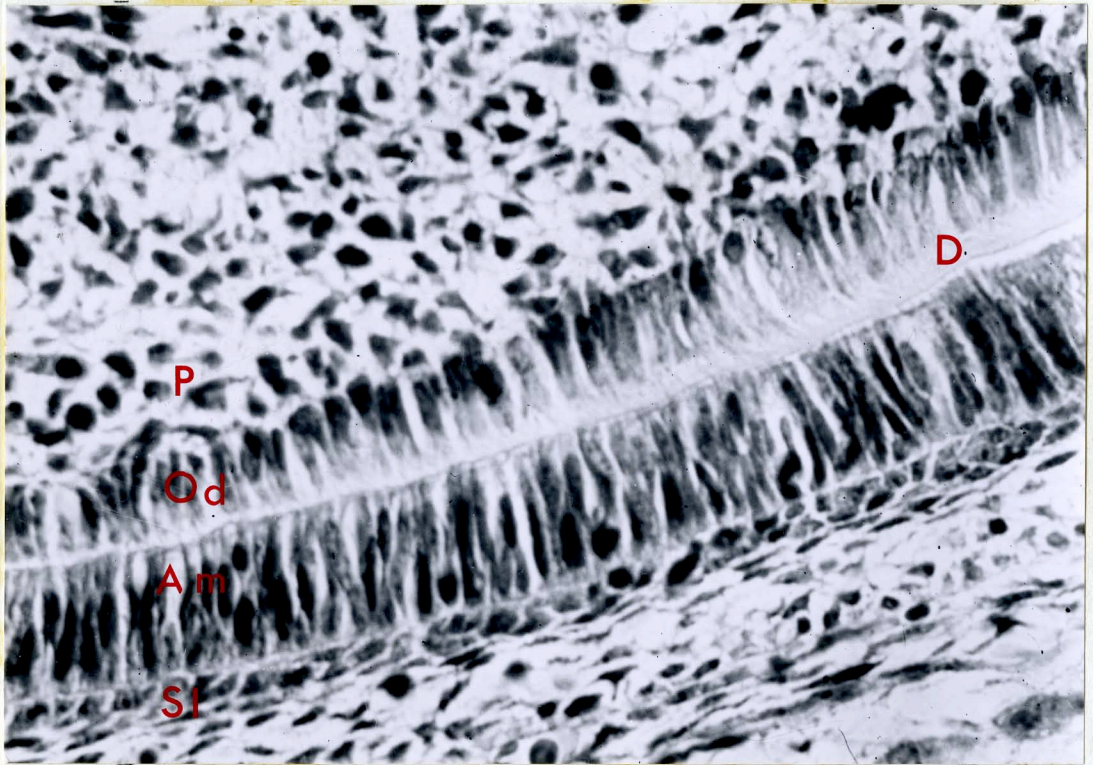   D - Dentin

FIGURE 19

APPROVAL SHEET

The thesis submitted by Michael L. Kiely has been read and approved by four 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.

_10 - 15 - 63_
Date

Lincoln Brown
Signature of Advisor

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