DISTRIBUTION OF GLYCOGEN STORAGE DURING ODONTOGENESIS IN FETAL DEVELOPMENT IN *Calomys callosus**

DISTRIBUIÇÃO DOS DEPÓSITOS DE GLICOGÊNIO DURANTE A ODONTOGÊNESE INTRA-UTERINA EM Calomys callosus

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SUMMARY

Animal cells store carbohydrates in the form of glycogen as a source of potential energy. This storage frequently occurs in embryonic tissue because it demands a quick energy source, mainly during cell differentiation and development. This study evaluated the distribution of glycogen storage during odontogenesis in fetal development in Calomys callosus, by using 12-20 day-old fetuses, 5 fetuses for each day. The heads of the fetuses were removed and fixed in 10% formaldehyde in PBS. The specimens were embedded in glycol-methacrylate; the ones that were 15 days or older were previously decalcified in EDTA. Histology slides were obtained for each day of development containing 3μm-thick semi-serial sections. These slides were divided into 2 identical groups, one of them was submitted to the P.A.S. reaction and the other group was first submitted to salivary amylase and immediately afterwards to the P.A.S. reaction (control group). Light microscope analysis of the initial phases of odontogenesis (12-15 days of development) revealed a more prominent P.A.S. reaction in the ectomesenchymal cells. At 16-17 days there was a marked prevalence in the follicle region; at 18-19 days the reaction was intense in the stellate reticulum and at 20 days, in the inner epithelium and in the dental papilla. According to the methodology developed and to the results obtained, it was concluded that a variation of the glycogen storage in the different regions of the dental germ occurs during odontogenesis, thus making the ***Calomys callosus*** *a suitable biological model for odontogenesis studies.*

UNITERMS: *glycogen; odontogenesis; tooth germ;* ***Calomys callosus****; PAS reaction.*

RESUMO

As células animais estocam carboidratos na forma de glicogênio como fonte de energia. Esses depósitos ocorrem amplamente nos tecidos embrionários para disponibilização rápida de energia, principalmente durante a diferenciação e o desenvolvimento das células. O presente trabalho avaliou a distribuição de depósitos de glicogênio durante a odontogênese intra-uterina em *Calomys callosus* utilizando fetos com 12 a 20 dias, sendo 5 fetos de cada dia. As cabeças dos fetos foram removidas e fixadas em formaldeído 10% em PBS. Os espécimes foram processados para inclusão em glicol metacrilato (Leica Historesin), sendo aqueles com mais de 15 dias previamente descalcificados em EDTA. Para cada dia de desenvolvimento obteve-se cortes semi-seriados com 3 μm de espessura. As lâminas foram divididas em 2 grupos, sendo um grupo submetido à reação P.A.S. e o outro submetido à amilase salivar e em seguida à reação P.A.S. (grupo controle). Ao microscópio de luz foi observada, nas fases iniciais da odontogênese (12-15 dias), reação P.A.S. mais evidente nas células ectomesenquimais e aos 16-17 dias a reação predominou na região do folículo dental. Aos 18-19 dias a reação intensificou-se no retículo estrelado e aos 20 dias nas células do epitélio interno e na papila dental. De acordo com a metodologia empregada e com os resultados obtidos, concluímos que ocorre variação dos depósitos de glicogênio nas diferentes regiões do germe dental ao longo da odontogênese e que o *Calomys callosus* representa um modelo biológico para estudos da odontogênese.

UNITERMOS: glicogênio; odontogênese; germe dental; *Calomys callosus*; reação PAS.

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INTRODUCTION

The tooth development process undergoes common stages in spite of the existence of different kinds of tooth morphologies. Odontogenesis begins with an interaction between the oral epithelium and the subjacent ectomesenchyme around the 38th day of human fetal development. This epithelial-ectomesenchymal interaction is responsible for regulating odontogenesis, as well as regulating the differentiation of the various cell lineages of the dental germ[24,1,25,11,22,26,13].

Tooth morphologic development is well known, having been the object of several studies in the last decade[22,13]. Much progress was made in identifying the molecules involved in odontogenesis; however, as yet little is known about the specific function of these molecules[23, 24, 1, 17, 11, 22, 26, 27, 13].

Animal cells store carbohydrates in the form of glycogen as an energy source. When required, the glycogen is broken down to yield glucose, which is the main energy source for metabolic processes[14,7,9,2,10,12]. Glycogen is widely found in embryonic tissues and its presence reflects a relatively anaerobic condition[16,17]. Some studies have identified glycogen deposits in precursor cells, which can be related to some kind of cellular interaction and/or to the production of extracellular matrix by differentiated cells[20,6,21,19,3,17,22,13].

The first study that described the presence of glycogen in the stellate reticulum of the developing fetal guinea-pig incisor was carried out in 1896 by Creighton apud[5]. The occurrence of glycogen in oral epithelial structures was confirmed[5,17], but the functional significance of these glycogen deposits for development has remained undetermined[17].

Glycogen was identified in several dental germ components, however, doubts still remain regarding its localization and characteristics[17]. The high concentration of glycogen in fetal tissues is justified by the occurrence of rapid changes, which demand a quickly available energy source[18,20].

Calomys callosus is a rodent frequently used as a biologic experimental laboratory model due to its advantages over other laboratory animals, because it is very handy and suitable for research[15]. The odontogenesis in the *Calomys callosus* is similar to that in other rodents[4], however their glycogen distribution during the odontogenesis has not been investigated. The present study evaluated the distribution of glycogen deposits during odontogenesis in the fetal development of first molars in the *Calomys callosus*.

MATERIALS AND METHODS

In this study 10 female and 5 male rodents, mating in a 2:1 proportion were used. The presence of a vaginal cork indicated the first day of pregnancy. From the 12th to the 20th day of pregnancy, one female rodent per day was anaesthetized, had its fetus removed and beheaded. The heads were fixed in 10% formaldehyde in 0.1M PBS for 24 hours and embedded in glycol methacrylate (Historesin – Leica). The 16 day-old or older ones were previously decalcified in EDTA 7%.

Three μm thick frontal sections of the heads were obtained with a microtome in the first molar region. The sections were initially submitted to acetone treatment for 30 minutes, later these slides were separated into the 2 groups and kept for 1 hour in a kiln at 37°C. One of the groups had distilled water placed over the sections and the other, salivary amylase (control). Afterwards the sections were submitted to the Periodic Acid Schiff (P.A.S.) histochemical reaction in order to identify the glycogen deposits, according the following protocol: first, the specimens were oxidized in 0.4% periodic acid for 10 minutes, then in Schiff reactive for 30 minutes, next in sulfurous water for 2 minutes and finally in Meyer Hematoxylin for 45 minutes. Salivary amylase reactions were carried out for each age as a control.

RESULTS

At 12 days of development, a weak P.A.S reaction was detected for both the primary epithelial band and ectomesenchyme. At the bud stage (13-14 days) a weak reaction was observed both in the epithelial bud and dental lamina; the ectomesenchyme showed a more intense reaction (Fig. 1A). The bud-cap transition stage (15 days) presented a more intense reaction than the previous stages, particularly with regard to the staining in ectomesenchymal cells, in enamel organ, in dental lamina and oral epithelium (Fig. 1B). The P.A.S. positive reaction was observed at the cap stage (16 days) in the dental follicle region, in dental lamina and in oral epithelium. It was also weak in the epithelial cap and absent in dental papilla (Fig. 1C). The same was observed in the early bell stage (17 days), in addition to the reaction P.A.S. in stellate reticulum of the enamel organ (Fig.1D). At the bell stage (18-19 days) a positive P.A.S. reaction was observed in the stellate reticulum (Fig. 2A and 2B) and dental follicle and a weaker staining in the dental papilla (Fig. 2C and 2D). At the early crown

stage (20 days) P.A.S.-positive cells were found in pre-ameloblasts in the region of future cusps (Fig. 3A and 3B). The dental papilla cells exhibited intense reaction (Fig. 3C and 3D) while the stellate reticulum cells showed a weaker staining (Fig. 3B).

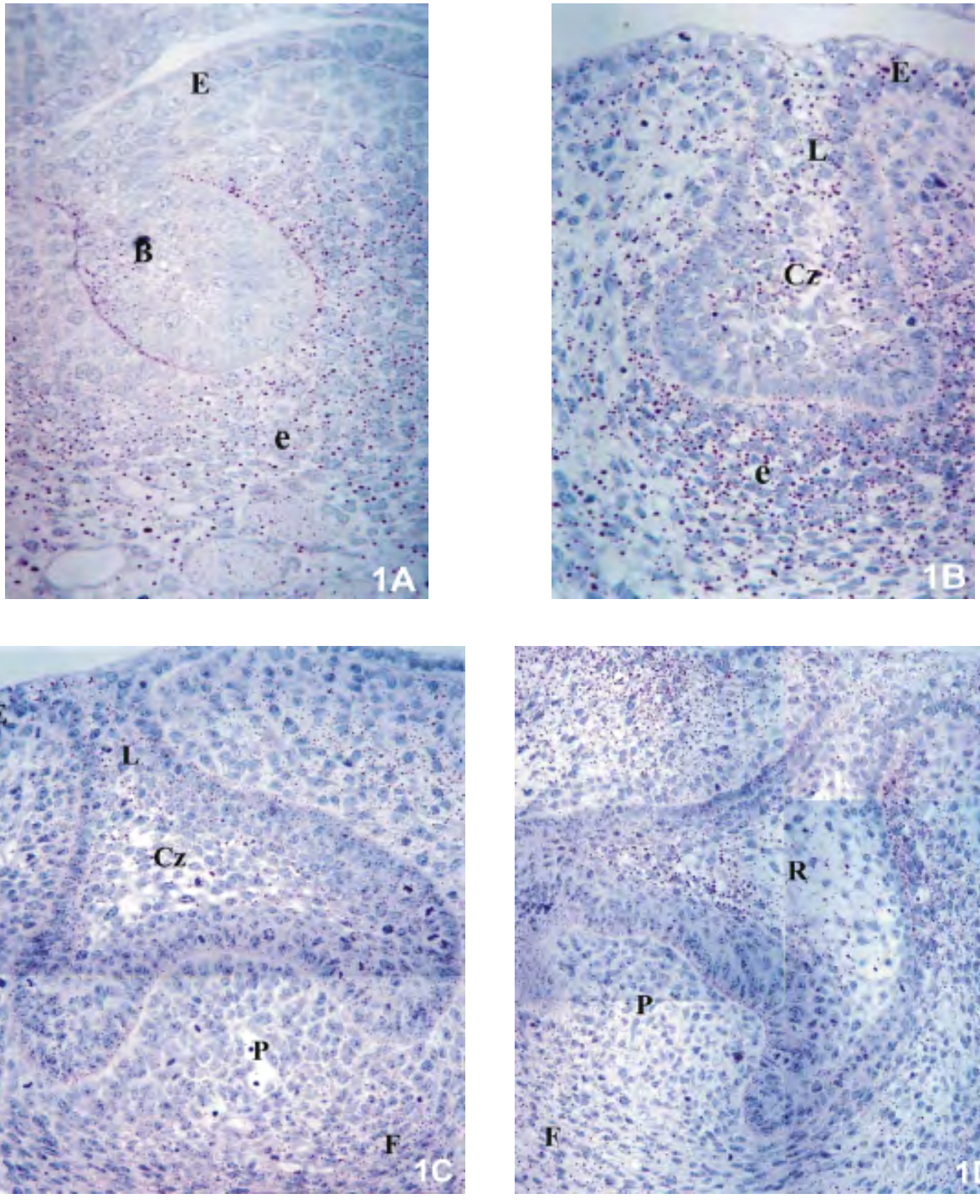

Figure 1 – Photomicrograph of first molar dental germ. 1A - Bud stage at 14 days of development, showing evidence of positive P.A.S. reaction (in red) in condensed ectomesenchyme cells (e) around the epithelial bud (B). 1B - Bud-cap transition stage at 15 days of development, P.A.S. reaction in ectomesenchyme (e), in enamel organ (Cz), in dental Lamina (L) and oral Epithelium (E). 1C - Cap stage at 16 days of development, showing evidence of P.A.S. reaction in epithelial cap (Cz), in dental Lamina (L), in oral Epithelium (E) and dental Follicle (F). Dental Papilla (P). 1D - Beginning of bell stage, 17 days of development, P.A.S. reaction in stellate reticulum (R) and in dental follicle (F). Hematoxylin of Meyer; original magnification 40×.

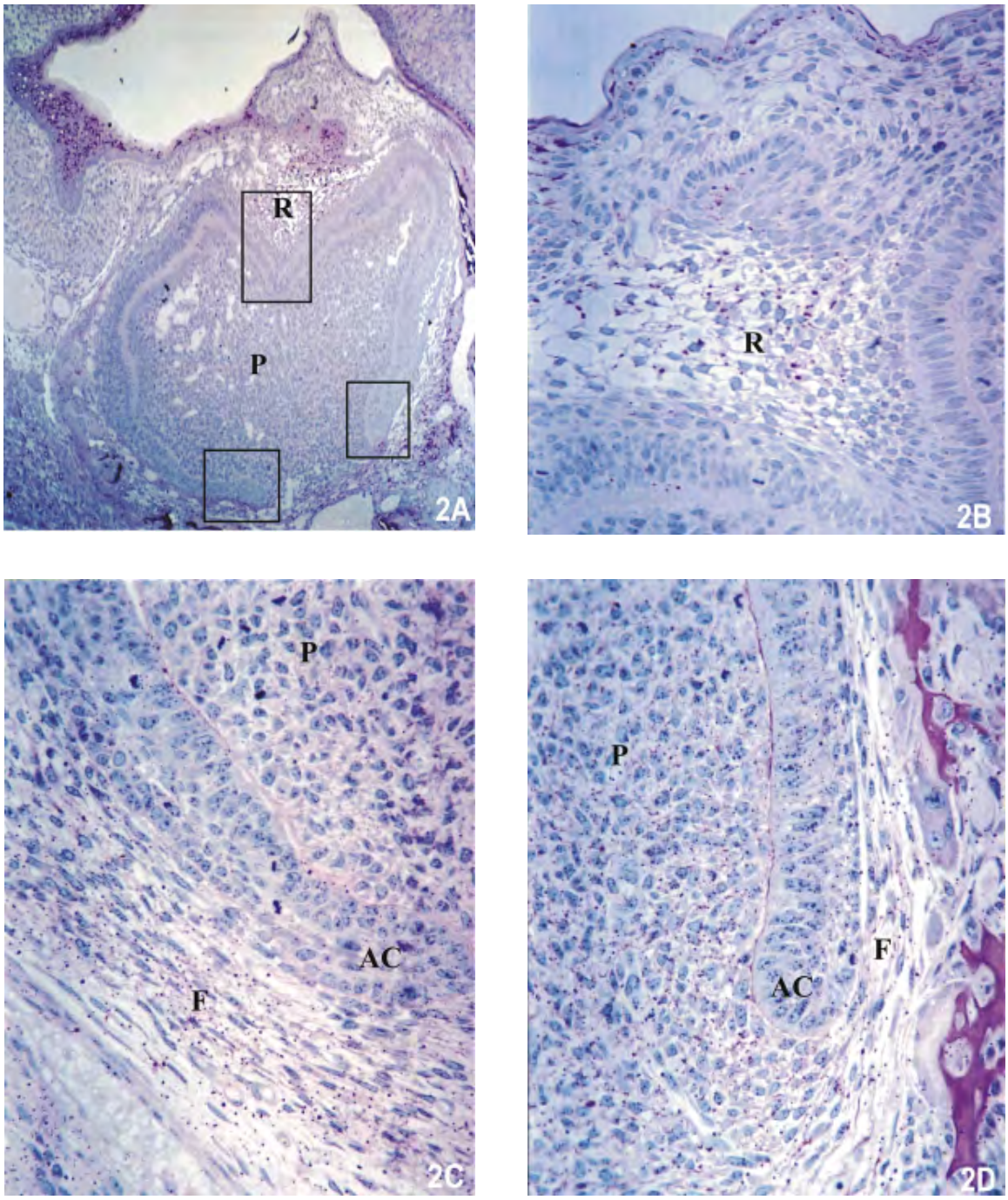

Figure 2 – Photomicrograph of first molar dental germ. 2A - Bell stage at 18-19 days of development. Stellate reticulum (R), dental papilla (P). 2B - Magnification of photomicrograph 2A showing evidence of P.A.S. reaction in stellate reticulum (R). 2C - Magnification of the region of the cervical loop (AC) showing positive P.A.S. reaction in dental follicle (F). Dental papilla (P). 2D - Positive P.A.S. reaction in dental follicle (F) and dental papilla (P). Hematoxylin of Meyer; original magnification 10× (2A); 40× (2B, 2C and 2D).

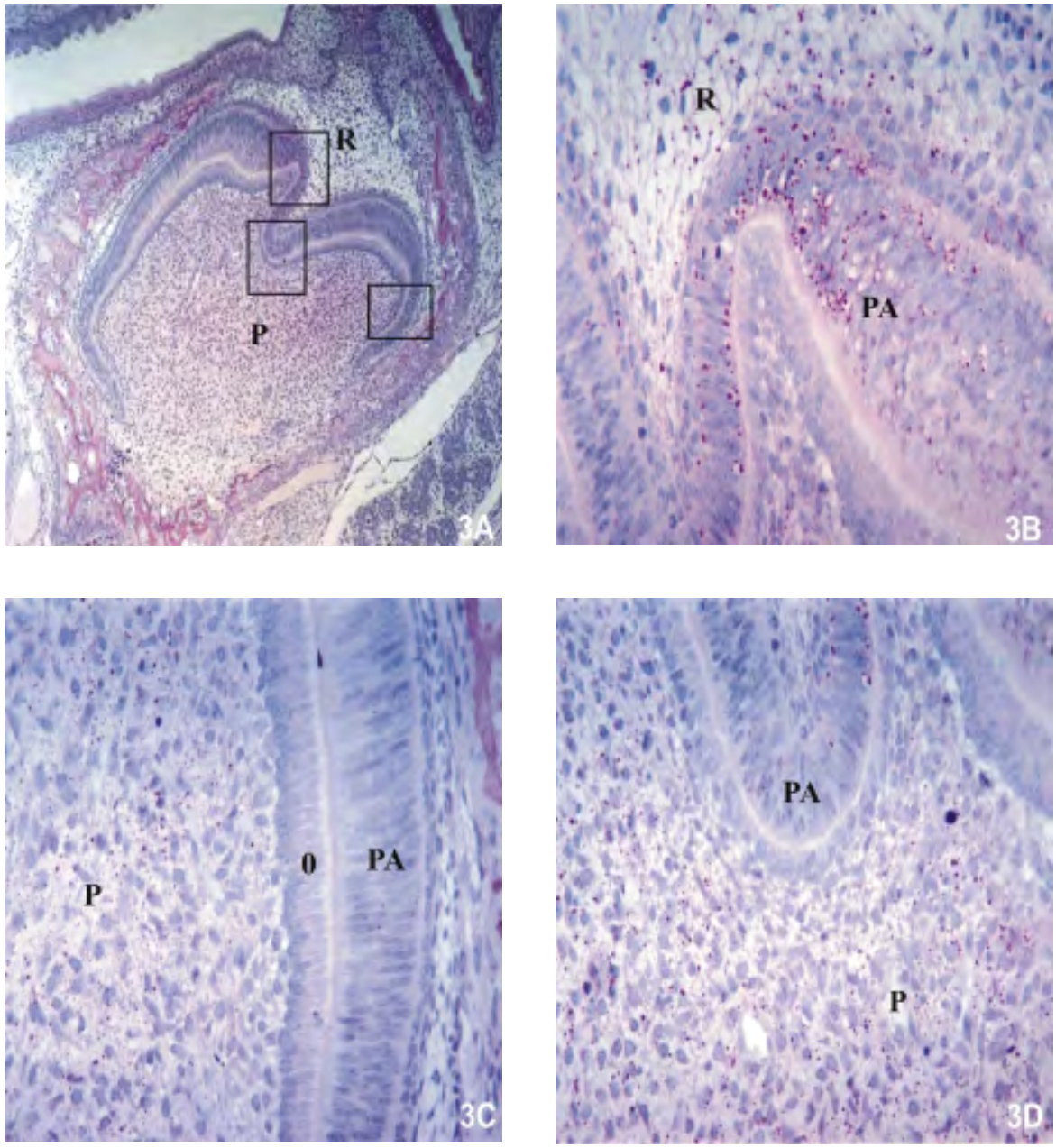

Figure 3 – Photomicrograph of first molar dental germ. 3A - Crown stage at 20 days. Dental papilla (P) and stellate reticulum (R). 3B - Magnification of photomicrograph 3A showing evidence of positive P.A.S. reaction (in red) in pre-ameloblast (PA) and in stellate reticulum (R). 3C - Positive P.A.S. reaction in dental papilla (P). Pre-ameloblast (PA), Odontoblast (O). 3D - Showing evidence of P.A.S. reaction in dental papilla (P) and pre-ameloblast (PA). Hematoxylin of Meyer; original magnification 10× (3A); 40× (3B, 3C e 3D).

DISCUSSION

No study has yet been conducted on the distribution of glycogen deposits during odontogenesis in *Calomys callosus*. This rodent has become an alternative experimental model because of the advantages it presents, such as easy handling, high productivity, year round reproduction and an apparent resistance to infections which are common to rats (*Rattus novergicus)*, mice (*Mus musculus*) and the guinea pig (*Cavia aperea*). They do not require large spaces or high cost to breed, since they are of a small size. These factors have contributed to a good adaptation of the *Calomys callosus* colony in laboratories, which has made them an appropriate biologic model species[15].

In cytochemical evaluations, the P.A.S. reaction is an important indicator of neutral polysaccharides, detecting carbohydrates, such as glycogen, starch and cellulose, as well as glycoprotein present in tissues. It is a classic cytochemical reaction, which mediates covalent bonds and promotes a characteristic magenta staining when in the presence of free or combined aldehydes in tissues.

In general, this kind of reaction is carried out in material embedded in paraffin, however, in this study the material used was embedded in glycol methacrylate. One of the advantages of this resin is the embedding process, which is performed at room temperature reducing morphologic changes in the material[8]. One disadvantage is that the resin hinders the reagent action on the material; therefore the first step of the modified P.A.S. technique with resin is to submit the sections to the action of acetone. The P.A.S.-positive reaction observed in present study was less intense than the one observed by Ohshima et al.[17] (1999), probably due to the embedding material. In the present study a control reaction with salivary amylase was carried out for each phase, moreover, it is well known that the other control reaction using another tissue (spongiotrophoblast) embedded in glycol methacrylate contains glycogen. Ohshima et al.[17] (1999) did not specify if the P.A.S. control reaction with salivary amylase was carried out for each phase of odontogenesis. They also did not specify the intensity of the P.A.S.-positive results for glycogen and other glycoprotein.

The distribution of glycogen storage observed in the developing teeth is in accordance with the findings of Ohshima et al.[17] (1999). Initially (12 to 15 days), the P.A.S.-positive reaction was more evident in ectomesenchyme cells, which may be due to their differentiation in the dental follicle. This prevalence in the follicle region peaked at 16-17 days. At the bell stage (18 and 19 days), large amounts of glycogen deposits were also observed in the stellate reticulum cells, since they probably store glycogen to supply the inner dental epithelial cells during their differentiation. The inner epithelial cells showed P.A.S. positivity on the 20th day of development, indicating relatively anaerobic conditions[16,17]. This finding can be associated to the differentiation of the inner epithelial cells in ameloblasts and the future lack of nutrition in the papilla, as a result of the odontoblast differentiation and dentine matrix production. These pools of glycogen are important to ameloblasts undergoing differentiation until they approach the dental follicle, which will be their new nutrition source. The large accumulations of stored glycogen in the dental papilla at the crown stage (20 days) must be related to the differentiation of these cells into odontoblasts and the future dental pulp cells.

The distribution and intensity of glycogen deposits are varied, since the presence of glycogen appears to precede cell function. It increases in intensity in areas in which the functions of cell division and maturation occur. The erratic distribution of glycogen occurs in accordance with the cell state of activity or rest, variations in cell functions and energy requirements in different species, which may be responsible for the differences in glycogen content between the human and rat species[18].

CONCLUSION

According to the methodology developed and to the results obtained, it was concluded that the storage distribution varied in different regions of the dental germ during the different odontogenesis phases. The detection of glycogen deposits in the dental germs did not significantly vary in material embedded in glycol methacrylate or in paraffin. *Calomys callosus* can be used as a biologic model in developing tooth studies (odontogenesis).

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