Histol Histopath (1987) 2: 129-133

Histology and Histopathology

Autoradiographic investigation of circadian rhythms in alveolar bone periosteum and cementum in young mice*

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Summary. This report presents circadian rhythms in cell proliferation of alveolar bone periosteum and cementum of the maxillary first molars of male 5-week-old BNL, Swiss albino mice which were maintained on a 12 hr light/dark cycle. Mice were injected with ^{3}H-TDR (luCi/gm. body wt.) 1 hr prior to sacrifice and killed every 3 hrs for 24 hrs starting at 9 a.m. Maxillae were decalcified, routinely processed histologically and autoradiographs prepared. Cell labeling indices of alveolar bone and cementum mesial to the first molar were determined. Alveolar bone periosteal and cemental cells show circadian rhythm in their DNA synthetic processes. Peaks in percent labeling exhibit higher values than previously reported for nontraumatized, normal dental periosteum and cementum. While the outer periosteum reveals a single 24 hr peak (6 p.m.), inner periosteum and cementum reveal two ultradian peaks 9 to 12 hrs apart involving both light and dark periods. Rodents are nocturnal, but high peaks are also evident in the light periods, consequently, not all peaks are synonymous with the period of animal activity and feeding. Although the single daylight peak of the outer periosteum may indicate growth of that surface at night to about noon, the double peaks exhibited by inner periosteum and cementum indicate light/dark, continuously active surfaces in terms of DNA synthesis and growth.

Key words: Circadian rhythms - Autoradiography - Alveolar bone - Cementum - ^{3}H-Thymidine

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* This investigation was supported in part by USPHS Research Grant DE-03014 from the National Institute of Dental Research, National Institutes of Health.

Introduction

Studies of the possible rhythmic activity of bone, cartilage and synovial cells and tissues have generated clear evidence of the existence of diurnal circadian variations (Simmons, 1962, 1963, 1964, 1968, 1974, 1979; Simmons and Nichols, 1966). The chronology of fracture healing was also investigated (Simmons et al., 1977). The evidence for such circadian rhythms has been obtained from rabbits, rats and mice: cell division has been studied using ^{3}H-thymidine, collagen matrix production using ^{3}H-proline and ^{14}C-glycine, glycosaminoglycan synthesis using ^{35}S, as well as other biochemical procedures. More recently, a number of studies have also revealed the existence of seasonal, as well as diurnal, variations in chondrogenic cells of the condylar cartilage of the mandible and epiphyseal cartilage of the tibia (Oudet and Petrovic, 1977, 1978, 1981, 1982).

Dental tissues have also been studied as to their rhythmicity. The existence of circadian patterns in the formation and mineralization of dentin and lamellar bone in rabbits was first demonstrated by Okada and Mimura (1938, 1940) using lead acetate markers. Miani et al. (1964, 1966) and Miani and Miani (1968) studied the calcification of dentin in rat and dog teeth using tetracycline labeling methods. Their results indicated rhythms associated with the feeding schedule. Domm and Kiely (1968), Michaeli and Weinreb (1968), Gasser et al. (1972), and Kiely and Domm (1973) reported that processes involving tooth eruption, as well as growth and attrition in rat incisors exhibit circadian rhythms. Furthermore, ultradian rhythms (periods less than 24 hours) were also demonstrated by Rosenberg and Simmons (1980a,b) in the calcium content of dentin in growing rabbit incisors, using electron microprobe and lead acetate techniques.

Studies of the rhythmicity of the dental supporting cells

and tissues of the oral cavity have received relatively less attention. DNA synthesis was studied in rat epithelial tissue and rat incisor enamel epithelium (England and Burke, 1968; Gasser et al., 1972; Kiely and Domm, 1973; Kiely and Wilde, 1974; Chiba et al., 1976); Mantell (1973) reported on circadian rhythm in the mitotic activity of rat incisor pulp. Circadian and ultradian rhythms in the gingival epithelium (Sandhu et al., 1986) and periodontal fibroblasts (Singh et al., 1986) have also been reported in young mice. The present initial study is part of a larger investigation aimed at a study of the circadian rhythms in the supporting oral tissues of young and aging animals.

Materials and methods

A total of 24 male 5-week-old Brookhaven National Laboratory (BNL), shortlived, Swiss albino mice were used in this study. The BNL mouse has a median lifespan of less than one year, but can survive with difficulty up to two years of age. The animals were maintained at 70° ± 3°F on a 12 hour light/dark cycle, with standard rat and mouse chow and water *ad libitum*. All mice were injected subcutaneously with 1 μCi/gm. body weight of tritiated thymidine (^{3}H-TDR) having a specific activity of 1.9Ci/mM 1 hr prior to sacrifice. The animals were killed every 3 hrs over a 24 hr period starting at 9 a.m.

Both maxillae were removed, fixed for 24 hrs in 10% neutral buffered formalin, washed, decalcified in EDTA and routinely processed histologically. Autoradiographs were prepared from 5 μm paraffin sections using Kodak NTB-3 liquid emulsion and a 16 day exposure. The final preparations were stained with hematoxylin.

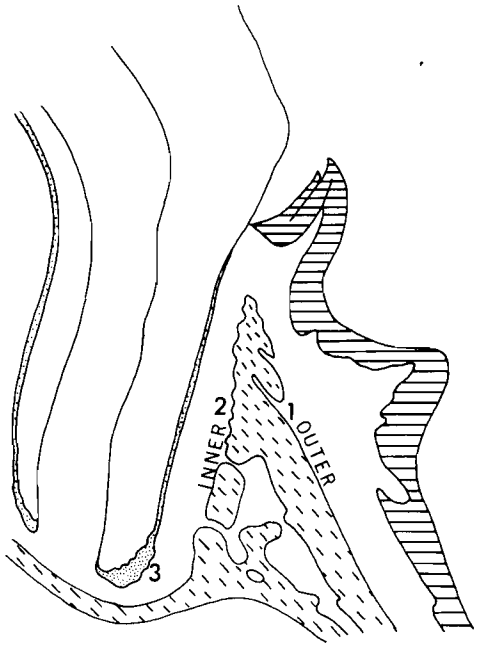
Labeling indices of alveolar bone (outer = mucosal surface; inner = periodontal surface) and cementum mesial to the first molar were determined as % labeling ± standard deviation (Fig. 1). Each value was derived from a minimum of 200 cells per animal. Statistical analyses were performed using a TRS-80 Model III microcomputer, and Student's t-statistic was used to evaluate changes in labeling index.

Fig. 1. Schematic diagram of the mesial aspect of the maxiliary first molar of a mouse. 1, Outer periosteum; 2, Inner periosteum; 3, Cementum.

Results

The data are presented in Table 1 and Figures 2 and 3.

In the outer alveolar bone periosteum facing the mucosa, a major peak occurred at 6 p.m. (2.05 ± 0.14%) followed by a statistically non-significant rise at midnight (0.40 ± 0.06%). The inner alveolar bone periosteum facing the periodontium, on the other hand, revealed two significant high peaks; one at 3 p.m. (2.28 ± 0.15%), and the second peak at 3 a.m. (3.01 ± 0.17%). No statistical difference exists between the two peak values. By comparison, the cemental cells exhibited two significant lower peaks and a third non-significant elevation. The statistically significant peaks occurred at 3 p.m. (0.75 ± 0.09%) with a $p \leqslant 025$, at midnight (1.17 ± 0.11%) and a non-significant elevation at 6 a.m. (0.63 ± 0.8%).



MOUSE MAXILLARY FIRST MOLAR

FIG. 1

Table 1. Percent labeling (m% ± S.D.) of alveolar bone Periosteum and cementum of 5-week-old mice

TIME	9 a.m.	12 noon	3 p.m.	6 p.m.
PERIOSTEUM				
Outer	0	0	1.0 ± .10	2.05 ± .14
Inner	0	0.37 ± .06	2.28 ± .15	0
CEMENTUM	0	0	0.75 ± .09	0
TIME	**9 p.m.**	**12 midnight**	**3 a.m.**	**6 a.m.**
PERIOSTEUM				
Outer	0	0.40 ± .06	0	0
Inner	0.80 ± 0.9	0.79 ± .09	3.01 ± .17	0.56 ± .08
CEMENTUM	0.29 ± .05	1.17 ± .11	0	0.63 ± .08

The light cycle is from 6 a.m.to 6 p.m.

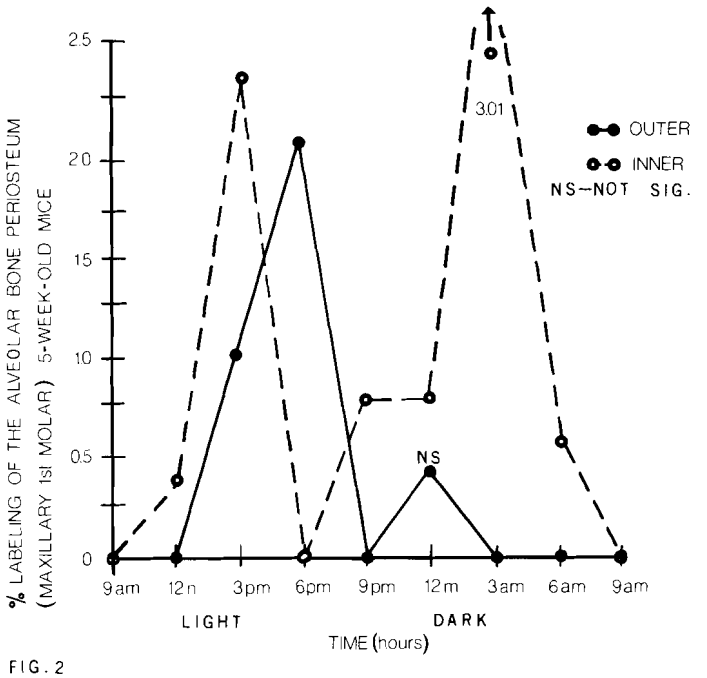


Fig. 2. Circadian rhythms in the ^{3}H-TDR labeling activity of the alveolar bone in 5-week-old mice.

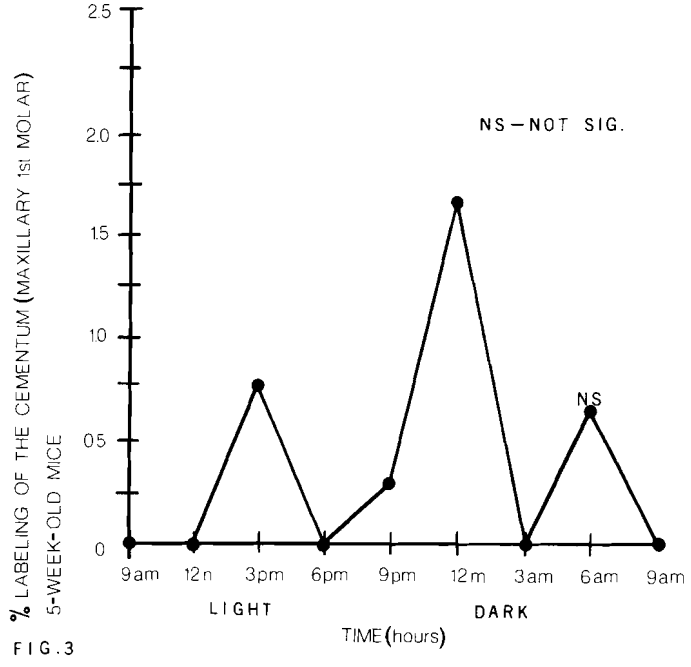


Fig. 3. Circadian variations in ^{3}H-TDR activity of 5-week-old mouse cementum.

Discussion

Previous autoradiographic studies by Tonna et al. (1969) of ^{3}H-thymidine labeling in 5-week-old rat alveolar bone periosteal (outer and inner) and cemental cells revealed labeling indices of 4.3, 1.9, and 0.8% respectively. Similar investigations by Tonna and Stahl (1973), using the BNL short-lived mouse tissues reported significantly lower values of 1.0, 0.6, and 0.3% respectively. The lower percent labeling of mouse tissues is explained on the basis that, aside from existing species difference, the 5-week-old mouse is biologically a significantly older animal than the rat of similar chronological age. In our previous studies of both rats and mice, all animals were sacrificed between 9 and 10 a.m., a period of low labeling of tissues compared to peak values obtained in the present study namely: 2.05% (6 p.m.) for outer periosteum; 2.28% (3 p.m) and 3.01% (3 a.m.) for inner periosteum and 0.75% (3 p.m.) and 1.17% (midnight) for cementum. In each case the % labeling is significantly higher than that reported in the previous studies. In terms of the proliferative response, parodontal tissues fall into three % labeling levels. The gingival epithelial tissues represent the high % labeling compartment, the supporting connective tissues including the periodontium constitute the low-high to medium % labeling compartments while the periosteum and cementum the low-medium to low % labeling compartments (Tonna and Stahl, 1973). The present study reveals peak % labeling as being significant even in tissues reported to exhibit low labeling indices. Consequently, the tissue potential for cell replenishment of alveolar bone periosteum and cementum under normal conditions of growth and maintenance is higher than reported in earlier studies.

Studies of the response of these tissue compartments in both rats and mice to trauma revealed increased cell labeling to significantly higher levels than normal (Tonna et al., 1969; Tonna and Stahl, 1973). Studies of circadian rhythms in traumatized dental tissues are not yet available, the possibility exists that the % labeling peaks will also be higher than reported or that trauma may induce a state overriding circadian rhythms changing the chronology of the phenomenon, as well as its normal levels.

The specific diurnal events recorded in the present study reveal DNA labeling of periosteal tissues during the light period, peaking at 6 p.m. in the outer periosteum and 3 hours earlier (3 p.m.) in the inner periosteum. However, a distinct difference appears to occur between these compartments in that the inner periosteum reveals a second dark period of DNA labeling peaking 12 hours (3 a.m.) after the day peak. Both peaks for the inner periosteum are of equivalent statistical magnitude. By comparison, the first peak exhibited by the cementum is in register with the afternoon peak (3 p.m.) of the inner periosteum while the second peak occurs at midnight, three hours perior to the second peak of the inner periosteum. The appearance of two peaks in the inner periosteum and cementum reveals ultradian rhythms within the circadian

cycle. Ultradian events were recorded during rabbit incisor growth and mineralization by Rosenberg and Simmons (1980a,b). It must be pointed out that rodents are nocturnally active, consequently, the mitotic rhythm is reciprocal to that of the human (Simmons, 1979). Similar double peaks in DNA synthetic activity (daytime and night-time peaks) have been recorded in the gingival epithelial cells (Sandhu et al., 1986) and in the periodontal ligament (Singh et al., 1986) of the 5-weeks-old mouse.

In studies of DNA synthesis in endochondral ossification, the most active period for growth occurred during the light part of the cycle (Simmons, 1968, 1979; Simmons and Nichols, 1968; Oudet and Petrovic, 1977). Although the percentage of cells synthesizing DNA remained relatively steady during the day, ^{3}H-thymidine labeling was significantly lower at the onset of the dark period. The results were reserved for matrix synthesis (Simmons, 1979). In cartilage and bone, peak cell RNA synthesis and active matrix synthesis occured late in the afternoon, approximately when DNA synthesis decreased. Collagen synthesis in bone metaphyses coincided with the period of maximum osteoclast cell numbers, about midday (Simmons and Nichols, 1966). These authors suggested that the intensity of bone formation and resorption are coincidental processes, and that active linear bone growth requires an integrated activity of all cells in the growth apparatus. It was also shown in humans by Jubiz et al. (1972), and in rats by Stromberg and Simmons (1981), that circulating immunoreactive parathyroid hormone levels are higher at night. It was, therefore, not surprising to find the DNA synthesis peaks for both the periosteum and cementum occurring in the light period. Presumably this will be followed by active matrix synthesis during the animals' active dark period, however, the second set of DNA synthesis peaks (between midnight and 3 a.m.) indicates a more complex circadian phenomenon involving two significant ultradian events. Double burts of DNA synthesis appear to be natural to these tissues, but not at all sites (e.g., outer periosteum of the alveolar bone). To complicate matters further, Miani et al. (1964, 1966) and Miani and Miani (1968) using tetracycline markers reported that in rat and dog teeth the circadian rhythms of dentin calcification appeared to be related to the feeding schedule. A possible relationship between circadian variations in dental tissues and rhythmic activity of adrenergic neurons has been suggested (Larsson and Linde, 1971).

The present results point to the existence of circadian and ultradian rhythms in periosteal and cemental tissue compartments. They also point out the possibility of ultradian rhythms in a tissue which may be out of phase with other dental supporting tissues (Sandhu et al., 1986; Singh et al., 1986). Consequently, studies are needed which monitor periods longer than 24 hours. A better understanding of the circadian, ultradian, as well as seasonal rhythms and their phasing will thus be generated which will hopefully provide us with a knowledge of the significance of these events to the growth, development, maintenance and aging of the supporting dental tissues. The present results from a significant baseline for our future studies of the normal effects of aging on the cell proliferative rhythms of dental bone and cementum, as well as necessary information to the assessment of histopathological changes which have an effect on cell proliferative potentials.

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Accepted November 18, 1986.