GROWTH OF DOG TOOTH GERMS AND CHANGE IN CONTENTS OF P, Ca AND Mg

BY

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INTRODUCTION

The problem on the metabolism through which tooth may grow is of interest not only in the biochemical field because of its characteristic properties as a specific organ, but also from clinical point since it can be the most basic knowledge to the problems of the dental clinics.

From this standpoint, a series of the studies have been planned in the Department of biochemistry, School of Dentistry, Tokyo Medical and Dental University, e.g., Sasakı1,2) has already investigated on the respiration of tooth germs as well as on the substances capable of its substrates, Fujisaki3) has extensively tried further studies on effects of inhibitors on respiration and activity of dehydrogenase to analyze the mechanism of the metabolism of this tissue. In all the cases of these studies, they employed tooth germs of permanent teeth obtained from 1-2 month-dogs. However, from the above studies or even in literatures nothing has been clarified practically in what growing stage these tooth germs are or how they may develop.

The authors precisely observed how tooth germs develop as dog grows, because such investigation might offer clear basis to the further studies on the metabolism by using dog tooth germs. Simultaneously, the investigation was extended to see how phosphate, Ca and Mg are incorporated into tooth germs in the process of its growth and co-workers Kato et al.4) successively examined by using P23 to what fractions of tooth germ the phosphate is distributed.

Such studies might contribute something to shed light on the respiration and metabolism of various organic substances of tooth germs as well as on the change in phosphate and Ca which vary with growth of the tissue corresponding to the metabolism.

MATERIALS AND METHODS

Tooth germs examined were obtained from dogs ages of which extend over the range of 10-70 days after birth. (The date of birth for each dog

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was accounted as the 1st day and was always ascertained).

Dogs were fed with mother dogs until they were killed for experiments (or until previous days in 2-3 cases). They generally eat foods prepared for mother dogs other than mother’s milk, e.g., vegetables or fish cooked with rice and barley.

In almost all the cases, dogs were brought to the laboratory and sacrificed immediately or on the following day in 2-3 cases (they were fed with rice mixed with miso-soap).

They were weighed immediately before the experiments followed by intravenous injection or intracardial puncture with 5 cc of 2.5% Ravonal. After separation of maxillary bone from mandibular bone, tooth germs of first molars (M1) and canines (C) were gouged out from the lower jaw and those of first molars (M1) and fourth premolars (P4) from the upper jaw.

Tooth germs gouged out were separated into soft parts and calcified parts, each of these two parts was weighed on a cover glass weight of which was known and wet weight of each part was obtained by subtracting weight of each cover glass. Individual part was placed in an oven and dried at 100-150°C for 90 minutes and weighed again after cooling in a desiccator for 20-30 minutes.

Soft parts of tooth germs thus obtained were put into pyrex oxidation tube with pyrex ebullition capillaries as well as with 1 cc of 5N H2SO4 and heated. 30% H2O2 was dropped when required and heating was continued until solution turned colorless. About 2 cc of H2O was added after completion of wet incineration, tubes were allowed to stand for 10 minutes in a boiling water bath, solutions were transferred quantitatively to graduated test tubes to make the total volume 20 cc, and P, Ca and Mg were determined with each solution.

P was determined by the FisKE-SUBBAROW’s method5).

Ca and Mg were measured by the chelate titration method6) with EDTA and murexide and phthaleine complexon were used as indicators.

RESULTS AND DISCUSSION

It seems very unreasonable to use dogs for such experiments because those available for experiments are generally cross-breeds though unavoidable to obtain tooth germs of favorable size readily and also economically. Therefore, the authors expected considerable deviation of observed values. It was tried to use litter mates as many as possible, yet there is no idea about to what extent such effort can prevent the deviation of experimental data.

Fig. 1 shows the relationship between body weights and days after birth. Figures in this Fig. are numbers of groups and the same figures signify litter
mates.

Actually body weights might probably increase along sigmoid curve as usual growth curve, but deviation is observed this much with dogs sacrificed for the experiments.

From another aspect, it could be said that body weights of dogs collected for this experiment fairly agreed to this extent. However, it is not clear at present what kind of relationship is observable between body weight and growth of tooth germ.

Wet weights of tooth germs were plotted against days after birth, which is shown in Fig. 2.

It seems probable that it has a tendency shown by broken lines though each point shows considerable deviation, i.e., every line looks like the first half of sigmoid curve indicating that growth of M₁ of the lower jaw is fastest followed by that of M₃ and P₄ of the upper jaw (curves for these two teeth rise almost parallel) and C of the lower jaw shows the slowest growth.

Figs. 3-6 illustrate the growth of tooth germs separated into soft and calcified parts. In these cases, dry weights were indicated.

In Fig. 3, dry weights of soft parts—especially for M₁ of the lower jaw which shows the most rapid growth—revel the growing process along fairly obvious sigmoid curve. M₁ and P₄ of the upper jaw grow to the same extent

![Graph](image-url)

Fig. 1. Body weight and days after birth for dogs used for experiments (each figure shows a number of group and the same figures signify ‘litter mates’).
and C of the lower jaw seems to show the slowest growth also in this case.

In order to analyze these growth curves more clearly, weights were plotted on logarithm scale as shown in Fig. 4, i.e., the first half of each sigmoid curve shown in Fig. 3 gives straight line since this region is an exponential. Moreover, for M₁ of the lower jaw the first 40 days are the logarithmic period of growth and transferred to the stationary period from 50 days and thereafter, for M₁ and P₅ of the upper jaw the logarithmic period extends over 50 days after birth followed by the stationary period approximately from 60 days after birth, and C of the lower jaw seems to be retained at the logarithmic period even after 70 days. The slopes of curves for the logarithmic period are not so different comparing M₁ of the lower

![Fig. 2. Weights of tooth germs and days after birth.](image1)

![Fig. 3. Dry weights of soft parts and days after birth.](image2)
jaw with $M_1$ or $P_4$ of the upper jaw respectively except that $C$ of the lower jaw gives fairly slow curve.

The above findings have led us to conclude that observing the growth of the soft part these tooth germs grow with similar rate though the period is slightly shifted. A similar phenomenon was observable with the growth of the calcified part which is illustrated in Fig. 6 below.

Fig. 5 shows how dry weights of the calcified parts increase with growth. At a glance, it looks as if they showed exponential curves, but it seems probable that each of them belongs to the first half of sigmoid curve since they are transformed to those shown in Fig. 6 when weights are plotted on the

![Graph showing growth of soft part of tooth germ.](image)

Fig. 4. Growth of soft part of tooth germ.

![Graph showing dry weights of calcified parts and days after birth.](image)

Fig. 5. Dry weights of calcified parts and days after birth.
logarithm scales, i.e., every tooth germ likely shows the logarithmic period approximately in the first 50 days followed by the stationary period. Only C of the lower jaw gives a straight line for longer duration extending over 60 days after birth. This probably means that calcification is slowest also in this tooth germ.

In Fig. 4, the soft parts of \( M_1 \) and \( P_4 \) of the upper jaw show little slower growth compared with that of \( M_1 \) of the lower jaw but Fig. 6 shows that in the calcified part difference is less obvious than that in the soft part.

Comparing Fig. 4 with Fig. 6 the following facts are observed, i.e., \( M_1 \) of the lower jaw shows that weight of the soft part is superior to that of the calcified part in the initial period, both weights become identical about 30 days after birth and weight of the calcified part rapidly surpasses the soft part thereafter showing obvious increase even 50 days after birth when weight of the soft part begins settling to the stationary state.

Similarly the soft parts of \( P_4 \) and \( M_1 \) of the upper jaw are also heavier than their calcified parts but become identical about 40 days after birth and their calcified parts markedly increase to surpass the soft parts thereafter. These show a tendency similar to that of \( M_1 \) of the lower jaw though the growth is slightly slower. (but in \( P_4 \) and \( M_1 \) of the upper jaw the transition stage from logarithmic period to stationary period for the soft part is coincident with that for the calcified part differently from \( M_1 \) of the lower jaw).

On the other hand, growing state of \( C \) of the lower jaw is considerably different from that of the above three, i.e., weight of the calcified part becomes approximately equal to that of the soft part already 10 days after birth and the calcified part grows much more rapidly than the soft part thereafter, but the logarithmic period for the soft part is still lingering even

![Graph showing the development of calcified part](image)

Fig. 6. Development of calcified part.
60-70 days after birth while the growth of the calcified part is transferred from the logarithmic stage to the stationary stage.

The authors attempted to obtain the ratio of dry weight of the calcified part to the total dry weight of the tooth germ as an indicator for calcification and tentatively named “calcification coefficient”.

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\text{Calcification coefficient} = \frac{\text{dry weight of the calcified part}}{\text{dry weight of the calcified part} + \text{the soft part}}
\]

The change in this value is shown in Fig. 7, i.e., the calcification coefficients for M₁ and P₄ of the upper jaw increase also along the sigmoid curve, but those of M₁ and C of the lower jaw show somewhat different behavior.

In other words, M₁ and P₄ of the upper jaw show very slight calcification 10-22 days after birth but calcification develops very rapidly thereafter giving a value 0.8 as a calcification coefficient 70 days after birth, while the calcification of M₁ of the lower jaw fairly develops already in the first 10 days followed by slower development reaching about 0.8 60 days after birth. C of the lower jaw shows fairly rapid calcification giving calcification coefficient 0.5 already 10 days after birth, also increases with considerable rapidity thereafter and finally gets to 0.8 approximately 50 days after birth, but increasing rate drops markedly from this period.

Such findings probably indicate that in this tooth germ calcification develops in comparatively early period though growth of the soft part is slow.

As described above, minute investigations revealed that each tooth showed individual behavior in the process of formation, which should be further clarified also from the metabolic observation.

Fig. 7. Calcification coefficient of tooth germ.
Further attempt was to find relationship between growth of tooth germ, the soft and calcified parts separately, and water content.

Fig. 8 shows relationship between days after birth and ratios of wet weights to dry weights for the soft parts. Water content of the soft part increases with its growth and slightly decreases after certain period. Maximum water content is found 40-60 days after birth. The stage of calcification to which such period corresponds is not so obvious.

It was also impressed that $M_4$ of the lower jaw showed a little different behavior among the 4 kinds of tooth germs as given in Fig. 8.

On the other hand, water contents of the calcified parts were observed by plotting ratios, dry weight/wet weight, against days after birth as shown in Fig. 9 as contrast to Fig. 8.

But no clear tendency is observable in this case unlikely to what was found in the soft tissue.

As to the water contents of $M_4$ of the lower jaw and $P_4$ and $M_1$ of the upper jaw, it looks as if they increased temporarily and decreased again.
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thereafter. However, it is not so trustful since cases belonging to 30-70 days period are not so enough to evidence it.

Another attempt was to observe how Ca and P, the main components of the tooth, behaved in tooth germs which showed individual states in their growing processes as described above.

In this case, enhancement of the calcified part could be regarded as increase of Ca and P,* but it is considered that Ca and P may be incorporated into the soft part of tooth germ prior to deposition of Ca and P in the calcified part. Such concept led us to observe Ca, Mg and P in the soft part following each stage of the growing process.

Fig. 10 shows Ca contents of the soft part (expressed by % in dry weight of tissue) plotted against days after birth. According to this Fig. it is very difficult to deduce any clear tendency at each stage because of considerable deviation of points, but it could be roughly concluded that all of 4 kinds of tooth germs show enhancement of Ca content with simultaneously increased deviation 40-60 days after birth. The content in this stage is 0.5-3% which shows that tooth germ does not contain specially high Ca content since the pulp generally gives about 2% of Ca content, though Ca content of the dog skin is approximately 0.04%.

Moreover, Eichler et al. have investigated concerning Ca contents of the soft parts of tooth germs obtained from 5 dogs (28-92 days after birth).

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\begin{array}{cccc}
\bar{M}_1 & \bar{C} & M_1 & P_4 \\
\end{array}
\]

![Fig. 10. Growth of soft part and Ca content.](image)

* As for the problem about how the composition of the calcified part changes with growing stage, co-workers Irie et al. have already reported**.
They reported that incisors show 0.260-0.113% content and molar teeth contain 0.112-0.062% respectively. These values agree fairly well with those obtained by the authors if the water content is taken into consideration since % of their data was calculated from the wet weight.

Mg contents are illustrated in Fig. 11 which shows no clear tendency but it seems likely that Mg increases also about 40-60 days after birth and is accompanied by simultaneous enhancement of deviation. The content shows about 1% which means that tooth germs show not so exceptionally high content since Mg content of the pulp is 0.7% according to the literature, though the dog skin shows only about 0.03% for Mg content. Ca and Mg contents gave no clear tendency in the growing process as mentioned above. On the other hand, P content showed very obvious tendency as indicated in Fig. 12, i.e., C of the lower jaw and M₁ and P₄ of the upper jaw show a peak value in the first 10 days respectively with marked deviations which become less with simultaneous lowering of the peaks showing about 1% 40 days after birth and the deviation gives a slight increase again in the 50-60 days period. M₁ of the lower jaw also shows a tendency similar to the above three though deviation in the first 10 days is much less than the above. P content in the 40 days period is approximately 1% which corresponds to a value reported for pulp in the literature. The skin likely gave a less value (0.15%) . According to the paper by Eichler cited above, P contents of dental sacs are 0.208-0.104% for molar teeth and 0.23-0.140% for incisors, while in their later paper they reported 0.0723% for a mean value of 14 dogs. Taking water content into consideration, their values almost agree with those obtained by the authors since their values were calculated from wet weights.

From the above findings, it is concluded that the soft parts of tooth germs show considerably high P content in the initial period of calcification.

Fig. 11. Growth of soft part and Mg content.
Fig. 12. Growth of soft part and P content.

when calcification is still slight, P content is gradually lowered with development of calcification and is finally settled to a value which corresponds to the content in the pulp being accompanied by some fluctuations in values. On the other hand, Ca and Mg contents in the soft part are not so high in the initial stage of calcification, increase with considerable fluctuation as calcification develops and may also reach the content in the pulp with lessening of fluctuation.

According to Eichler et al., majority of Ca in the soft part of tooth germ is not ultrafilterable and ultrafilterable portion shows a content the same with that in the plasma. Most of the P component is also non-ultrafilterable suggesting that it is probably colloidal P. Ultrafilterable P is also estimated to be several times the ultrafilterable P in plasma.

In order to deduce how the above phenomena are practically significant for the formation of tooth, simultaneous investigation is still desired to clarify the behaviors of various substances other than those described above.

**Summary**

Tooth germs of the permanent teeth (M₃ and C of the lower jaw, M₁ and P₄ of the upper jaw) were gouged out from dogs whose ages are in the range of 10-70 days, each of them was weighed after separation into calcified part and soft part, and contents of Ca, Mg and P of the soft part were determined. The results of the above investigations were as follows:

1) Body weight as well as weight of tooth germ increases along a sigmoid curve, though dogs employed were cross-breeds. Out of 4 kinds of the above tooth germs, M₁ of the lower jaw increases markedly in the earliest stage followed by P₄ and M₃, and C grows most slowly.
2) Increasing state of dry weights of both soft part and calcified part showed obvious transition from logarithmic period to stationary period in the growing process. More precise observation revealed that individual tooth germs showed comparatively different growing states to one another, especially C of the lower jaw grew very differently from the other three.

3) Ratio \( \frac{\text{dry weight of the calcified part}}{\text{dry weight of total tooth germ}} \), so-called calcification coefficient, also clarified specific growing process of individual tooth germs.

4) Change in water content of the soft part as well as of the calcified part with growth of the tooth germ is that the soft part shows a temporary increase of water content and decreases again with growth, and the calcified part also shows a similar tendency though not so obviously.

5) P content of the soft part shows a high value in the early period lowering with development of the calcification and finally settles down to P content of the pulp with slight deviation during development.

On the other hand, contents of Ca and Mg are very slight in the initial period, increase with considerable deviation as calcification develops and probably reach their contents in the pulp.

References