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Fibroblast cell population kinetics in the mouse molar periodontal ligament and tooth eruption

K. A. S. PERERA* AND C. H. TONGE

**Department of Oral Biology, School of Dentistry, University of Leeds, Leeds, LS2 9LU, and Department of Oral Anatomy, The Dental School, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4BW.*

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INTRODUCTION

Tooth eruption is a complex process involving the interplay of several tissues and probably involving various kinetic and metabolic events within the periodontium. However, as reported and discussed in a previous publication (Perera & Tonge, 1981) there is a close relationship between periodontal ligament fibroblast proliferation and migration and tooth eruption.

From the viewpoint of fibroblast cell population kinetics and tooth eruption, there are two contentions and both theories of tooth eruption are equally intriguing. Firstly, migration of periodontal ligament fibroblasts from an apical progenitor zone (in several discrete cell layers with different velocity profiles) to more occlusally placed zones is thought to bring about tooth eruption in the continuously erupting rat incisor (Zajicek, 1974). Secondly, during the periodontal ligament cytogenesis, periodontal ligament fibroblast migration occurs but the cells are arranged in discrete linear fashion and tooth movement is thought to be brought about by the actual contraction of linearly arranged fibroblasts acting on the tooth surface (Ness, 1964). Both these contentions of migration theory demand active proliferation and migration of periodontal ligament fibroblasts with tooth development and eruption. The reason why cells of the periodontal ligament should show such differential rates of migration and/or special arrangement is certainly of interest.

In any kinetic study it is essential to know the type of cell population under investigation. The classification in general use is that of LeBlond (1964) which divides tissues (depending on the ability to incorporate ^{3}HTdR and also on the degree and duration of ^{3}HTdR retention) into stable, expanding or renewing cell populations. However, based on three kinetic parameters (cell inflow (influx), cell outflow (efflux) and cell proliferation) a simple and more practical classification has emerged (Gilbert & Lajtha, 1965; Lala, 1971; Wright, 1975). These are: (*a*) *Steady state*, in which the population size (the cell number) remains unaltered. This does not imply a static situation but rather a dynamic equilibrium between cell input (by cell birth or by inward migration), and cell output (by cell death or by outward migration). All healthy surface epithelia fall into this category and are thus in a steady state; (*b*) *Growing*, where cell input is higher than the cell output and, con-

* Reprint requests to Dr Perera.

sequently, the population size increases as in growing animals and tissues; (*c*) *Decaying*, where output exceeds input and results in a decline in the population size. Present day cell kinetic research depends on this type of cell population typing.

A fundamental problem arising from these cell population considerations is the type of kinetic model applicable to the developing molar periodontal ligament fibroblasts. This requires the identification of the developing periodontal fibroblasts as a steady state system or as an expanding (growing) system kinetically.

The analysis of cell proliferation in a growing population like the developing molar periodontal ligament has its own problems. However, in a kinetic study, Roberts & Jee (1974) and Roberts, Chase & Jee (1974) showed that the molar periodontal ligament does behave and respond kinetically to various stressing stimuli. Further, in an autoradiographic study (Perera & Tonge, 1981) of developing and erupting molars of young experimental mice, we have demonstrated the existence of a progenitor area with increased proliferating fibroblasts in the apical zone and migration of these cells into other zones (middle and cervical) with a peak proliferative and migratory activity in the 12 days old group, which is also the time that tooth eruption takes place in the mouse. Thus a close relationship has been established between periodontal ligament fibroblast proliferation and migration and molar tooth eruption. The present paper describes a study of fibroblast cell population kinetics within the molar periodontal ligament to establish whether there is a cytokinetic basis for the proliferation and migration associated with tooth eruption.

MATERIALS AND METHODS

Animals

One hundred and twelve young mice in four groups 10, 12 16 and 20 days old, respectively, were used. Each group consisted of 26 experimental and 2 control animals and all its members were of approximately the same weight. These were housed and treated as reported previously (Perera & Tonge, 1981).

Tritiated thymidine (^{3}HTdR), specific activity 1·9 ci/mM, was given by intraperitoneal injection in doses of 1 μci/gm body weight. Injections were given (whenever possible) between 9 and 10 am. One animal from each group was killed at 1 and 2 hours, and then at 2 hourly intervals up to 50 hours after injection using an overdose of pentobarbitone Na. Two unlabelled animals were used as controls for each of the four groups.

Histology

The mandibles were removed, fixed, demineralised and processed for paraffin wax embedding in exactly the same way as described previously (Perera & Tonge, 1981). Serial sections were cut in the mesiodistal plane at 6 μm from the periodontal ligament of the mandibular first molars, one section in five being retained.

Autoradiography

Autoradiographs of both the experimental and control sections were prepared according to the Liquid Emulsion dipping technique (Rogers, 1973). The exact procedure followed was described in the previous communication (Perera & Tonge, 1981). Control non-radioactive sections were processed identically and simultaneously with the labelled sections and two fogged experimental slides were included in each exposure box. This provided a check for both latent image fading

and positive and negative chemography (Rogers, 1973) which helped to establish that the silver grains seen over the experimental tissue were due to radioactivity and were not artefactual. The developed autoradiographs were stained with haematoxylin and eosin.

Fraction of labelled mitoses of periodontal ligament fibroblasts

The fraction of labelled mitoses of the periodontal ligament fibroblasts in the apical zone was scored at time periods from 1 to 50 hours after injection, in the four groups of animals. The counting procedure followed was essentially the same as that used for the labelling index determination described previously (Perera & Tonge, 1981). At least one hundred mitotic figures from each experimental stage were examined and the number of labelled mitoses was divided by the total number of mitoses counted and multiplied by 100 to determine the percentage fraction of labelled mitoses (PLM). Each mitosis with three or more grains over it was considered to be labelled. In addition, observed labelling and mitotic indices were determined separately by counting 3000–4000 apical cells in some groups, especially in the 16 days old group, because it was realised that these values would be necessary to calculate some parameters (cell generation time, T_c) in this group.

A graph of the percentage fraction of labelled mitoses (y-axis) versus time (x-axis) after the injection of ^{3}H-thymidine was made. The duration of cell cycle and its phases from the plot of percent, LM/M (PLM) against time was estimated using the 50% intercept method. This involved intercepting the y-axis of the percentage labelled mitoses (PLM) curve at the 50% (or 0·5) level of mitotic labelling by a line parallel to the time axis. The time interval from ^{3}H-thymidine injection to the 0·5 level of PLM was $t_2 = TG_2 + 0{\cdot}5$ M on the initial wave of labelled mitoses. T_s was the time interval between the 0·5 levels on the ascending and descending portions of the initial peak. T_c was the time interval between the peaks of the first two waves of labelled mitoses.

Because of the uncertainty of the type of kinetic system which must be assumed in estimating various kinetic parameters, cell cycle characteristics together with other parameters of the periodontal ligament fibroblasts were estimated for the apical zones of the periodontal ligament of animals in the four groups assuming:

(A) A steady state cell renewal system.

(B) An exponentially growing periodontal ligament fibroblast population.

Additionally, kinetic parameters were also estimated using the Gilbert computer programme.

Estimation of areas under the PLM curves

In order to measure the number of initially-labelled cells which give rise to a second mitosis, the relative sizes of the areas in the PLM curve enclosed under each wave of labelled mitoses were measured (Mendelsohn & Takahashi, 1971). These areas were found by plotting the PLM points from each group on a graph paper containing 1 mm squares. A line was hand drawn to include a best fit of all points, and the number of squares under each wave was counted. Since the PLM returned to near zero between each wave of labelled mitoses, there was no great problem in defining what area lay under each wave. For each group, the area in squares under the second wave (A_2) was divided by the area in squares under the first wave (A_1) to give a ratio of sizes.

Statistical method

In this study the data are expressed as either median or mean (arithmetic mean) values of the individual values. In evaluating median values real differences are implied only if they are of the order of 20 % (Baserga & Wiebel, 1969; Wright, 1975). Mean values plus or minus one standard error of the mean were determined for some parameters. When a difference in the mean of two groups of data was evident, its significance was usually assessed by the student '*t*' method (Snedecor & Cochran, 1971). In all tests a probability '*P*' value (of the event occurring by chance) of less than 0·05 was regarded as significant.

There are certain errors inherent in the counting procedures due to the different sizes of a mitotic figure and an interphase nucleus and also due to the phenomenon of self absorption.

Correction factors are available for application to the problems of size differentials and self absorption (Simnett & Heppleston, 1966). However, since most values in this study are comparative, these problems should be serious only when and if absolute values of labelling and mitotic index are necessary.

Computer analysis of PLM curves

One difficulty which arises when analysing PLM curves is that of fitting a curve to the data points. A hand drawn curve, heuristically fitted, is generally used to read off the median phase durations at the 0·5 level; fitting the curve by eye is difficult and has led to many rather over-optimistic curves. Furthermore, this method limits the worth of the data which are being collected. Graphical methods, therefore, are imprecise.

Some workers have used a multicompartmental approach to obtain sets of linear differential equations (Barrett, 1966; Takahashi, 1968; Trucco & Brockwell, 1968; Bronk, 1969), and automatic optimisation techniques have been applied to these methods by Barrett (1970) and Steel & Hanes (1971) to estimate the best values for the cell cycle parameters. These methods, however, suffer from lack of estimates of the standard errors of the parameters.

Gilbert (1972) proposed that when γ distributions are used to describe the durations of the phases of the cell cycle, the Laplace transform of a PLM curve can be described by simple analytical functions. Briefly, to find the cycle parameters which best fit a set of data, a Laplace transform is obtained by integrating the data numerically for values of the angular frequency. Then, by a least squares method, the optimum theoretical Laplace transform curve is found using these new observational values; standard errors of parameters are also obtained. The theoretical PLM curve with time is generated by numerical integration of an inverse transform.

Fitting of PLM curves

The PLM is a continuous curve and can be represented mathematically by a function $f(t)$ for all values of time, though this function may not be capable of an explicit expression in simple terms. The Laplace transform of such a function is obtained by integrating the data numerically for values of the angular frequency (W), and was given by Gilbert (1972).

$$F(s) = \int_0^\infty f(t) \exp(-st).dt \; re(s) > \gamma_0,$$

where $f(t)$ is a real function of a variable t, say time, which has finite values for all positive t and which vanishes for all negative t.

For all values of the complex quantity

$$s = \gamma + iw$$

The inverse Laplace transform is given by

$$f(t) = \frac{1}{2\pi}\int_{-\infty}^{\infty} F(\gamma+iw).\exp(\gamma t+iwt).dw \quad \gamma > \gamma_0$$
$$= \frac{1}{\pi}\int_{0}^{\infty} re\,[F(\gamma+iw).\exp(\gamma t+iwt)].dw$$

$F(s)$ is an analytical function of a complex variable s and itself has a real and an imaginary component.

The analysis is best carried out by computer and was written in FORTRAN and run on an IBM 360/67 Computer. The programme generated mean values for the parameters of the cell cycle with standard errors and percentage coefficients of variation with standard errors (mean value ± S.E.; *C.V.* ± S.E.). An attempt was, therefore, made to fit the PLM curves using the Gilbert programme.

RESULTS AND INTERPRETATION OF KINETIC PARAMETERS

Fraction of labelled mitoses

Table 1 shows the percentage fraction of labelled mitoses related to various times after the injection of ^{3}HTdR in the apical zones of the 10, 12 and 16 days old groups of mice. In the 20 days old group, mitotic figures were so scarce that it was decided to exclude this group from the analysis. Figures 1, 2 and 3 show the individual PLM curves for the apical zones of the 10, 12 and 16 days old groups, respectively. A comparison of these PLM curves is shown in Figure 4: they were fitted by visual approximation. In all the three groups there were well defined first peaks, but a second peak was obtained only in the 10 and 12 days old groups. The median values for some phase durations were read off at the 0·5 level of mitotic labelling. Using this method the shortest values for T_s were found in the apical zone of the 12 days old group (8·6 hours compared to 11·1 hours in the 10 days old group and 13·05 hours in the 16 days old group). Similar values were also obtained for T_c. However, in the 16 days old group and, therefore, in the absence of a second peak in the PLM curves, T_c (and hence T_g) could not be measured and had to be calculated. Superimposed upon this problem was the apparent uncertainty of the kinetic system that must be assumed for the periodontal ligament fibroblast cell population. Therefore, when estimating kinetic parameters, periodontal ligament fibroblast population was considered separately as forming:

(1) A steady state system.

(2) An exponentially growing system.

If we assume that all cells are cycling, and the growth fraction (I_p) is 1, then T_c can be calculated for the two systems.

Steady state population

Wright (1925) postulated that in an ideal steady state condition, the number of cells present in any stage of the cell cycle is proportional to the time spent in that stage; in other words the frequency with which cells in any one phase are found is proportional to the duration of that phase.

Table 1. *Calculated percentages labelled mitoses (PLM) values per animal for each experimental stage in the three groups of mice*

Time (hours)	10 days old	12 days old	16 days old
1	5·10	9·90	2·00
2	25·00	31·23	14·98
4	60·04	70·01	37·51
6	82·20	90·33	75·11
8	94·00	99·10	89·02
10	97·51	85·51	94·51
12	85·50	40·00	96·50
14	55·03	25·56	91·53
16	36·01	16·51	70·10
18	31·75	14·33	42·50
20	22·23	8·01	38·00
22	16·52	5·51	35·33
24	13·01	2·47	29·50
26	6·50	3·10	28·50
28	2·01	3·75	25·01
30	2·75	4·25	20·12
32	1·73	5·03	9·10
34	2·51	11·11	3·41
36	8·50	38·00	—
38	15·22	54·33	—
40	28·00	49·11	—
42	43·31	31·10	—
44	46·20	28·51	—
46	40·00	19·50	—
48	20·01	8·50	—
50	11·51	3·51	—

These latter statements indicate several simple relationships between phase durations and frequency and are given by Cleaver (1967).

$$\frac{N_m}{N_0} = T_m/T_c \tag{1}$$

$$\frac{N_s}{N_0} = T_s/T_c \tag{2}$$

where N_m/N_0 and N_s/N_0 are the fraction of cells in mitosis (mitotic index) and in DNA synthesis (labelling index) respectively; T_m, T_s and T_c are the mitotic, DNA synthesis and cell cycle time respectively. Similarly fraction of cells in pre-DNA synthesis and in post-DNA synthesis can be worked out.

Exponentially growing population

In an exponentially growing population, where all cells are cycling and there is no cell loss, the population density falls with age in the cell cycle. This is because there is a preponderance of young cells in the population as with each mitosis two g_1 cells are produced. Consequently the height of the distribution at the beginning of g_1 is double that in mitosis.

Equations which have been specially derived for the exponential growth distribution are available and given by Johnson (1960), Smith & Dendy (1962) and Cleaver (1967).

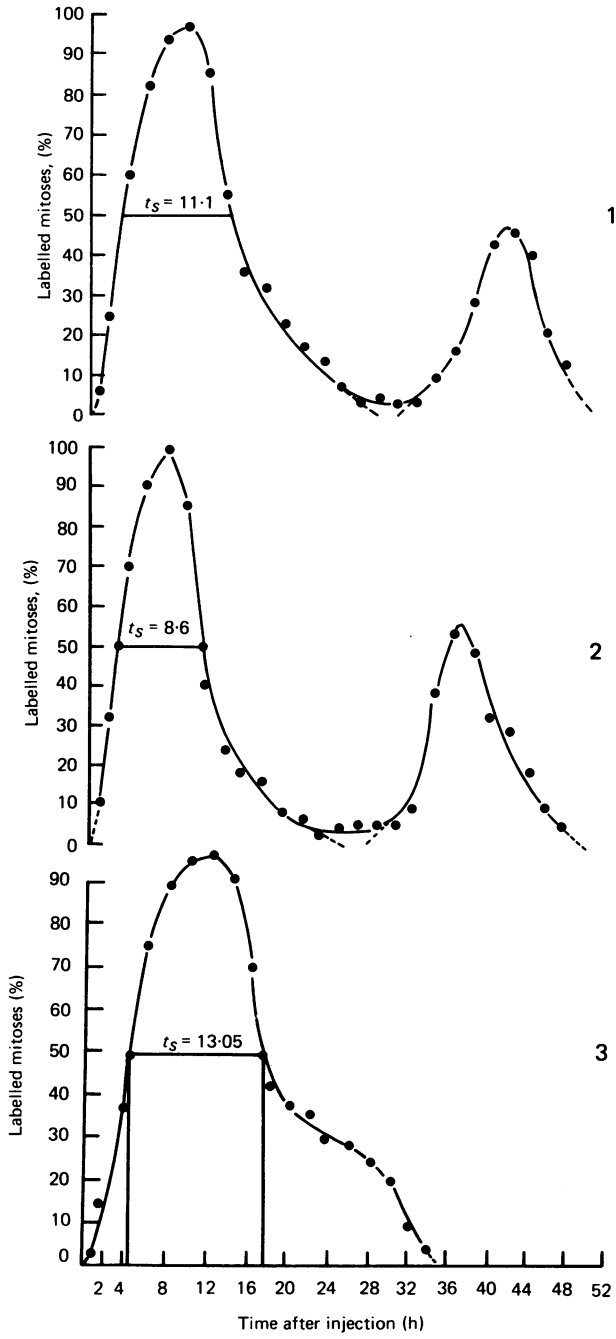

Fig. 1. The percentage fraction of labelled mitosis (PLM) curve for the apical zone of the molar periodontal ligament of the 10 days old mice, with the line fitted by visual approximation – data from Table 1.

Fig. 2. The percentage fraction of labelled mitosis (PLM) curve for the apical zone of the molar periodontal ligament of the 12 days old mice, with the line fitted by visual approximation – data from Table 1.

Fig. 3. The percentage fraction of labelled mitosis (PLM) curve for the apical zone of the molar periodontal ligament of the 16 days old mice, with the line fitted by visual approximation – data from Table 1.

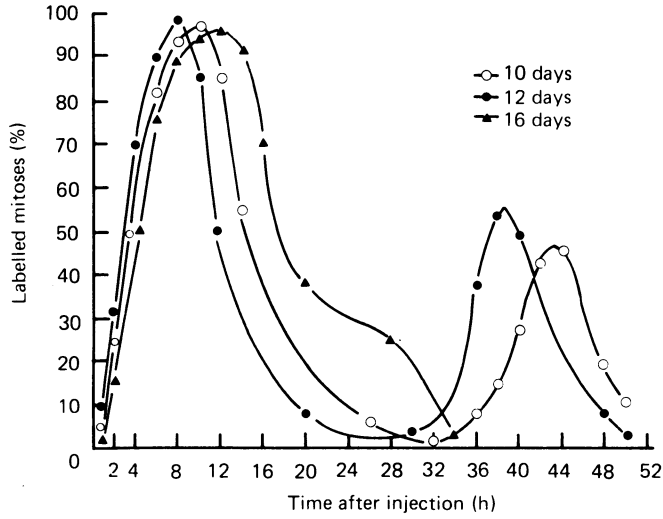

Fig. 4. PLM curves: 10, 12 and 16 days old groups. The percentage fraction of labelled mitosis curves for the apical zones of the molar periodontal ligament of mice 10, 12 and 16 days old compared.

$$\frac{N_m}{N_0} = \text{In } 2\,\frac{T_m}{T_c} \tag{3}$$

$$\frac{N_s}{N_0} = \exp\,(t_2 \text{ In } 2/T_c)\,\exp\,(T_s \text{ In } 2/T_c - 1) \tag{4}$$

where

$t_2 = (T_{g_2} + \frac{1}{2}T_m)$; $T_c = (T_m + T_{g_1} + T_{g_2} + T_s)$;
In 2 = log $_e$2 = 0·693; exp = exponential.

If growth fraction (I_p) is less than unity then these values will be apparent cell cycle times $T_c(a)$. Cell cycle parameters were determined for the PDL fibroblast cell population in the three groups using above formulae.

Estimation of kinetic parameters of periodontal ligament fibroblasts assuming a steady state system

(A) 10 *days old group*

Figure 1 shows the PLM curve for the apical zone in the 10 days old group. The first wave reached 97·51 %, 10 hours after the injection, then fell, with a slightly less gradient, to a minimum of 2·01 % at 28 hours. Although the gradient of the ascending limb of the second wave was somewhat similar to that of the first, it reached a maximum of only 48 % at 43·4 hours. However, the points in the descending limb of the second wave showed some degree of scatter.

Estimates for the duration of the phases of the cell cycle made through the 50 % intercept were T_s 11·1 hours; $(T_{g_2} + \frac{1}{2}T_m)$ 3·4 hours; T_c 33·4 hours. There were 90 mitotic figures among the 1125 labelled cells scored in animals killed within the first 6 hours. Since the number of progenitor cells in relation to the mitosis and labelled cells was the same, an estimate of the duration of mitosis could be made from:

$$\frac{T_m}{T_s} = \frac{N_m}{N_s}, \text{ from equations 1 and 2}$$

$$\frac{T_m}{T_s} = \frac{90}{1125}$$

Therefore

$$T_m = 11{\cdot}1 \times \frac{90}{1125} = 0{\cdot}88 \text{ hours.}$$

Therefore

$$T_{g_2} = 3{\cdot}4 - 0{\cdot}44 = 2{\cdot}96 \text{ hours}$$

and

$$T_{g_1} = T_c - (T_s + T_{g2} + T_m)$$
$$= 33{\cdot}4 - (11{\cdot}1 + 2{\cdot}96 + 0{\cdot}88) = 18{\cdot}46 \text{ hours.}$$

The growth fraction (I_p) for this group can be calculated from:

$$I_p = \frac{LI \text{ obser}}{LI \text{ theor}}$$

$$LI \text{ observed} = \frac{N_s}{N_0} \text{(obser)} = \frac{1125}{4000} \times 100 = 28{\cdot}12\,\%,$$

$$LI \text{ theoretical} = \frac{N_s}{N_0} \text{(theor)} = \frac{11{\cdot}1}{33{\cdot}4} = 0{\cdot}332 = 33{\cdot}2\,\%,$$

Therefore

$$I_p = \frac{28{\cdot}12}{33{\cdot}2} = 0{\cdot}8469 = 84{\cdot}69\,\%.$$

(B) 12 *days old group*

Figure 2 illustrates the PLM curve for the apical zone in the 12 days old group. The curve reached a peak value of 99·10 % at 8 hours and fell to a minimum of 2·47 % at 24 hours. Although the gradient of the ascending limb of the second wave was comparable with that of the first, the peak value of the second wave reached only 55 % at 38·5 hours. The descending limb of the second wave reached a minimum of 3·51 % at 50 hours, with points showing a greater degree of scatter. Estimates for the duration of the phases of cell cycle made through the 50 % intercept were: T_s 8·6 hours; ($T_{g_2} + \frac{1}{2}T_m$) 3 hours; T_c 30·5 hours.

In this group there were 91 mitotic figures among the 1000 labelled cells scored in animals killed within the first 6 hours.

From this, an estimate of the duration of mitosis was made as in the 10 days old group:

Therefore

$$T_m = 8{\cdot}6 \times \frac{91}{1000} = 0{\cdot}78 \text{ hours.}$$

Therefore

$$T_{g_2} = 3 - 0{\cdot}39 = 2{\cdot}61 \text{ hours,}$$

and

$$T_{g_1} = T_c - (T_s + T_{g_2} + T_m)$$
$$= 30{\cdot}5 - (8{\cdot}6 + 2{\cdot}61 + 0{\cdot}78) = 18{\cdot}51 \text{ hours.}$$

The growth fraction (I_p) was calculated from:

$$LI \text{ observed} = \frac{N_s}{N_0} \text{(obser)} = \frac{1000}{4000} \times 100 = 25\,\%.$$

$$LI \text{ theoretical} = \frac{N_s}{N_0} \text{(theor)} = \frac{8{\cdot}6}{30{\cdot}5} = 0{\cdot}281 = 28{\cdot}1\,\%,$$

therefore

$$I_p = \frac{25}{28{\cdot}1} = 0{\cdot}889 = 88{\cdot}9\,\%.$$

(C) 16 *days old group*

Figure 3 shows the PLM curve for the apical zone in the 16 days old group. No recognisable pattern could be discerned in the percentage labelled mitoses in animals studied after 34 hours, so these observations were not used in the analysis. The percentage of labelled mitoses reached a maximum value of 96·50 % at 12 hours and fell to 3·41 % at 34 hours. The second wave of labelled mitoses was not obtained in this group possibly due to the specialisation of cells or cells entering the G_o phase (phase of proliferative rest). Using the 50 % intercept on the PLM curve, T_s 13·05 hours and $(T_{g_2}+\frac{1}{2}T_m)$ 4·35 hours were estimated.

In tissues where it is not possible to obtain the second wave of labelled mitoses, phases of the cell cycle may be estimated on the basis of Wright's hypothesis (1925). This holds for all tissues in steady state, but not for cells in exponential growth.

The observed mitotic and the labelling indices were 1·5 and 19 % respectively in animals killed within the first 4 hours. By Wright's hypothesis,

$$\frac{T_c = T_s \times N_c}{N_s} \qquad \text{(equation 2)}$$

where N_c is the number of progenitor cells and N_s the number of labelled cells. Therefore

$$T_c = \frac{13{\cdot}05 \times 100}{19} = 68 \text{ hours.}$$

Similarly

$$T_m = \frac{T_s \times N_m}{N_s} = \frac{13{\cdot}05 \times 1{\cdot}5}{19} = 1{\cdot}02 \text{ hours,}$$

$$T_{g_2} = (T_{g_2}+\tfrac{1}{2}T_m)-\tfrac{1}{2}T_m = 4{\cdot}35-0{\cdot}51 = 3{\cdot}84 \text{ hours,}$$
$$T_{g_1} = T_c-(T_s+T_{g_2}+T_m) = 68-(13{\cdot}05+3{\cdot}84+1{\cdot}02),$$

thus

$$T_{g_1} = 50{\cdot}09 \text{ hours.}$$

Estimation of kinetic parameters of the periodontal ligament fibroblasts assuming an exponentially growing cell population

(A) 10 *days old group*

The estimates for the duration of cell cycle and its phases from the plot of PLM against time (Fig. 1) using the 50 % intercept were T_s 11·1 hours, $(T_{g_2}+\frac{1}{2}T_m)$ 3·4 hours and T_c 33·4 hours.

The observed labelling index was 28·12 % and the mitotic index 3 %. The growth fraction of the fibroblast cell population was derived from the ratio of observed/theoretical labelling index, the theoretical value being derived from the equation (Cleaver, 1965):

$$\frac{N_s}{N_0}(\text{theor}) = \exp\frac{t_2 \ln 2}{T_c} \exp\left(\frac{T_s \ln 2}{T_c}-1\right) \qquad \text{(equation 4)}$$

where

$$t_2 = (T_{g_2}+\tfrac{1}{2}T_m) \text{ and } T_c = (T_m+T_{g_1}+T_{g_2}+T_s).$$

These values are 28·12 and 50·70 %, giving a growth fraction (I_g) of 0·5546 or 55·46 %.

The mitotic index was corrected to 5·41 % on the grounds that only 55 % of the

total cell population was proliferating. Fraction of cells in mitosis (N_m/N_0) in an exponentially growing population is given by Johnson (1960) and Cleaver (1965).

$$\frac{N_m}{N_0} = \log_e 2 \frac{T_m}{T_c} \qquad \text{(equation 3).}$$

Substituting the corrected mitotic index in this equation, the value is 2·607 hours. Thus,

$$T_{g2} = (T_{g2} + \tfrac{1}{2}T_m) - \tfrac{1}{2}T_m = 2{\cdot}097 \text{ hours},$$

and

$$T_{g_1} = T_c - (T_s + T_{g2} + T_m) = 17{\cdot}59 \text{ hours}.$$

(B) 12 *days old group*

The estimates of the duration of cell cycle and its phases from the plot of PLM against time (Fig. 2) using the 50 % intercept were T_s 8·6 hours ($T_{g2} + \frac{1}{2}T_m$) 3 hours and *Tc* 30·5 hours.

The observed labelling index was 25 % and the mitotic index 3·02 %. As in the 10 days old group the theoretical labelling index was derived from the equation 4.

These values were 25 and 48·9 % giving a growth fraction (I_p) of 0·5112 or 51·12 %.

The mitotic index was corrected to 5·907 % on the grounds that only 51 % of the total cell population was proliferating.

Substituting the corrected mitotic index in the equation 3 T_m value was 2·59 hours. Thus,

$$T_{g2} = (T_{g2} + \tfrac{1}{2}T_m) - \tfrac{1}{2}T_m = 1{\cdot}705 \text{ hours},$$

and

$$T_{g_1} = T_c - (T_s + T_{g_2} + T_m) = 17{\cdot}605 \text{ hours}.$$

(C) 16 *days old group*

The estimates for the duration of cell cycle and its phases from the plot of PLM against time (Fig. 3) using the 50 % intercept were T_s 13·05 hours and ($T_{g2} + \frac{1}{2}T_m$) 4·35 hours. However, due to the absence of the second wave, the value for T_c had to be calculated and the previously calculated value of 68 hours was used in the following estimation.

The observed labelling index was 19 % and the mitotic index 1·5 %. The theoretical labelling index was calculated as in the 10 and 12 days old groups using equation 4.

These values were 19 and 45 %, giving a growth fraction (I_p) of 0·422 or 42·2 %.

The mitotic index was corrected to 3·55 % on the grounds that only 42·2 % of the total cell population was proliferating.

Substituting the corrected mitotic index in the equation 3 T_m value is 3·482 hours. Thus

$$T_{g2} = (T_{g_1} + \tfrac{1}{2}T_m) - \tfrac{1}{2}T_m = 2{\cdot}609 \text{ hours},$$

and

$$T_{g_1} = Tc - (Ts + T_{g_2} + T_m) = 48{\cdot}85 \text{ hours}.$$

Interpretation of kinetic estimates

The values and other calculated parameters assuming a steady state system are given in Table 2.

The median values for T_{g_1}, T_{g_2} and T_m would appear to be very similar in the 10 and 12 days old groups. However, the values for these parameters in the 16 days old group were quite different and they were the longest. In evaluating estimates of cell kinetic parameters the real difference should not be implied unless they are of the

Table 2. *Median values for kinetic parameters in the apical zones of the developing periodontal ligaments of* 10, 12 *and* 16 *days old mice assuming a steady state system*

Animal group	Cell cycle parameter					
	T_s	T_g	T_{g2}	T_m	T_c	I_p
10 days old	11·10	18·46	2·96	0·88	33·4	0·84
12 days old	8·60	18·51	2·61	0·78	30·5	0·88
16 days old	13·05	50·09	3·84	1·02	68·0	—

Table 3. *Median values for kinetic parameters in the apical zones of developing periodontal ligaments of* 10, 12 *and* 16 *days old mice assuming an exponential growth system*

Animal group	Cell cycle parameter					
	T_s	T_g	T_{g_1}	T_m	T_c	I_p
10 days old	11·10	17·59	2·09	2·60	33·40	0·55
12 days old	8·60	17·60	1·70	2·59	30·50	0·51
16 days old	13·05	48·85	2·60	3·48	68·00	0·42

order of 20 % (Baserga & Wiebel, 1969). To what extent this could be applied to the cell kinetics in the periodontal ligament is not known. However, on this criterion T_s in the 12 days old group is significantly lower than that in the 10 or 16 days old groups. On the other hand, T_c values in the 12 days old group would not differ significantly from values obtained for T_c in the 10 or 16 days old groups. On the same basis, there is no significant difference between the values for T_{g1}, T_{g2} and T_m between the 10 and 12 days old groups. However, T_{g_1}, T_{g2} and T_m values in the 16 days old group is significantly longer than that for either the 10 or 12 days old groups. Table 3 shows the alternative estimates of these parameters assuming an exponential growth system in the periodontal ligament.

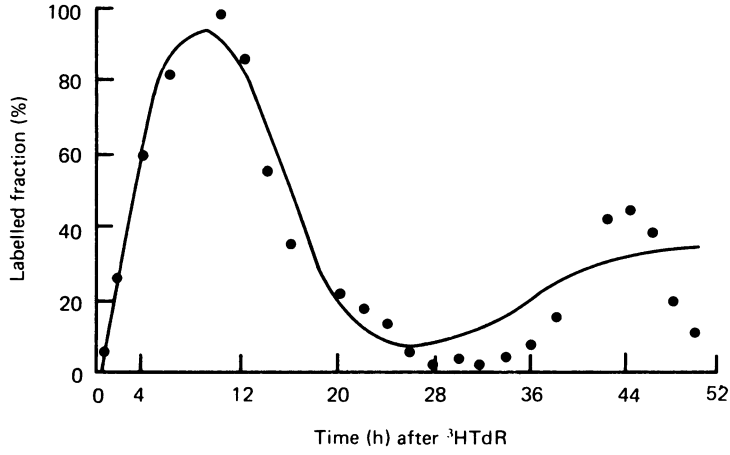

Fig. 5. Theoretical PLM curve: 10 days old group. The percentage fraction of labelled mitosis (PLM) curve for the apical zone of the molar periodontal ligament of the 10 days old mice with the line fitted by the Gilbert computer programme.

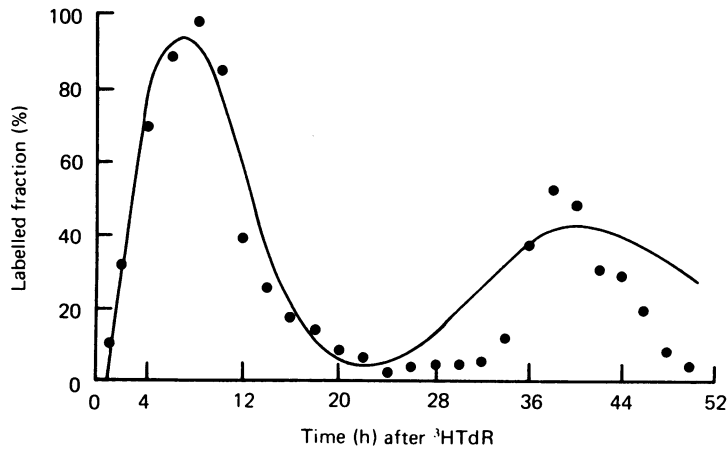

Fig. 6. Theoretical PLM curve: 12 days old group. The percentage fraction of labelled mitosis (PLM) curve for the apical zone of the molar periodontal ligament of the 12 days old mice, with the line fitted by the Gilbert computer programme.

Although T_m values are longer in comparison with the values obtained assuming steady state conditions, the values for T_{g_1} and T_{g_2} in the 10 and 12 days old groups are somewhat shorter.

It is difficult to draw definite conclusions about the importance of these differences, since the median values obtained above (by both systems) give no information concerning the variability of the data.

An attempt was, therefore, made to fit the above PLM curves using the Gilbert programme. The simulated curves with data points drawn from a summary print out from the line printer are presented in Figures 5 and 6 for the 10 and 12 days old groups. The programme generated the original data points and the fitted curve in graphical and numerical form. The particular value of the Gilbert method is that standard errors are generated and are valuable in assessing the worth of the data. However the standard errors generated are on the minimal side.

It is apparent in both cases that although the fit over the first peak is acceptable, the second wave produced by the computer does not closely follow the experimental data points. As the programme assumes a stationary population, the failure of the computed curve to fit the data adequately could be interpreted as evidence of cell migration from the apical zone to other zones.

The mean values for phase durations are given in Table 4; T_m is not included, as the programme regards mitosis as instantaneous, and the time taken for mitoses is

Table 4. *Mean values for kinetic parameters in the apical zones of the periodontal ligaments of* 10 *and* 12 *days old mice from the Gilbert computer programme*

	Cell cycle parameter				Coefficient of variation		
Animal group	T_s	T_{g_1}	T_{g_2}	T_c	CV_c	CV_{g_2}	$CV_s \pm g_2$
10 days old	12·61 ± 0·15	26·07 ± 1·68	3·65 ± 0·17	42·33 ± 1·58	33·00 ± 3·21	55·70 ± 9·05	28·61 ± 2·70
12 days old	10·08 ± 0·31	20·80 ± 0·90	3·01 ± 0·18	33·90 ± 0·82	23 ± 23·2·00	50·84 ± 9·06	29·02 ± 3·64

Table 5. *Calculated values for cell birth rates (K_b) and potential doubling times (t_{pd}) of the apical fibroblasts in the* 10 *days and* 12 *days old mice*

Type of cell population	Parameter	Calculated value	
		10 days old	12 days old
Steady state	$K_b = I_p/Tc$	0·02516	0·02885
Exponential growth	$K_b = \ln(1+I_p)/Tc$	0·01312	0·01351
Steady state	$t_{pd} = T_c/I_p$	39·76	34·65
Exponential growth	$t_{pd} = \ln 2/K_b$	52·82	51·30

incorporated in T_{g_1} and T_{g2}; hence these phases are over-estimated by $\frac{1}{2}T_m$. Mean values for T_s are $12{\cdot}61 \pm 0{\cdot}15$ and $10{\cdot}08 \pm 0{\cdot}31$ for the 10 and 12 days old groups respectively.

Similarly, mean values for T_{g1} are $26{\cdot}07 \pm 1{\cdot}68$, $20{\cdot}81 \pm 0{\cdot}90$ for the two groups. Although this suggests a significant difference between the corresponding values of T_s and T_{g_1} in the two groups, the results for T_c and T_{g2} show no significant difference between the two groups. The fact that coefficients of variation are very large (Table 4) indicates that there is great variability in individual cell cycle times and consequently reduces the significance of these estimates. Although analysis is rendered difficult because of variability in individual cell cycle times. T_s values for the apical zone decreased in the 12 days old group in comparison with the 10 and 16 days old groups. This may have been accompanied by similar decreases in T_c, and consequently have increased the growth fraction in the apical zone in the 12 days old group.

Birth rates and potential doubling times for periodontal ligament fibroblasts for the apical zones of 10 and 12 days old groups suggest a high rate of cell production in these zones; the magnitude of these cell production rates (birth rates) is given in Table 5 for both systems.

However, like the median values, evaluation of these estimates is difficult because it is not certain to what extent the real difference could be implied from these values.

Estimation of areas under PLM curves

The ratio of wave sizes (A2/A1) were 0·343 and 0·533 for the 10 and 12 days old groups, respectively. Calculation of this ratio was not possible in the 16 days old group due to the difficulty of obtaining the second wave. These values (A2/A1) were interpreted to mean that the percentages of initially labelled fibroblasts which give rise to a second mitosis were 34% for the 10 days old group and 53% for the 12 days old group.

DISCUSSION

Fibroblast cell population kinetics in the progenitor (apical) zone of the molar periodontal ligament has established a definite cytokinetic basis for the proliferation and migration associated with molar tooth eruption. In particular this has demonstrated a reduction in the values for cell cycle parameters (Table 2, 3 and 4) in all the groups but with a maximum reduction in the 12 days old group. Thus, the estimated cell kinetic data have confirmed the following proposition put forward previously (Perera & Tonge, 1981); that the apical zone, with the highest prolifera-

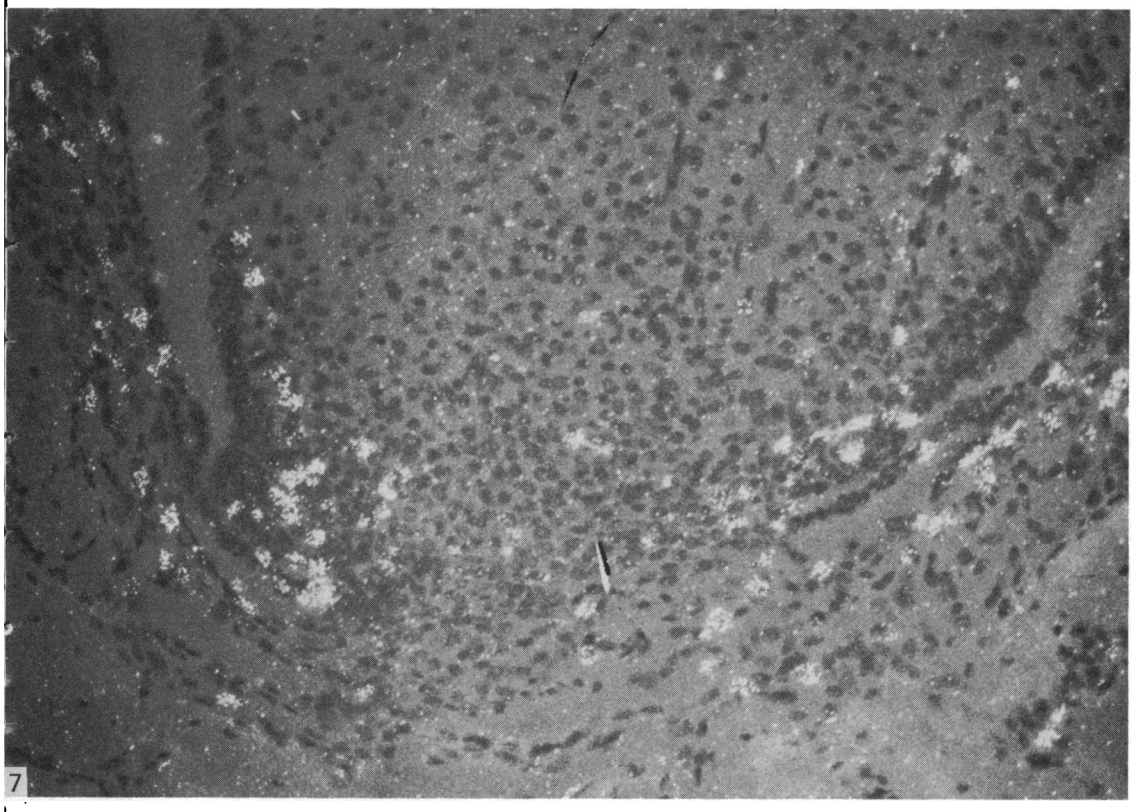

Fig. 7. Autoradiograph: D-G reflected light. A high power (×220) autoradiograph of the developing periodontal ligament (mesial aspect) of the first molar tooth, viewed under dark ground reflected light. Labelled and dividing cells are quite evident within the periodontal ligament and also within the pulp adjacent to the root.

tive rate, appears to be feeding the middle zone which in turn feeds cells to the cervical zone. Nevertheless, middle and cervical zones also showed proliferative activity. The whole sequence was highest in the 12 days old group, high in the 10 days old group and low in the 16 days old group and virtually insignificant in the 20 days old group. This suggests that the periodontal ligament behaves and functions as a 'stimulated tissue' during this period 10–16 days of age with a peak kinetic activity at 12 days of age. This is interesting because 12 days of age is the approximate time of tooth eruption in the mouse.

The values for cell cycle characteristics (T_c, T_s, $T_{g2}+\frac{1}{2}T_m$) obtained in this investigation are in reasonable accord with values obtained by Roberts, Chase & Jee (1974) in an orthodontically stimulated periodontal ligament system. These workers studied the cell cycle characteristics of the periodontal ligament fibroblasts of rat molar teeth at different time intervals of post-stimulation (8, 27 and 36 hours) by subjecting teeth to orthodontic tooth movement using rubber bands. They showed that the values for cell cycle parameters tend to reduce significantly, especially in the 27 hours post-stimulation group. The observation of a similar reduction in values of cell cycle parameters in the present investigation, i.e. the reduction of T_s and T_c values in all the groups but with a maximum reduction in the 12 days old group, suggests that the periodontal ligament functions as a stimulated system during this

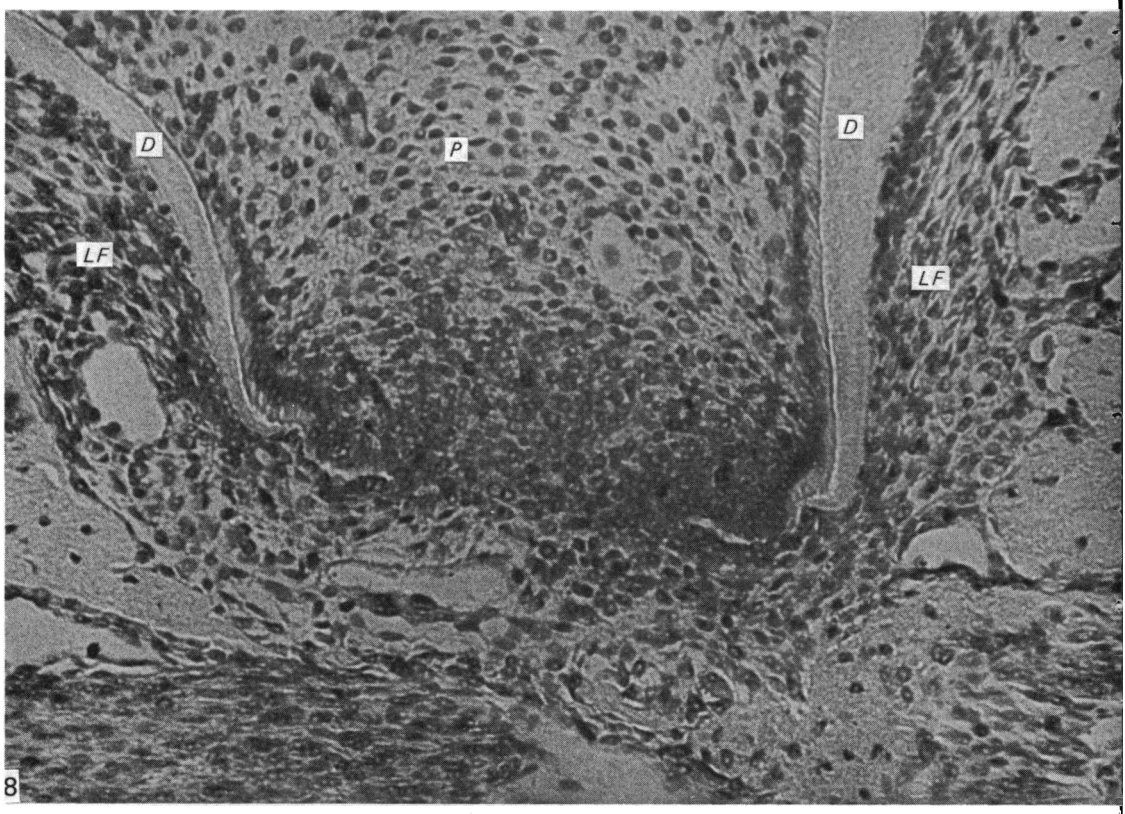

Fig. 8. Autoradiograph: transmitted light. Autoradiograph similar to Figure 7 but viewed under transmitted light for detail tissue and cell identification. Labelled and dividing cells are seen with dark silver grains. *D*, dentine; *LF*, labelled and dividing fibroblasts; *P*, pulp.

period. This means that an increased rate of fibroblast cell production is brought about by the reduction of the DNA synthesis time (T_s), cell generation time (T_c) and possibly by the reduction in other parameters as well. Thus fibroblast proliferation and migration is a direct consequence of the kinetic (stimulated) behaviour of the periodontal ligament.

It is not possible in this study to estimate the cell migratory rate (cell loss rate) as it is not possible to calculate growth rate (kg) of the proliferating population, in this instance the periodontal ligament, from the data. Nevertheless the parameter (t_{pd}), which is the doubling time which would be anticipated if no cell loss or gain occurred within the population and is calculated from the relation $t_{pd} = \text{In}2/K_b$ or $t_{pd} = T_c/I_p$, would give a rough approximation as to the extent of gain or loss. For an accurate theoretical prediction this value should be compared with the doubling time (t_d). However, the values for t_{pd} (Table 5) in the 12 days old group are less than those for the 10 days old group, suggesting that increased cell loss is occurring from the apical zone of the 12 days old group compared with that of the 10 days old group. The extent to which this is occurring can only be appreciated by calculating the cell loss factor.

It is essential to identify the cell population under investigation, in this instance the developing molar periodontal ligament, in kinetic terms. For conditions of steady

state to be satisfied, for each cell generated one cell should move out of each cell compartment, while in exponential growth the dividing cells double their number after an interval equal to the duration of the cell cycle.

Although there is hardly any evidence in the literature as to the exact kinetic behaviour of the molar periodontal ligament fibroblasts, a few studies have explored the fibroblast cell population in the developing periodontal ligament in experimental animals. Zajicek (1974) showed that the periodontal ligament fibroblasts of the continuously erupting incisor behave as a kinetic system with cell production at the apical base and migration towards the occlusal direction at different velocities. Ten Cate (1972) demonstrated cell proliferation in the apical progenitor zone and suggested migration, implying a steady state system for the fibroblast proliferation and migration within the molar periodontal ligament. However, the models used in the present investigation consist of developing periodontal ligament of 10, 12, 16 and 20 days old mice and these models could be looked upon as forming expanding populations. Consequently, cell kinetic parameters were estimated assuming both the exponential and steady state systems separately in an attempt to identify the correct system applicable to periodontal ligament fibroblast proliferation.

The two sets of estimates derived for cell cycle phases have presented some difficulties in deciding which values are representative of the real fibroblast cell population in the developing molar periodontal ligament. However it is interesting that although growth of the periodontal ligament during these stages appear to be exponential, cell migration would fit more closely with a steady state cell renewal system. Further, the low growth fraction (I_p) values for 10 and 12 days old groups, derived by assuming an exponential system (Table 3), suggest either the inapplicability of this system or actual very low proliferative fractions in these groups. The high I_p values, derived by assuming the steady state system (Table 2), appear to fit with the observations of high labelling and mitotic index distributions. Evaluation of all the available evidence suggests that in a tissue like molar periodontal ligament, with a high degree of specialisation and where increased number of cells are being removed from the proliferative phase with time (increased decycling probability), steady state system is the correct kinetic model applicable.

There were some difficulties in analysing the PLM data. Especially in the 16 days old group, no direct reading of T_c was possible because of the absence of a second wave; this indicates that either the cell cycle time (T_c) is quite long and in excess of the experimental period of 50 hours (which is very unlikely) or that there is so much variation in the g_1 phase that randomisation throughout the phases of the cell cycle occurs very quickly or that cells are being removed from the proliferative phase (decycle) due to specialisation. In this instance the third alternative might well be the case. Superimposed upon this problem was the apparent inability of the Gilbert programme to analyse the data adequately and the possible reasons for this inability have been given earlier. However the failure of the computed curve to fit the data adequately has been interpreted as further evidence of cell migration from the apical progenitor zone.

Maximum cell production and migration in the 12 days old group is further substantiated by the data concerning the ratio of wave sizes (areas) of PLM curves. These have shown that the percentage of initially labelled fibroblasts which give rise to a second mitosis wave was considerably higher (53 %) in the 12 days old group compared with that of the 10 days old group (34 %).

The findings of this study have confirmed the apico-occlusal migratory model

reported previously (Perera, 1978; Perera & Tonge, 1981) and have also identified the kinetic type of the developing molar periodontal ligament. Further, a definite cytokinetic basis has been established for the periodontal ligament fibroblast proliferation and migration. This means that the developing molar periodontal ligament functions as a 'kinetic system' during the period of 10–16 days after birth with a peak kinetic activity at the 12th day, which is also the age at which molar tooth eruption takes place in the mouse. Thus fibroblast proliferation and migration are major causative factors responsible for tooth eruption. Based on these results the following mechanism for tooth eruption is proposed.

Proposed multifactorial concept of tooth eruption

Fibroblasts in the apical zone act as a stem cell compartment for the rest of the periodontal ligament. Proliferation takes place in the apical progenitor zone, the cells migrating from the apical zone to middle and cervical zones with a decreasing degree of proliferative activity. Because these fibroblasts migrate, then by definition apical cells have no input and may be regarded as stem cells originating from the ectomesenchymal cells. The middle zone may be regarded as a dividing transit compartment receiving cells from the stem cell compartment and subsequently feeding the cervical zone. Cells in these zones then specialise to provide secretory fibroblasts and possibly osteoblasts and cementoblasts, but these must be regarded as dividing transit compartments since there was a measurable proliferative rate over the first few hours after the injection of thymidine (Perera & Tonge, 1981). Structural integrity is maintained by proliferating cells from all the zones and collagen formed by the secretory fibroblasts.

The apico-occlusally directed migration implies that fibroblasts continuously change their position within the periodontal ligament. This involves a continuous turnover of intercellular bonds, rapid turnover of collagen in the periodontal ligament which in turn requires a similar pattern of turnover on the part of the supporting alveolar bone. The metabolic studies reported elsewhere (Eastoe, 1967; Melcher & Correia, 1971; Kameyama, 1973; Ten Cate & Deporter, 1974; Perera, Beynon & Tonge, 1974, 1975; Perera, 1976) clearly established a fast turnover of both collagen and supporting bone in the periodontium. However, these metabolic changes in the periodontal ligament and supporting bone associated with tooth movement are neither direct causes (in the sense of prime movers) nor direct effects of tooth movement. Nevertheless these are essential, mutually dependent and accompanying processes of tooth movement.

The mechanism of tooth eruption proposed here suggests that the periodontal ligament behaves and functions as a stimulated (kinetic) system during the time of tooth eruption. Fibroblast proliferation and migration (apico-occlusal migration) is a direct consequence of this kinetic (stimulated) behaviour of the periodontal ligament. This mechanism implies some ultrastructural or other special features on the part of the migrating fibroblasts for them actively to pull the tooth axially while they migrate. This has been demonstrated in the periodontal ligament fibroblasts of the rat at least in one study (Beertsen, Everts & Hooff, 1974). However, in the molar tooth the whole process is a time-limited phenomenon, while a similar but continuous mechanism is proposed in the case of continuous eruption.

Tooth eruption is visualised as a complex process involving many developmental, metabolic and kinetic events in the periodontium. However, it is concluded that in such a multifactorial concept periodontal ligament fibroblast proliferation and migration are major causative factors responsible for tooth eruption.

SUMMARY

Fibroblast cell population kinetics in the developing molar periodontal ligament was investigated in 10, 12, 16 and 20 days old mice by autoradiography after the administration of [^{3}H]thymidine. Labelled mitoses, in number per unit area, were counted over the apical zones of the sections and percentage labelled mitoses (PLM) curves were drawn. Median values for some phase durations were read off at the 0·5 level of mitotic labelling. In determining other kinetic parameters the periodontal fibroblast population was considered separately as (1) a steady state system, (2) an exponentially growing system. An attempt was made to estimate mean values for these parameters using the Gilbert Computer programme. The programme generated the original data points and the fitted curve in graphical and numerical form together with the mean values and standard errors. The fact that the Gilbert programme assumes a stationary population in its theoretical PLM curve analysis was used to establish the kinetic type of the periodontal fibroblast population.

The present study has demonstrated that in the growing periodontal ligament where cell specialisation and migration occurs steady state system is the kinetic model applicable. Failure of the computed PLM curves to fit the data adequately confirmed the fibroblast migration (apico-occlusal migration) from the apical zone to other zones. A definite cytokinetic basis for the periodontal ligament fibroblast proliferation and migration was established. Accordingly, fibroblast proliferation and migration takes place by a reduction in the DNA synthesis time (T_s), cell generation time (T_c) and possibly by a similar reduction in other parameters. Maximum reduction in these parameters is associated with the peak proliferative and migratory activity in the 12 days old group which is also the time that tooth eruption takes place in the mouse. Thus fibroblast proliferation and migration are major causative factors responsible for tooth eruption. Based on these results, a mechanism for tooth eruption is proposed.

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