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A Histochemical Study of Tooth Development in the Chinese Hamster (Cricetulus griseus)

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A HISTOCHEMICAL STUDY OF TOOTH DEVELOPMENT IN THE CHINESE HAMSTER (CRICETULUS GRISEUS)

(TITLE)

BY

ROBERT B. DASZKIEWICZ

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THESIS

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Have Examined a Thesis Entitled

Histochemical Study of Tooth Development

in the Chinese Hamster (Cricetulus griseus)

Presented by

Robert B. Daszkiewicz

A Candidate for the Degree of Master of Science

And Hereby Certify That, In Their Opinion, It Is Acceptable

ABSTRACT

The normal morphological development of the Chinese hamster (Cricetulus griseus) during the last week of gestation was studied. A review of the literature reveals no distinct study of tooth development in this species. An identical gestation period is found in the mouse (Mus musculus) making direct comparison between the embryos of the same age of the two species feasible. During this investigation, it was found that morphological tooth development in the Chinese hamster lagged behind that of the mouse by about 2 days. On day 14 of gestation in the Chinese hamster, the oral epithelium is stratified along the free margin of the jaws. This is the earliest indication of odontogenesis of the development of molars. Included in this description is the thickening and elongation of the dental lamina, appearance of enamel organs and proliferation of these parts through cap and bell stages.

In addition to morphological development, a consideration of some of the molecular components of developing teeth in the Chinese hamster using histochemical techniques is undertaken.

Four histochemical methods were used to show the chemical constituents of the teeth. The four methods used in this study were Lehman's polychrome, pyronine-methyl green (P/MG), periodic acid-Schiff (PAS), and hematoxylin

and eosin. These procedures revealed the histochemistry of many areas during prenatal tooth development. Lehman's polychrome stain showed a good overall view of the types of chemical components present in the samples. The P/MG method was used along with the Lehman's polychrome method to distinguish areas of DNA and RNA. The PAS stain tested samples for carbohydrates, including glycogen. Although the results for this method were not very distinct, they do coincide somewhat with the results of previous investigators of other rodents. The eosin-hematoxylin stain distinguished cytoplasmic areas, staining pink, while the nuclei stain dark blue.

Additional chemical aspects of tooth development are found in the definitive text on oral histology and embryology by Orban (1972).

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INTRODUCTION

General features of tooth development in mammals, including man, are well known, Orban (1972) and Arey (1965).

Teeth are derived from two distinct embryonic tissues: (1) ectoderm which gives rise to the enamel organ and subsequently the enamel of the tooth; and (2) mesenchyme, the precursor of all the other parts of the tooth and supporting structures.

The first structures to appear in tooth development are collectively called the dental anlagen. At first these structures consist of a continuous ridge of tissue, one for each jaw, which appear as solid proliferations of the oral epithelium extending into the underlying mesenchyme. These laminae are positioned at right angles to the surface epithelium. They extend around the arch of each jaw and are termed the labiodental laminae (Fig. 1).

When the labiodental lamina is differentiated, it is made up of two separate parts. One part consists of a vertical labial ingrowth which marks off the future lip and vestibule. This is termed the labial lamina. The second part continues as a lingual prolongation of epithelium which eventually gives rise to the enamel organs. This lingual prolongation is known as the dental lamina (Fig. 1 and Fig. 3).

Enamel organs arise as bud-like outgrowths of the dental laminae at sites corresponding to the location of the future deciduous teeth (Fig. 2). These outgrowths appear first as solid structures. Later the distal epithelium of these buds invaginates and the cavity which forms is filled with mesenchyme. In this manner, they serve as a form for the crown of the developing tooth.

After the labial laminae and the dental laminae appear, differentiation and growth become very important in the development of the tooth. This is best represented in Figs. 3 and 4. The changes which occur leading to the stage of development in Fig. 3 are as follows:

(1) The epithelial bud or tooth germ becomes removed from the oral epithelium, but is still attached to it by a narrow strand of cells called the dental lamina.

(2) The mesenchyme at the base of the epithelial bud undergoes rapid proliferation. During this process, a condensation of mesenchymal cells, known as the dental papilla, causes a depresssion in the distal wall of the epithelial bud which is now called the early enamel organ.

When the mesenchymal cells which lie against the epithelium of the tooth germ begin to proliferate, they cause an indentation in the base of the germ. The outer surface cells, those unaffected by the indentation, are called the outer enamel epithelium. That layer of cells which overlies the advancing dental papilla are called the inner enamel epithelium. The tissue which occupies the space between these two layers becomes modified and known as the stellate

reticulum (Fig. 4).

As development of the enamel organ proceeds, the mesenchymal cells continue to invaginate into the epithelial mass to the extent that the enamel organ assumes the shape of a bell or inverted goblet. The dental lamina becomes reduced in size. In this stage of development, a fourth layer appears (Fig. 5). It is adjacent to the inner epithelium and is termed the stratum intermedium. The following tissues can now be seen in the enamel organ from the outside inward: (1) outer epithelium, (2) stellate reticulum, (3) stratum intermedium and (4) the inner epithelium located at the border of the embryonic pulp chamber (Fig. 6).

A thorough review of the literature reveals that there has been no study of the tooth development of the Chinese hamster (Cricetulus griseus). However, detailed features of tooth development in the mouse (Mus musculus) are described by Cohn (1957) and Rugh (1968). The formation of dentin and enamel of the crown, with special reference to enamel free areas, was observed by Gaunt (1956). In addition, material is available on the development of rat dentition by Wislocki and Sognnaes (1950) and Reith (1957).

Both of the above-mentioned species have gestation periods of twenty-one days, which makes direct comparison between the embryos of the same age of the two species feasible.

This study involves an investigation of the normal morphological development in the Chinese hamster during the last two weeks of gestation (14-21 days). It also involves a consideration of some of the molecular components of developing teeth in the Chinese hamster using histochemical techniques.

Wislocki and Sognnaes (1950) provide an investigation of histochemical reactions of normal teeth of different stages of development from man, monkey and the rat. Additional chemical aspects of tooth development are found in the definitive text on oral histology and embryology by Orban (1972).

MATERIAL & METHODS

All the animals used in this investigation came from populations in the Animal Room of The Department of Zoology, Eastern Illinois University. Dr. E. B. Krehbiel kindly allowed the use of the Chinese hamsters. Representative samples of both Chinese hamsters (Cricetulus griseus) and the mouse (Mus musculus) were taken.

Both the mouse, Allen (1922) and Rugh (1968), and the Chinese hamster have similar estrous cycles, Asdell (1964). For aging purposes, the occurrence of a vaginal plug, Bronson, Dagg and Snell (1966) and Rugh (1968), was used to indicate day zero in staging all embryos. Development was allowed to continue until day 14. Thereafter, samples of embryos of

the Chinese hamster consisting of one litter of pups were collected everyday from day 14 to day 21 with the exception of day 15. A significant decrease in the stock population prevented collection of 15-day old embryos.

In addition to the Chinese hamster material prepared in this study, some 18-day mouse material was also prepared. Day 18 of gestation was picked because it represents the midpoint of development between days 14 and 21, and for this reason would provide the best date for comparison with both earlier and later tooth development in the Chinese hamster.

The embryos were decapitated. Half of the head samples of each litter were split on a medial sagittal line; the remainder were unsevered. This procedure was carried out to ensure that a significant number of serial sections showing good molar development could be obtained with a minimum number of sacrifices of viable females from the population. However, it was also important to have undivided heads to ensure good incisor serial sections. All samples were fixed immediately in either Kahle's fixative, Henley and Costello (1957); formalin saline, Jones (1966) and Sumner and Sumner (1969); or Bouin's fixative, Jones (1966). Most of the samples were fixed in either Kahle's or formalin saline. A limited number of heads were fixed in Bouin's.

All material gathered was embedded in paraplast after the method of Jones (1966) and Sumner and Sumner (1969). Heads were cut serially at 10μ both transversely and sagit-

tally to give a clear representation of developmental changes. The material was then spread on slides by use of Mayer's albumen affixed as described in Jones (1966).

All material was stained by one of four different histochemical methods. These methods were conducted to show the chemical constituents of the teeth. The four methods used in this study were Lehman's polychrome, Lehman (1965); pyronine-methyl green, Gurr (1965); periodic acid-Schiff, Jones (1966) and Sumner and Sumner (1969); and hematoxylin and eosin, Humason (1972).

Much of the material studied was stained with Lehman's polychrome. This stain was designed as a screening stain for general molecular groups of nucleic acids, proteins and mucopolysaccharides. "Celestine blue stains nuclei steel blue and cytoplasmic RNA lavender. Naphthol yellow S stains histone, hemoglobin, keratin and other basic proteins or proteins rich in -SH groups yellow. Aniline Blue stains all mucopolysaccharides various shades of clear blue. Chromotrope 2R stains acid and neutral proteins scarlet. When two of the above molecular components are concentrated in the same cellular region, the multiple dye binding will result in complementary colors. For example, mitotic chromosomes appear green by virtue of celestine blue staining of nucleic acids and naphthol yellow S staining of chromosomal histones," Lehman (1965).

The periodic acid-Schiff reagent (PAS) method was used

to demonstrate certain carbohydrates. Periodic acid is a specific oxidant for 1,2-glycol groups (CHOH-CHOH), converting them to dialdehydes (CHO-CHO). The aldehydes which are formed can then be detected with Schiff's reagent. The staining of a component a magenta color is interpreted as a positive result, Sumner and Sumner (1969).

An eosin-hematoxylin method was used to distinguish cytoplasmic and nucleic areas, Humason (1972). For this study, Harris' hematoxylin was used and stained nuclei dark blue. Eosin which stained cytoplasmic areas pink or light red was the counter stain used.

OBSERVATIONS

The Chinese hamster has a dental formula of incisors 1/1; cuspids 0/0; premolars 0/0; and molars 3/3, making a total of 16 teeth. The first molar arises first, followed by the second and then the third. Development of upper jaw molars lags behind the lower jaw molars by half a day. Incisors begin to appear during the gestation period at day 16.

The dental lamina is the first normal dental structure to appear. Before day 14, there is probably no indication of differentiation of the dental lamina.

The following is a day-by-day description of the development of molars in the Chinese hamster.

Day 14:

The oral epithelium is stratified along the free margin of the jaws (Fig. 7). This is an early indication of odontogenesis. The dental lamina at this time consists of 2 to 3 layers of cuboidal cells. Both the oral ectoderm and the underlying mesoderm show numerous mitotic figures (Fig. 7).

Day 16:

The dental lamina thickens and elongates. The length is increased by anterior-posterior growth. Enamel organs for both the upper and lower first molars make their appearance at this time. Rapid cellular proliferation at specific sites on the surface of the dental lamina forms a club-shaped tooth bud, consisting of a ball of densely packed cuboidal cells connected to the dental lamina by a short, thick stalk (Fig. 8). Around the base of the bud are deeply staining mesodermal cells aggregated together.

Day 17:

The elongation of the dental lamina continues. Invaginations appear on the deep surfaces of the tooth buds of the first molars. This is the first indication of the cap stage (Fig. 9). This invagination is filled with deeply staining mesenchymal cells which initiate the formation of the dental papilla and vascular elements between the central cells of the enamel epithelium. Surrounding the enamel organ are two to three layers of flattened mesenchymal cells which can be

identified as the primordium of the dental sac.

Day 18:

Proliferation of the cells of the inner and outer enamel epithelium greatly increases the size of the enamel organs of the first molars (Fig. 10). Accumulation of intercellular substances between cells of the stellate reticulum enlarges its spaces and gives the cells of this region a loose distribution. The outer enamel epithelium thins out into a peripheral layer of low columnar cells lying upon a few layers of squamous cells. The inner enamel epithelium is comprised of a single layer of columnar cells whose nuclei show no uniformity in position. These cells increase in height during proliferation, but the position of their nuclei remains unchanged. A narrow eosinophilic zone present between the inner enamel epithelium and adjacent cells of the dental papilla marks the site of the future dentino-enamel junction. Proliferation of cells of the dental papilla continues to fill the expanding invagination of the enamel organ (Fig. 11). The dental sac is well defined at this date and consists of several layers of flattened cells oriented closely around the enamel organ. There is an increased vascularity both within the dental papilla and among cells adjacent to the dental sac. The cellular stalk connecting the enamel organ to the dental lamina is thinning out. Bone appears and begins to encircle the tooth germs of the first molar.

At this time, tooth buds for the second molars begin to appear (Fig. 12). The dental lamina extends posteriorly from these buds.

Day 19:

Growth of the enamel organs of the first molars is accentuated, particularly in their cervical region, causing an increase in the size of the pattern of the future crowns (Fig. 13). The stellate reticulum is also expanded, not only by proliferation of its cells, but also by an increase in the size of the spaces between them. A layer of flattened cells between stellate reticulum and inner enamel epithelium marks the first appearance of the stratum intermedium (Fig. 14). There is cell proliferation in both inner enamel epithelium and dental papilla, increasing their extent.

Day 20:

At this time, organs of the first molars are in the bell stage. They begin forming the pattern of the cusps and the outline of the future dentino-enamel junction (Fig. 15). The outer enamel epithelium is considerably reduced in thickness, consisting of one to three layers of cuboidal cells. Cells of the inner enamel epithelium continue to divide and slowly increase, almost doubling in height; numerous capillaries of the dental papilla are in close approximation to them (Fig. 16). The connection between the enamel organ and the dental lamina is nearly lost.

Day 21:

The crown pattern is almost completed in the enamel organs of the first molars (Fig. 17). Growth in the cervical region persists for a time after birth. Certain cells of the inner enamel epithelium transform into fully differentiated ameloblasts; these cells more than double their height and their nuclei orient farthest from the future dentino-enamel junction (Fig. 18). This cyto-differentiation of ameloblasts begins at high points corresponding to growth centers on the cusps. Some cells of the inner enamel epithelium do not differentiate but remain low columnar, forming a small group on each of the high points of the cusps. This group of cells, extending along an entire margin of each cusp near its apex, appears to invaginate pulpward forming one or more shallow depressions which disrupt the contour of the future dentino-enamel junction; the pulpal surface of this cord is convex. The invagination also seems to carry inward with it adjacent cells of the stratum intermedium and stellate reticulum. Cells from the dental papilla differentiate into odontoblasts, align themselves opposite ameloblasts and begin forming predentin (Fig. 18). The differentiation of odontoblasts also occurs next to the convex surface of the cord of undifferentiated cells of the inner enamel epithelium; this forms a small concavity of predentin along an entire margin of each cusp. Squamous cells of the stratum intermedium become more

cuboidal opposite ameloblasts undergoing cyto-differentiation but remain unchanged opposite the cord of undifferentiated cells.

At no time is any evidence of the third molar visible.

Incisor development is similar to that observed in the molar development. The incisor primordia is first seen at day 15, just posteriorly to the lip furrow band. Meckel's cartilage and inner and outer enamel epithelia are differentiated at day 16. On day 17, the inner and outer enamel epithelia are expanding, especially in the ventral incisors. Also at this time, the dental papillae first appear. By day 18, all of the above-mentioned structures have proliferated, ameloblasts and odontoblasts ring the papillae and the surrounding mesenchyme is being organized into bone. Alveolar bone formation is prominent, incorporating the abundant surrounding mesenchyme by day 20. Complete differentiation of the distal and intermediate portions of the incisors depends upon association with the proximal portions. This results in complete and integrated parts for the upper and lower incisors. Differentiation of all portions continues during birth on day 21 (Figs. 19 to 30).

Histochemistry

The observations of the histochemical aspects of molar development in the Chinese hamster follow in a day-by-day account.

Day 14:

With Lehman's polychrome stain, the cytoplasmic portions of the cells of the dental lamina stain lavendar and the nuclei stain blue. The chromosomes in the many mitotic figures stain dark green. This green staining is produced by a combination of the celestine blue staining of nucleic acids and naphthol yellow S staining of histones (Fig. 7).

The pyronine-methyl green (P/MG) procedure stains the cytoplasm of cells in the dental lamina red. Nuclei in these cells stain blue. Many bright red spots are present in the nuclei of cells in the dental lamina area. These are due to the staining of nucleoli. Numerous mitotic figures are evident in the sections using this stain. The chromatin material in these figures stains purple to blue-green depending on the amount of RNA in the individual nuclei (Fig. 31).

A weak stain reaction resulted with the PAS procedure for carbohydrates. Because of the low number of 14 day embryos, it was impossible to restain any samples for this particular day. The faint staining did show the cells of the dental lamina to stain a positive magenta color.

With eosin-hematoxylin, cytoplasmic areas of cells of the dental lamina stain pinkish, while the nuclei stain dark blue or purple. Many mitotic structures are present and stain purple (Fig. 32).

Day 16:

After using Lehman's polychrome stain, cells of the dental lamina appear the same as they do at day 14. The ball of densely packed cells within the club-shaped tooth bud stain much the same as the dental lamina. The mesenchymal cells immediately around the bud are aggregated very tightly and stain much darker than other cells in the same surrounding area. This may be because the closer aggregation of these cells makes the staining in this area appear darker or it might indicate the presence of mucopolysaccharides. Nuclei in these cells appear almost black, while cytoplasmic material stains purple. Once again, many mitotic structures are distinguishable and again, their chromosomes stain dark green (Fig. 8).

Samples stained with P/MG appear similar to the previous samples in the area of the dental lamina. The densely packed area within the tooth bud contains cells which stain positively for both DNA and RNA. There appear to be blue staining cells with darker staining blue nuclei in this area, but there also appear to be some cells with reddish staining cytoplasmic areas and blue nuclei. This may be due to difficulty in distinguishing single cells clearly. Many mitotic structures are visible and are staining very positively for DNA. With this stain, bone formation is not distinguishable (Fig. 33).

Fig. 34 shows a negative PAS reaction in the dental lamina area. There appears to be no staining in the cells making up the club-shaped tooth bud. The cells making up the oral epithelium along the free margins of the jaws stained positively for carbohydrates. This might help explain the positive reaction to the early forming dental lamina on day 14. The densely packed area within the tooth bud stains a positive magenta color for carbohydrates. The tightly aggregated mesodermal cells immediately around the bud stain negatively.

All of the above areas have pink cytoplasmic areas and dark blue nuclei when stained with eosin-hematoxylin stain (Fig. 35).

Day 17:

Use of the Lehman's polychrome stain yielded many areas staining identically with those in previous samples. The continually growing dental lamina, the densely packed area within the tooth bud, the many mitotic structures and the evident bone formation all stained identically to their respective areas found in the day 16 sample (Fig. 9). The now recognizable inner and outer enamel epithelium stain similarly to the dental lamina. The stellate reticulum cells are not packed tightly and appear to stain lighter than the areas mentioned above. Like the stellate reticulum, the very loosely packed flattened mesenchymal cells surrounding the tooth bud stain very light.

P/MG stains all areas similar to what was seen in earlier samples. Cells outside of the tooth bud appear to stain more intensely for DNA. In the area of the forming dental papilla, there seems to be an underlying layer of cells positive for RNA. Mitotic structures are visible. The chromosomes are positive for DNA, and are clearly within cells staining positively for cytoplasmic RNA. Bone formation is still questionable at this point (Fig. 36).

The only area positive to the PAS test is that of the oral epithelium. All other areas previously mentioned stain negatively for carbohydrates (Fig. 37).

The eosin-hematoxylin stain yielded similar results in all the previously mentioned areas as described at day 16.

Day 18:

Fig. 10 shows tooth formation in the cap stage with the use of Lehman's polychrome. Proliferation of inner and outer enamel epithelium is taking place. These cells are very tightly packed and appear to stain darker than in previous samples. This is possibly due to the tight packing of these cells. Accumulation of intercellular substances between cells of the stellate reticulum has enlarged this area (Fig. 11). The irregular shaped cells of this area stain positively for cytoplasmic RNA and the nuclei stain blue with the use of Lehman's polychrome. The dental papilla appears much like the stellate reticulum but is more densely packed with cells. An eosinophilic zone is

present between the inner enamel epithelium and the dental papilla, staining similarly to the two enclosing areas (Fig. 11). Mitotic figures are seen in the above areas and stain dark green as in previous samples. The cellular stalk, consisting of cells of the dental lamina, stains identically to the dental lamina in previous samples. Bone is now observed encircling the tooth germ outside what now is termed the dental sac in the area of the surrounding mesodermal layer. These cells stain positively for mucopolysaccharides (Fig. 10).

The P/MG stain appears to stain much more intensely on days 18 and 19. This is probably due to the staining procedure used in which the tissue samples from these two days were stained simultaneously in the same slide carrier. Something in the staining procedure caused these samples to stain more intensely. The staining of all areas was the same as in samples from day 17 but were much darker.

The carbohydrate test stains the same as previous samples with two exceptions. There appears to be a thin layer of carbohydrate material outlining the cap structure (Fig. 38). Bone formation is now distinguishable and stains positively with the PAS test.

Eosin-hematoxylin stains all areas as in previous samples (Fig. 39). The dental papilla seems to stain somewhat darker but this is probably due to the continual aggregation of cells in this area.

Day 19:

Tooth formation appears to be in the early bell stage (Fig. 13). Lehman's polychrome shows most areas to be the same as in previous descriptions. The stellate reticulum is still enlarging, with more spaces being evident between individual cells. In the area of the stratum intermedium, flattened cells between the stellate reticulum and inner enamel epithelium are starting to form (Fig. 14). These cells stain positively for cytoplasmic RNA and the nuclei stain positively for chromatin material.

Use of the P/MG stain was discussed at the day 18 level.

The PAS test and the eosin-hematoxylin test gave identical results as in previous descriptions (Figs. 40 and 41).

Day 20:

Tooth development is now in the bell stage. Lehman's polychrome stains all areas the same as in previous samples. The only differences are of a physical nature and have already been described (Figs. 15 and 16).

The P/MG stain used on these samples stains lighter and more normal than the previous two days' samples. Areas stain similarly to those described for the day 17 samples. Fig. 42 shows a space around the entire tooth structure. This space is the dental sac and does not stain. Also at this time, one can see odontoblasts starting to form. These

cells stain positively for RNA in the cytoplasm and positively for DNA in the nuclei (Fig. 43).

The PAS test for carbohydrates stains as described previously in all areas. The only positive results are in the oral epithelium and bone formation. In addition, there appears to be a positive staining layer at the junction between the dental papilla and the inner enamel epithelium. This is the site of the future dentino-enamel junction (Fig. 44).

There were no samples available for day 20 and day 21 using the eosin-hematoxylin stain. The results of all the previous samples tested would clearly indicate that these samples also would have stained in the same manner.

Day 21:

Three new areas are clearly seen in Figs. 15 and 16. The first is a thin layer of material which is positive for mucopolysaccharides after treatment with Lehman's polychrome. This thin layer of material probably is predentin. Odontoblasts and ameloblasts are clearly distinguishable as the second and third new areas, respectively. The cytoplasm of these cells stain positively for RNA and the nuclei stain positively for DNA (Fig. 16). There is much surrounding bone visible, staining sky blue. All other areas have been previously described in prior samples.

P/MG stain for this date confirms what is seen in the Lehman's polychrome samples (Figs. 45 and 46). The odonto-

blast and ameloblasts stain positively for cytoplasmic RNA. The nuclei of these cells show DNA present. Although these two types of cells stain similarly, the staining of the odontoblasts is more intense than that of the ameloblasts (Fig. 46). No staining is observed in the area where predentin is being laid down.

No sample was available for this day using the test for carbohydrates, PAS.

Staining of incisor samples results in the same pattern observed in the above molar descriptions. All areas of the incisors stain indentically with respective areas in the molars. See the descriptions of incisor developmental morphology and Figs. 19 to 30.

DISCUSSION

In this study, the development of the Chinese hamster dentition was investigated during the last week of prenatal life. It is apparent from this investigation that the Chinese hamster is a good source of material of all periods of tooth development. The relatively easy preparation and the smallness of the samples facilitated the making of serial sections, enabling a full comprehensive study of all phases of tooth development.

As was mentioned earlier, identical gestation periods made a direct, daily comparison between the tooth development of the Chinese hamster and that of the mouse feasible.

During this investigation, it was found that tooth development in the Chinese hamster lagged behind that of the mouse by about 2 days. This observation was determined by a comparison of the material of Chinese hamster prepared with that of the published material of the in-depth studies of the mouse by Cohn (1957) and Rugh (1968). Cohn (1957) described a dental formula for the mouse identical with that found in the Chinese hamster. The molars of the mouse arise in the same order as those in the Chinese hamster. In the mouse, the first is the largest and the third is the smallest. Highlights of Rugh's (1968) description of the development of mouse molars follows: "Before gestation day 12, there is no indication of the differentiation of the dental lamina. At day 12, there is a thickening of the oral epithelium denoting the beginning of the dental lamina. At day 13, there is a growth in length and thickness of the dental lamina. Day 14 yields tooth buds for the first molars. The molars are found to be in the cap stage at day 15. During day 16 and 17, there is much proliferation. The molars are in the bell stage on day 18. Day 19 has certain cells of the inner enamel epithelium transformed into fully differentiated ameloblasts. At day 20, one can view differentiation of the odontoblasts." Comparing Rugh's highlight with the descriptions within the text of this paper for the Chinese hamster, one sees them to be almost identical with

the exception of a 1-1/2 to 2 day lag of the Chinese hamster material behind that of the mouse material.

In addition to the material prepared in this study for Chinese hamster, some 18 day mouse material was also prepared. The 18 day time point was picked because it represented the midpoint of tooth development during gestation. This material was stained with Lehman's polychrome procedure and compared with similarly prepared material of the Chinese hamster. It was found that 18 day mouse material was in the same stage of development as material of the Chinese hamster at late day 19 or day 20 (Fig. 47). All areas of the morphologically similar embryos stained alike.

Cohn (1957) states, "the manner of growth, calcification and eruption are quite similar to those of human teeth." Later Cohn states, "because of the essential similarities in development and structure between the molars of mice and human teeth, mouse molars can be used to contribute to our understanding of human teeth." Arey (1965) and Orban (1972) are two texts that explain in some detail the development of teeth in humans. Orban's text is the definitive published work on the subject. A summary of early development of human teeth follows.

The dental anlagen which consists of a band of tissue in each jaw makes its appearance at approximately the sixth week of prenatal development. This occurs at about day 14

of gestation in the Chinese hamster. Soon thereafter the labial lamina is made up of a labial downgrowth and a lingual proliferation. This proliferation gives rise to the enamel organs. The labial lamina proliferation and the emergence of the enamel organs occurs at day 16 of gestation in the Chinese hamster.

The tooth germs arise as outgrowths of the dental laminae. They become invaginated by underlying mesenchyme, meanwhile remaining suspended by the dental lamina. Invagination of the tooth germ continues; accompanying this change, differentiation of the elements of the growing enamel organ eventually gives rise to four distinct cell layers which will be involved in the development of enamel. Tooth germ arises at day 17 of gestation in the Chinese hamster with differentiation continuing through day 21. During this same period, the growing enamel organ gives rise to four cell layers which will help develop enamel.

It is very evident that the above summary of human tooth development parallels the development described in this paper for the Chinese hamster. It seems clear that the teeth of the Chinese hamster can be used, as have the teeth of other rodents, to observe changes in the dental tissues when mechanical, hormonal or metabolic influences are placed upon them. These types of experiments would allow a better understanding of these types of disorders in human tooth development.

Cohn (1957) made two important observations in his study of dental development of the mouse. First that the enamel-free areas are initiated early in tooth development. Secondly, the cyto-differentiation of ameloblasts must take place for enamel to be formed. Similar observations were made by Gaunt (1956) in the mouse and by Addison and Appleton (1921) and Lefkowitz et al. (1953) in the rat.

A reasonable explanation for the failure of the non-formative ameloblasts to differentiate remains to be determined; nevertheless, this failure is evidence, even before birth, for the origin of the enamel-free areas on the mature cusps.

"The enamel-free areas typical of rodent molars are initiated before birth because of the failure of certain ameloblasts to undergo cyto-differentiation", Rugh (1968).

Squamous cells of the stratum intermedium appear to become cuboidal in shape when they are situated next to cells of the inner enamel epithelium undergoing cyto-differentiation. Reith (1957) stated that the cells of the stratum intermedium become stellate in shape rather than cuboidal. Only in the region of non-formative ameloblasts do cells of the stratum intermedium retain their squamous form. In agreement with Lefkowitz et al. (1953) and Gaunt (1956), these observations suggest that the stratum intermedium is concerned with cyto-differentiation of amelo-

blasts.

The procedures used in this study revealed the histochemistry of many areas during prenatal tooth development. Lehman's polychrome stain was chosen as the first stain used because of the many different types of molecular groups that it stains. This stain worked excellently to give a good overall view of the types of chemical components present in the samples. The P/MG method was used in addition to Lehman's method to allow areas of DNA and RNA to be distinguished. Both of these areas were stained positively with the Lehman's polychrome stain. The fact that both of these stains showed positive for DNA and RNA in the same areas at respective phases of developement, helps to authenticate their results.

The PAS stain is a test for carbohydrates, including glycogen. Glycogen has been described in detail in the fetal teeth of the rat, pig and man by a number of investigators, Horowitz (1942); Bevelander and Johnson (1946); Engel and Furuta (1942); Wislocki et al. (1948). All agree upon its presence in large amounts in the oral epithelium, dental lamina, outer enamel epithelium and stellate reticulum of fetal teeth. Reports vary on its presence in the stratum intermedium, ameloblasts, odontoblasts and pulp. Wislocki et al. (1948) used PAS in staining fetal human teeth and observed quantities of glycogen in the stratum intermedium, ameloblasts, odontoblasts and pulp. However,

in adult teeth of man and rhesus monkey, glycogen was not encountered in the enamel, dentin or pulp. On the other hand, it was plentiful in the pulp cells of the growing roots of adult guinea pigs' incisors.

At first glance, the results mentioned above seem to contradict the results obtained during the study. The PAS test did not seem to stain very many areas. The one place where there was a definite positive result at all times was in the oral epithelium of the Chinese hamster. Also, an outline of the cap structure at day 18 stained positively for carbohydrates. This outline is made up of the dental lamina, the outer enamel epithelium and the inner enamel epithelium. At day 16, the area within the club-shaped tooth bud stained positively for carbohydrates. This area corresponds to the area known as the stellate reticulum. Although the results for the test of carbohydrates in this study were not very distinct, they do coincide somewhat with the results of the authors mentioned above. It is important to note that the above authors did not all agree on the results of all areas.

Cohn (1957) gives the following summary of the sequence of postnatal events for the mouse occurring in the development of the molars. Amelogenesis or differentiation of the ameloblasts begins on the 1st to 2nd day in the first molars; on the 3rd to 4th day in the second molar and on the

11th to 12th day in the third molar. Eruption of the first, second and third molars into oral cavity takes place on the 16th to 17th day, 18th to 19th day, 28th to 29th day, respectively. Functional occlusion occurs on the 24th day, 25th day, 35th day in the first, second and third molars, respectively. The number of cusps (upper/lower) for these same molars are: 3/5, 3/4, 3/3; and the number of roots are: 3/2, 3/2, 3/1. It is most probable that the same, or very nearly the same, time schedule is incorporated in the postnatal development of the molars of the Chinese hamster with the exception of a 1-1/2 to 2 day lag.

This investigation shows a clear parallel in the prenatal dental development of the Chinese Hamster and the mouse. It is the author's opinion that the Chinese hamster can also be, as have the mouse and rat, used in dental developmental research.

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Figs. 1-6 are composite diagrams of tooth development after Orban and observations on Chinese hamster material.

Fig. 1 - Sagittal section through the jaws of an early embryo. Note the relation of the vestibule, lips, dental lamina, and tooth germ. V, vestibule; L, lip; DL, dental lamina; LL, labial lamina; UJ, upper jaw; LJ, lower jaw; TG, tooth germ; T, tongue.

Fig. 2 - Transverse section through the jaw of an embryo, showing the relation of the early tooth germ and the labial lamina. LL, labial lamina; TG, tooth germ; DP, dental papilla.

Fig. 3 - Transverse section through an early enamel organ. LL, labial lamina; DL, dental lamina; EO, enamel organ; DP, dental papilla.

Fig. 4 - Transverse section through an intermediate stage of a developing enamel organ. DL, dental lamina; OE, outer enamel epithelium; SR, stellate reticulum; IE, inner enamel epithelium; DP, dental papilla.

Fig. 5 - Transverse section through a late enamel organ. OE, oral epithelium; DL, dental lamina; DS, dental sac; EO, enamel organ; DP, dental papilla.

Fig. 6 - High magnification of area indicated in Fig. 5. UO, undifferentiated odontoblasts; IE, inner enamel epithelium; SI, stratum intermedium; SR, stellate reticulum; OE, outer enamel epithelium.

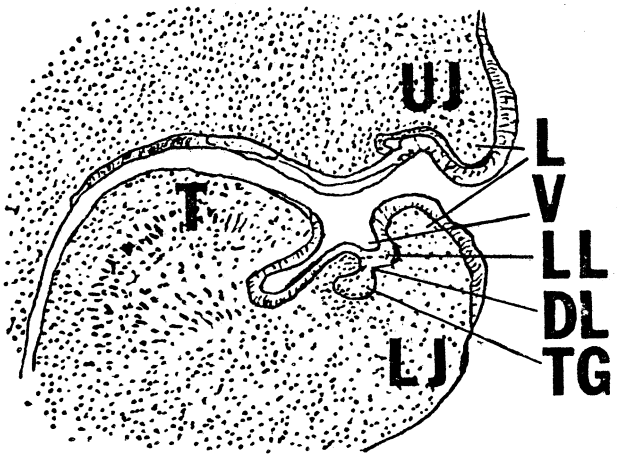
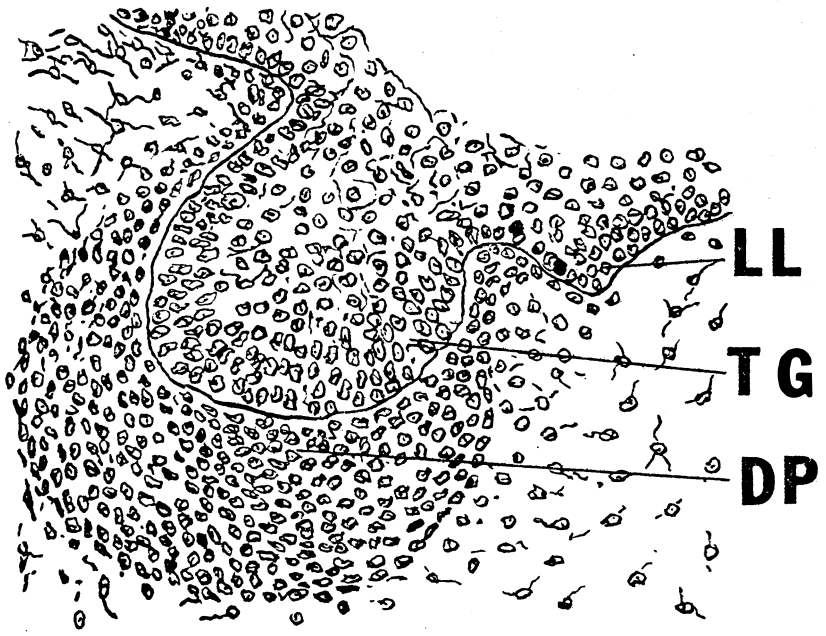


Fig. 1



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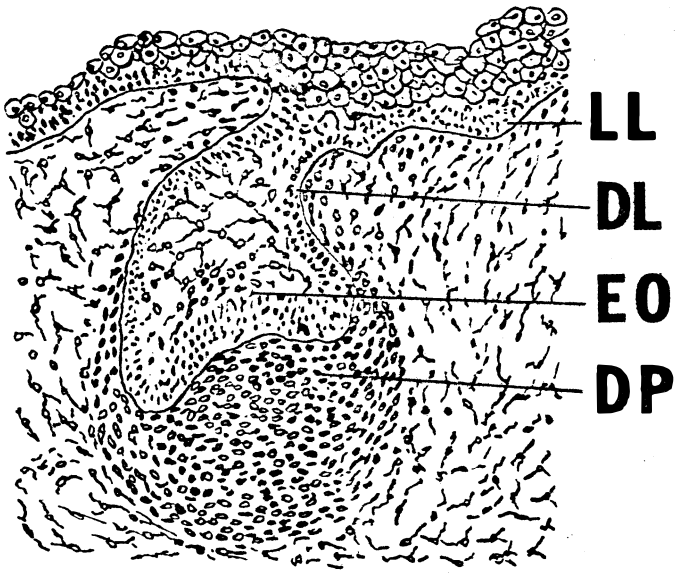

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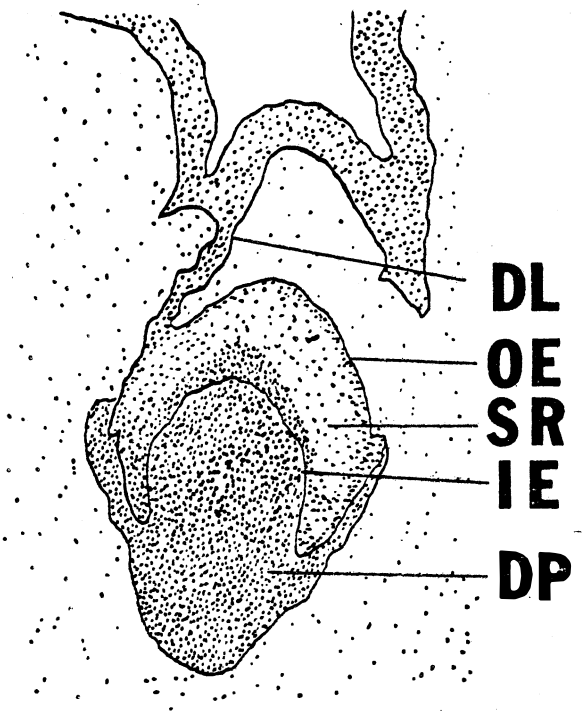
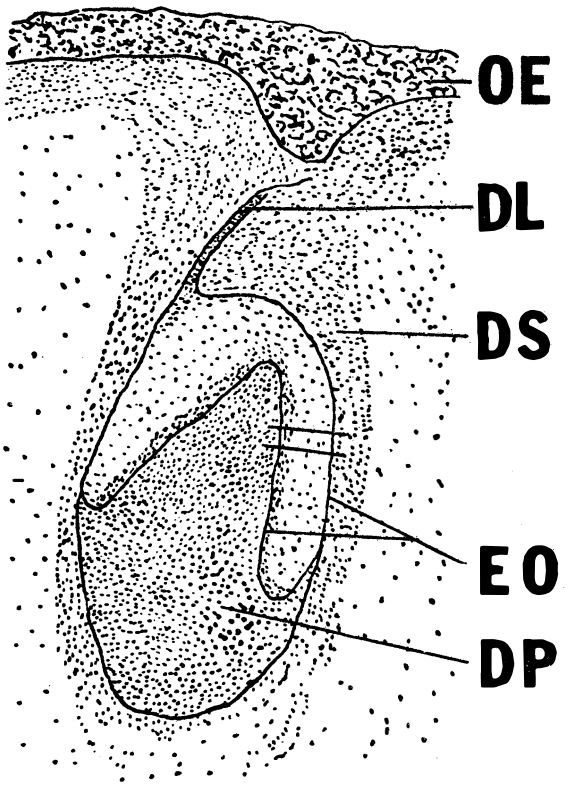


Fig. 4



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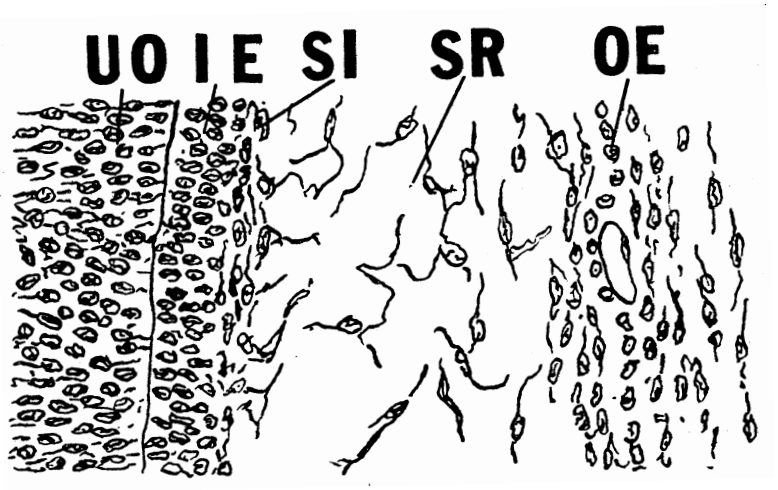


Fig. 6

Figs. 7-18 are sections of Chinese hamster molars stained with the Lehman's polychrome method.

Fig. 7 - Sagittal section through the jaw of a 14 day Chinese hamster embryo. Thickening of the oral epithelium in the region of the jaw denotes the beginning of the dental lamina. DL, dental lamina; OE, oral epithelium; MF, mitotic figures.

Fig. 8 - Transverse section through the upper right jaw of a 16 day Chinese hamster embryo. A club-shaped tooth bud of the first molar is present. DL, dental lamina; MF, mitotic figures.

Fig. 9 - Transverse section through the upper left jaw of a 17 day Chinese hamster embryo. Continued elongation of the dental lamina area is visible. DL, dental lamina; DP, dental papilla; EE, enamel epithelium; MF, mitotic figures; PDS, primordium of dental sac.

Fig. 10 - Transverse section through the right jaw of an 18 day Chinese hamster embryo. Cap stage of the first molars is present. DL, dental lamina; OE, outer enamel epithelium; BF, bone formation; DS, dental sac; T, tongue.

Fig. 11 - Magnification of area indicated in Fig. 10. IE, inner enamel epithelium; OE, outer enamel epithelium; DP, dental papilla; SR, stellate reticulum; DL, dental lamina.

Fig. 12 - Transverse section through the lower right jaw of an 18 day Chinese hamster embryo. A view of the second molar as a club-shaped tooth bud is present. DL, dental lamina; DP, dental papilla.

Fig. 13 - Transverse section through the left jaw of a 19 day Chinese hamster embryo. Accentuation of the cervical region of the enamel organ is shown. DL, dental lamina; DP, dental papilla; IE, inner enamel epithelium; SR, stellate reticulum; BF, bone formation; T, tongue.

Fig. 14 - Magnification of area indicated in Fig. 13. DP, dental papilla; IE, inner enamel epithelium; SI, stratum intermedium; SR, stellate reticulum.

Fig. 15 - Transverse section through the lower right jaw of a 20 day Chinese hamster embryo. The first molar is viewed in the bell stage. OE, outer enamel epithelium; IE, inner enamel epithelium; DP, dental papilla; SR, stellate reticulum; DS, dental sac; BF, bone formation.

Fig. 16 - Magnification of area indicated in Fig. 15. DP, dental papilla; IE, inner enamel epithelium; PD, predentin; OE, outer enamel epithelium; SR, stellate reticulum; C, capillaries.

Fig. 17 - Transverse section through the lower right jaw of a 21 day Chinese hamster embryo. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; SR, stellate reticulum; DS, dental sac; BF, bone formation.

Fig. 18 - Magnification of area indicated in Fig. 17. Ameloblasts and odontoblasts are shown. DP, dental papilla; O, odontoblast; PD, predentin; A, ameloblasts; SR, stellate reticulum.

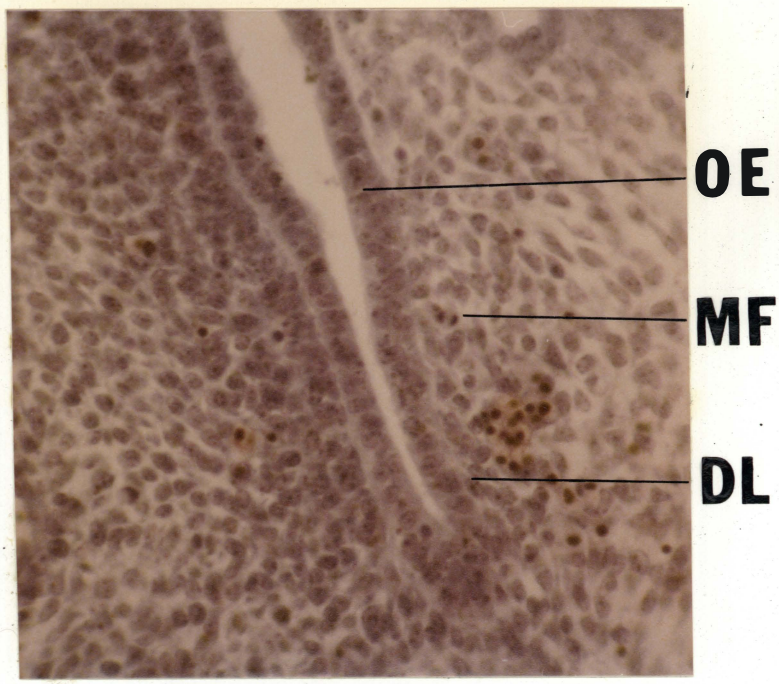
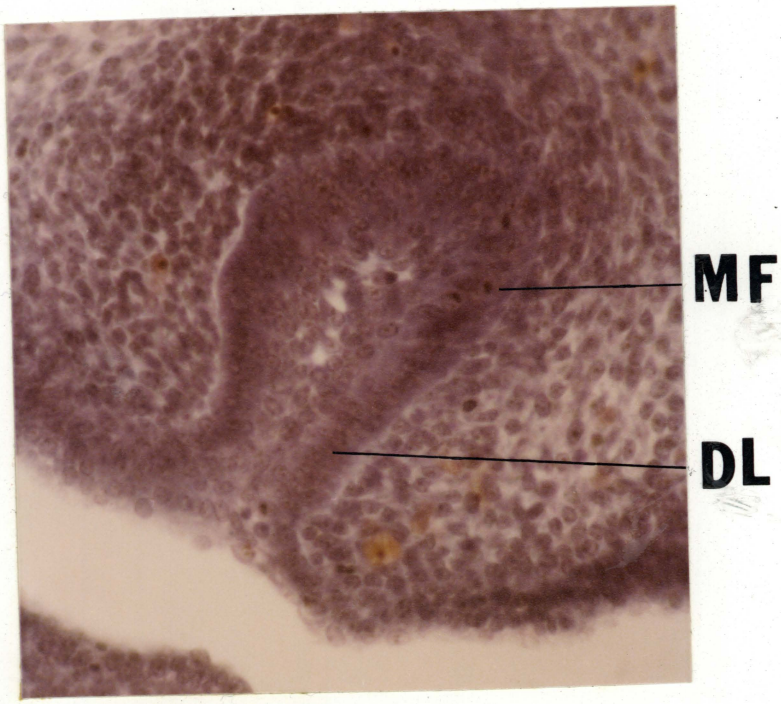


Fig. 7



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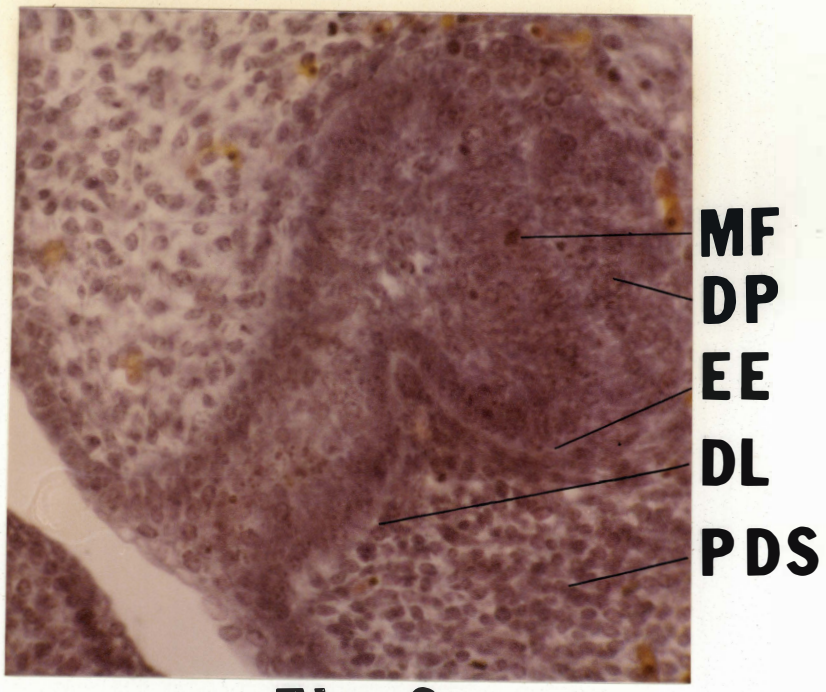

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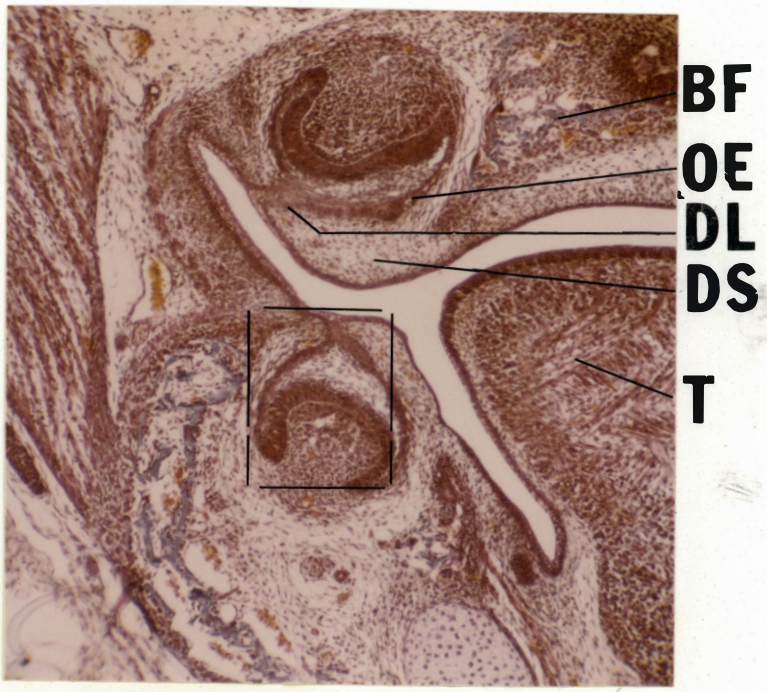

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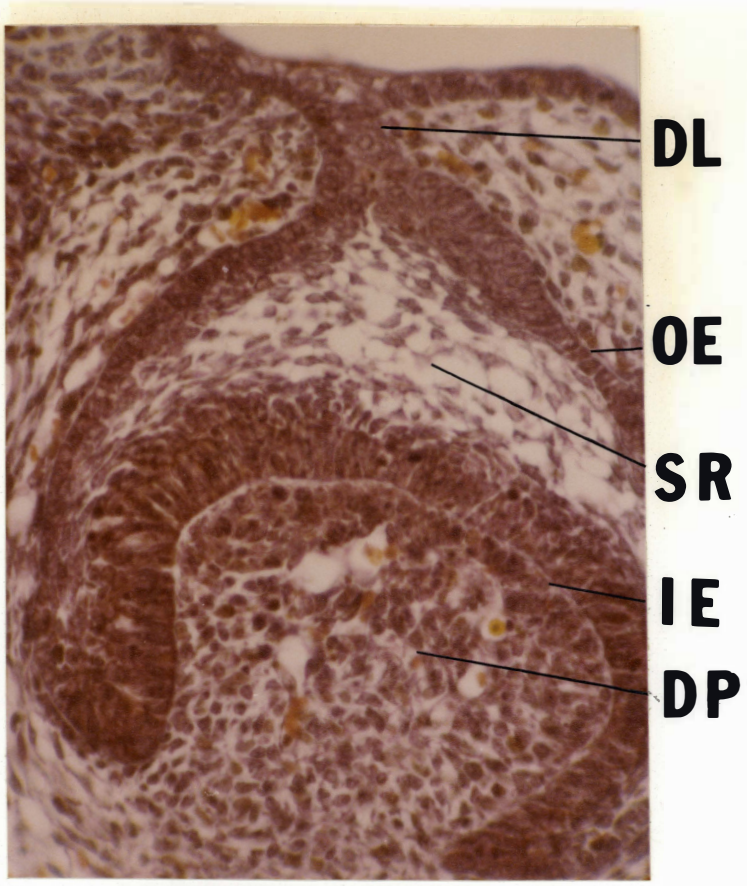

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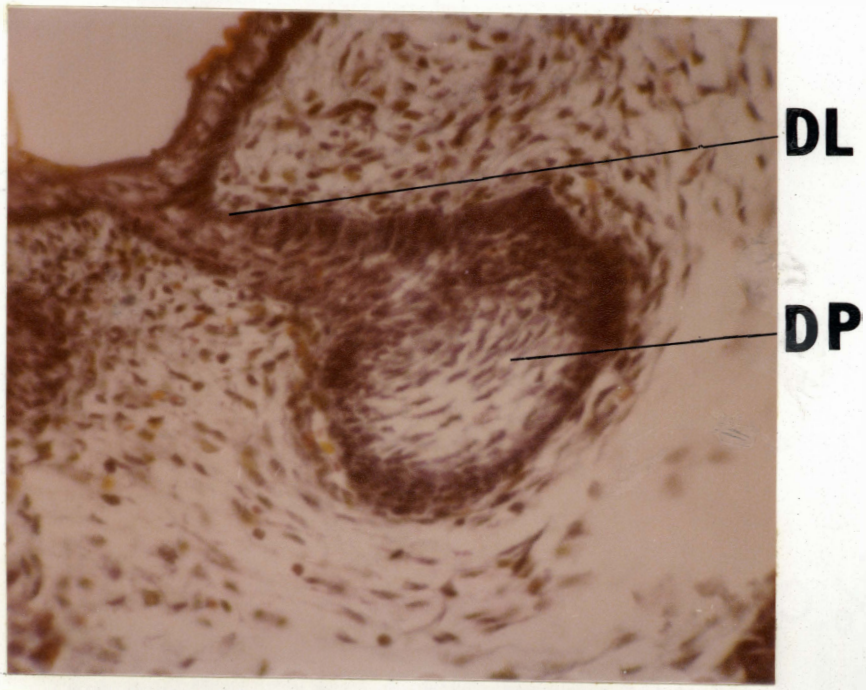

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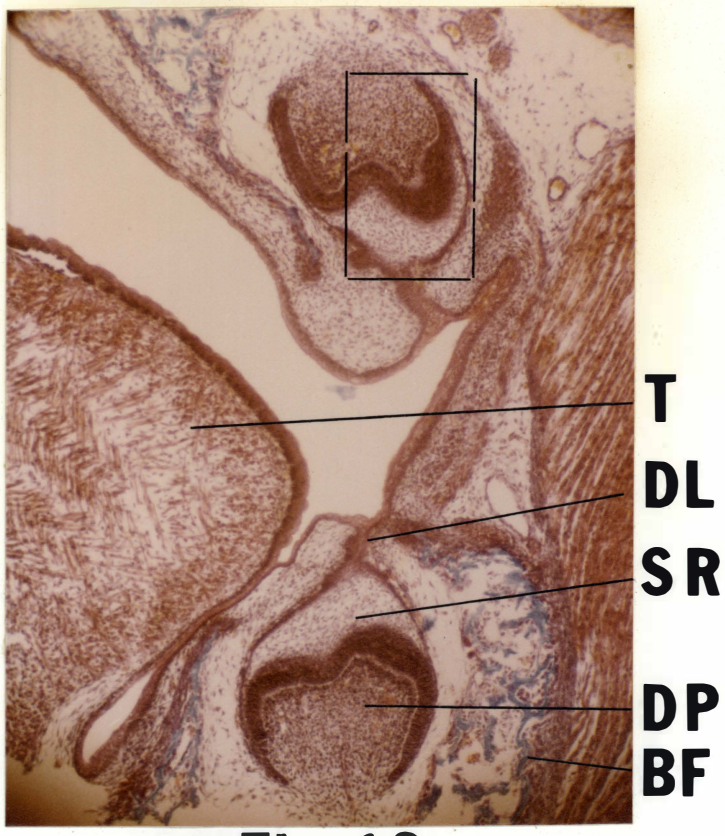

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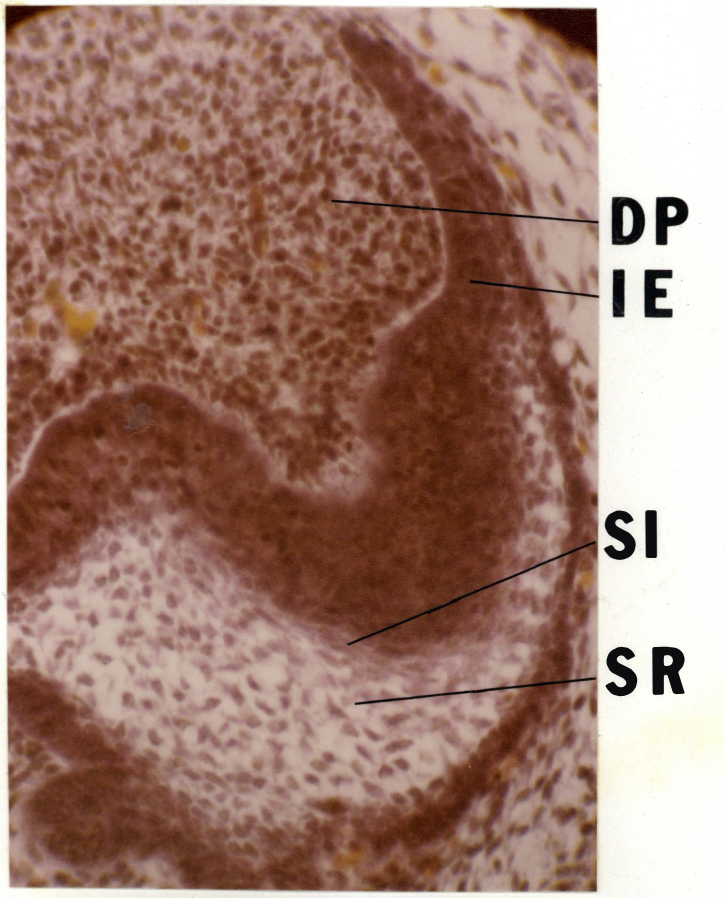

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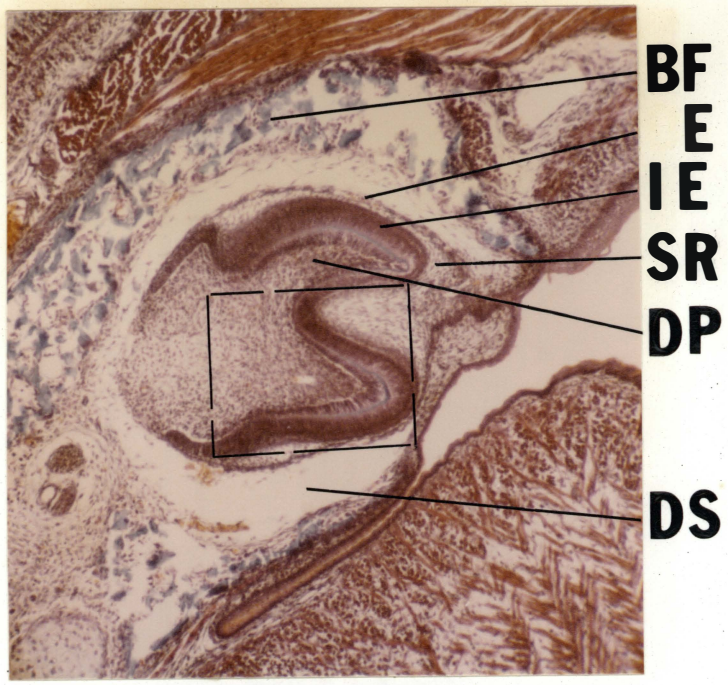

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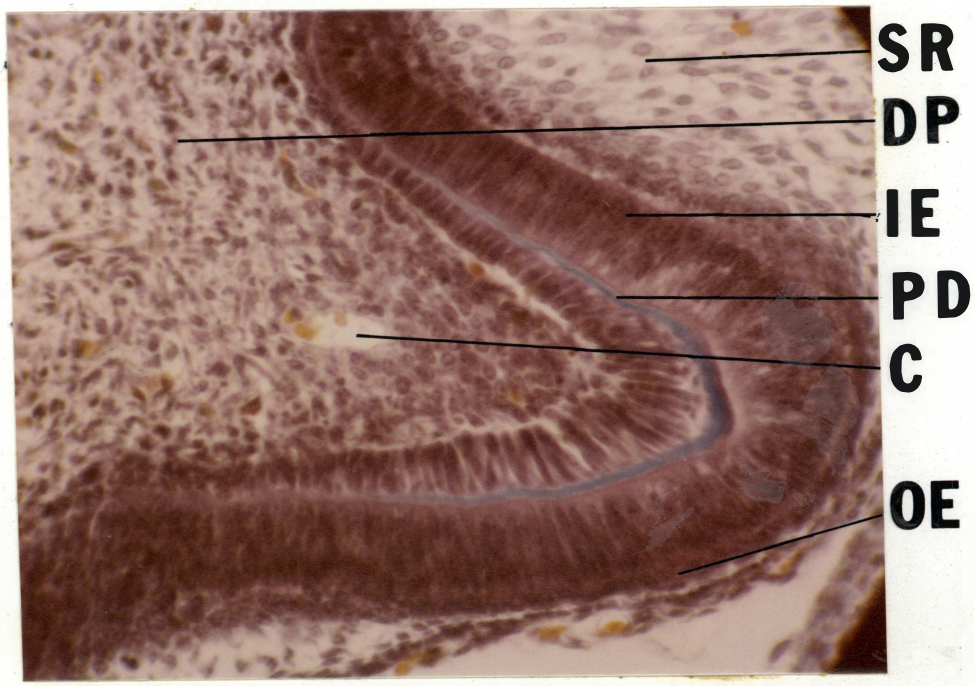

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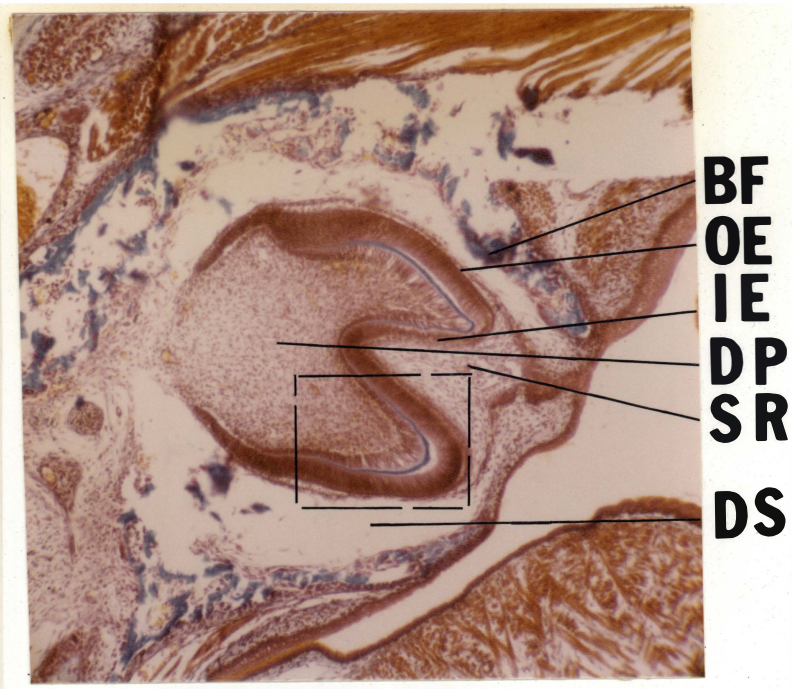

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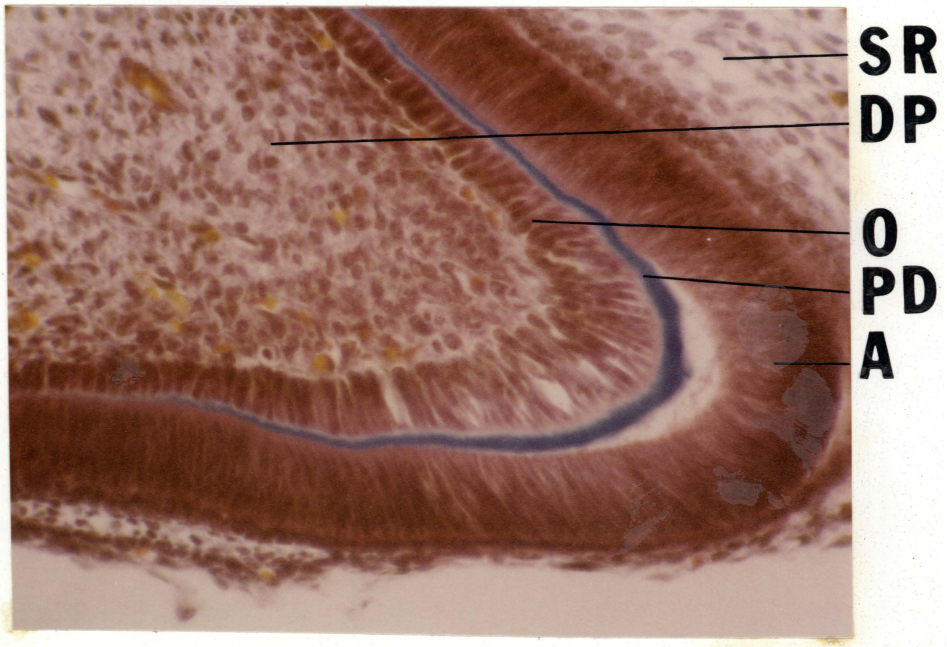


Fig. 18

Figs. 19-30 are sections of Chinese hamster incisors stained with the Lehman's polychrome method.

Fig. 19 - Transverse section through the lower left incisor of a 16 day Chinese hamster embryo. EO, enamel organ; LF, lip furrow band; MC, Meckel's cartilage; T, tongue.

Fig. 20 - Magnification of area indicated in Fig. 19. IE, inner enamel epithelium; OE, outer enamel epithelium; DP, dental papilla; MF, mitotic figures.

Fig. 21 - Transverse section through the lower right incisor of a 17 day Chinese hamster embryo. EO, enamel organ; LF, lip furrow band; MC, Meckel's cartilage; T, tongue.

Fig. 22 - Magnification of area indicated in Fig. 21. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; MF, mitotic figures.

Fig. 23 - Transverse section through the lower right incisor of an 18 day Chinese hamster embryo. EO, enamel organ; LF, lip furrow band; MC, Meckel's cartilage; T, tongue.

Fig. 24 - Magnification of the area indicated in Fig. 23. DP, dental papilla; O, odontoblasts; A, ameloblasts; OE, outer enamel epithelium; PD, predentin; BF, bone formation.

Fig. 25 - Transverse section through the upper left incisor of a 19 day Chinese hamster embryo. EO, enamel organ; MC, Meckel's cartilage; DS, dental sac; BF, bone formation; T, tongue.

Fig. 26 - Magnification of the area indicated in Fig. 25. DP, dental papilla; O, odontoblasts; A, ameloblasts; OE, outer enamel epithelium; PD, predentin; DS, dental sac; BF, bone formation.

Fig. 27 - Transverse section through the lower right incisor of a 20 day Chinese hamster embryo. EO, enamel organ; DS, dental sac; BF, bone formation; T, tongue.

Fig. 28 - Magnification of area indicated in Fig. 27. DP, dental papilla; O, odontoblasts; A, ameloblasts; PD, predentin; DS, dental sac; BF, bone formation.

Fig. 29 - Transverse section through the lower right incisor of a 21 day Chinese hamster embryo. EO, enamel organ; DS, dental sac; BF, bone formation.

Fig. 30 - Magnification of area indicated in Fig. 29. DP, dental papilla; O, odontoblasts; A, ameloblasts; OE, outer enamel epithelium; PD, predentin; DS, dental sac; BF, bone formation.

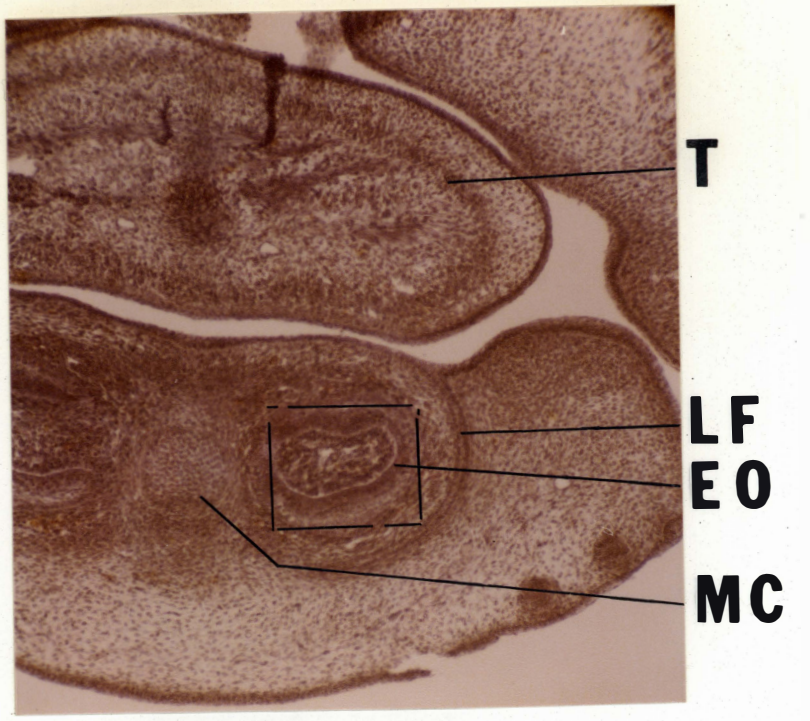
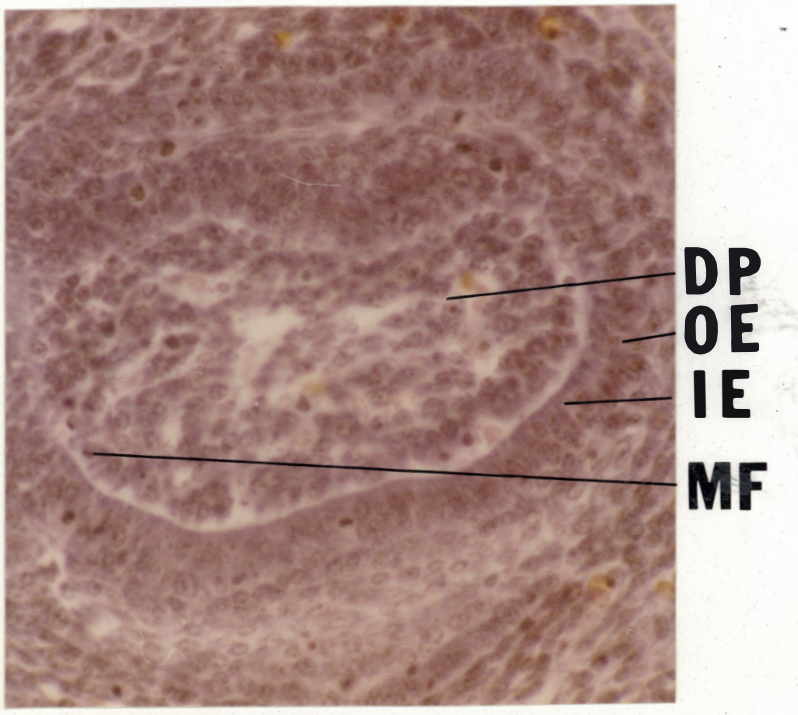


Fig. 19



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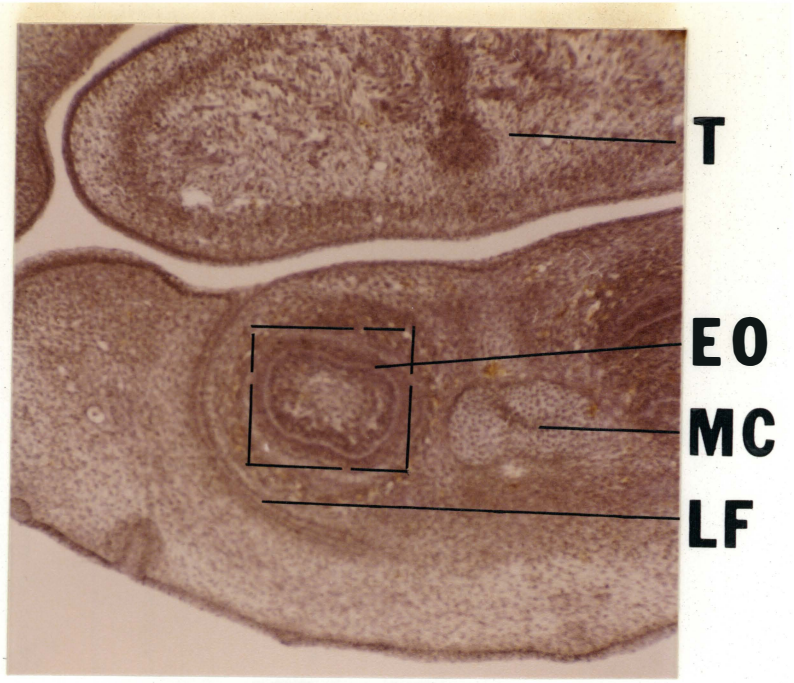
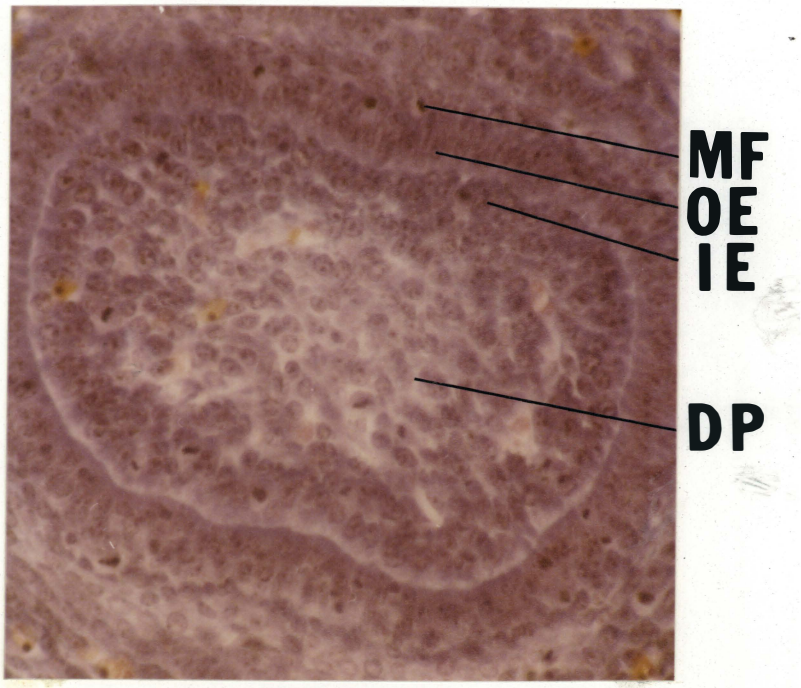


Fig. 21



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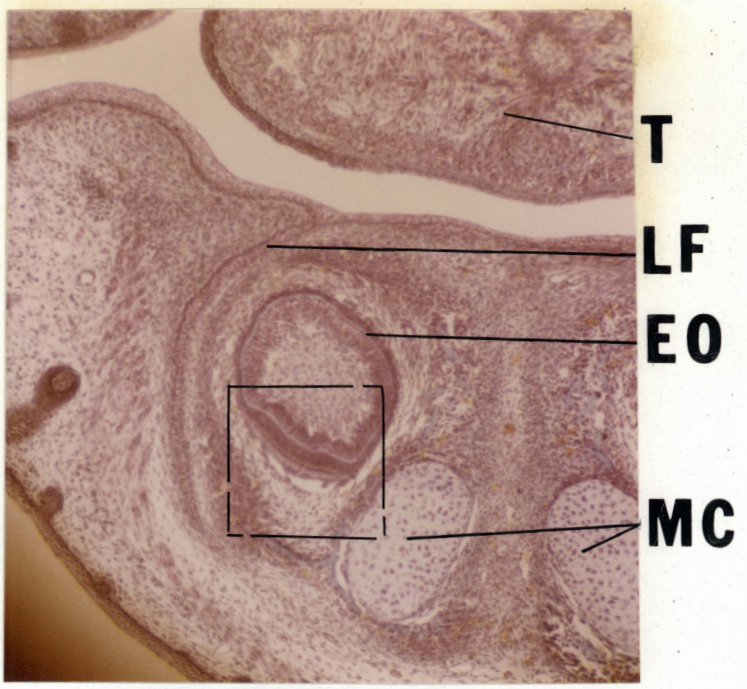


Fig. 23

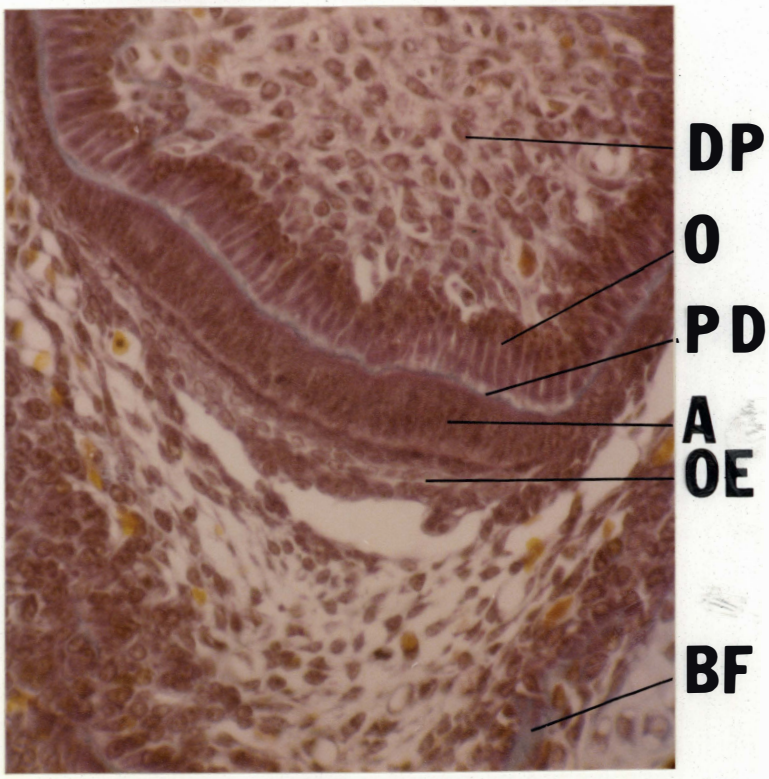


Fig. 24

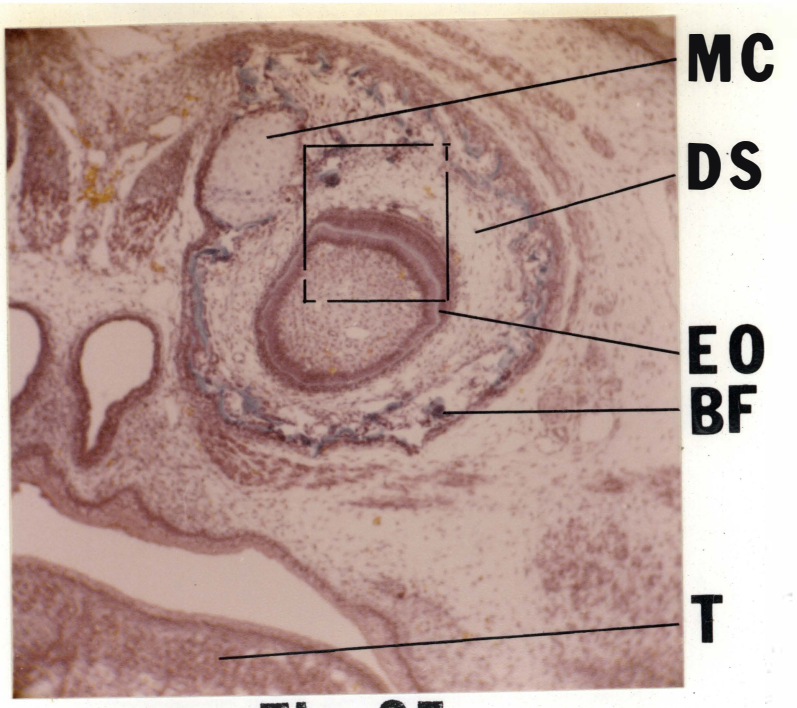
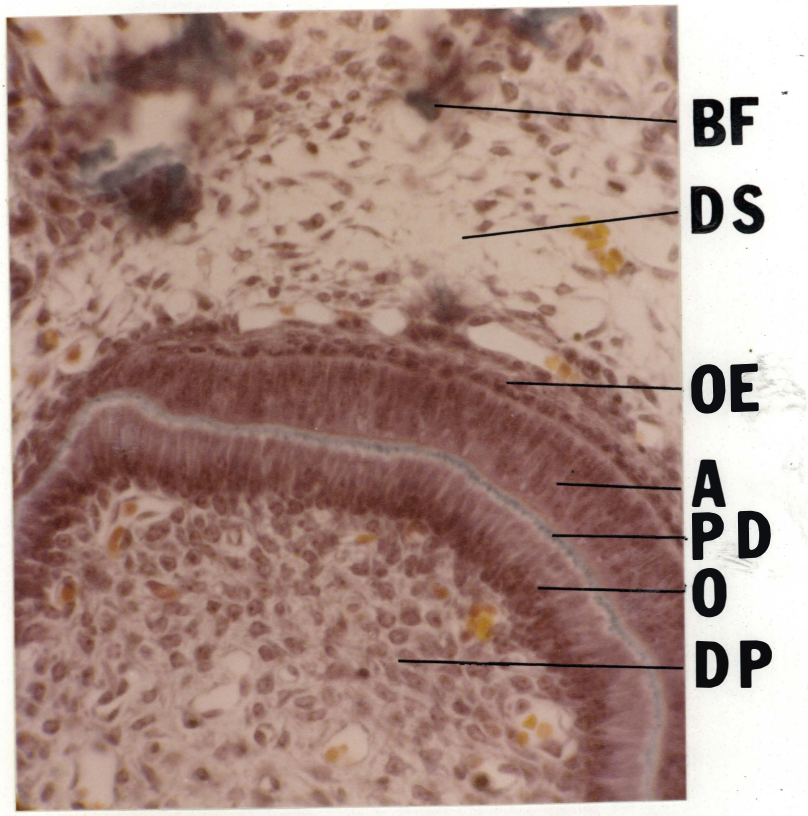


Fig. 25



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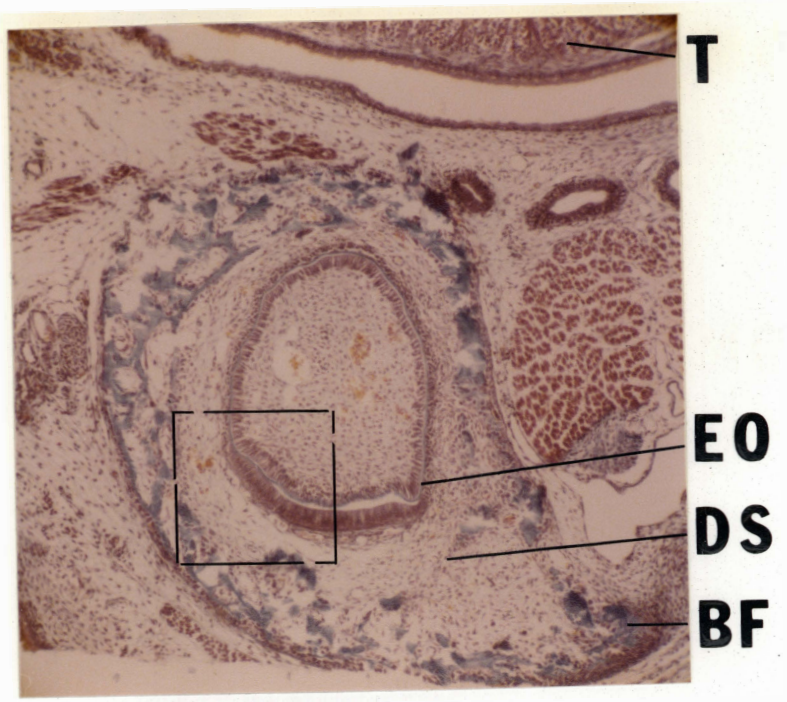

Fig. 27

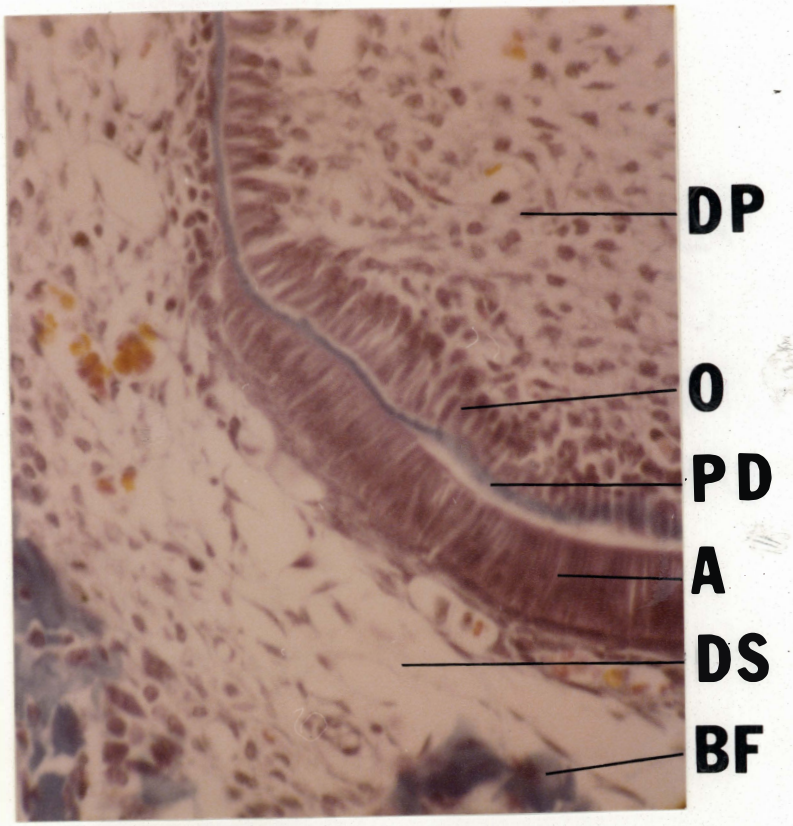

Fig. 28

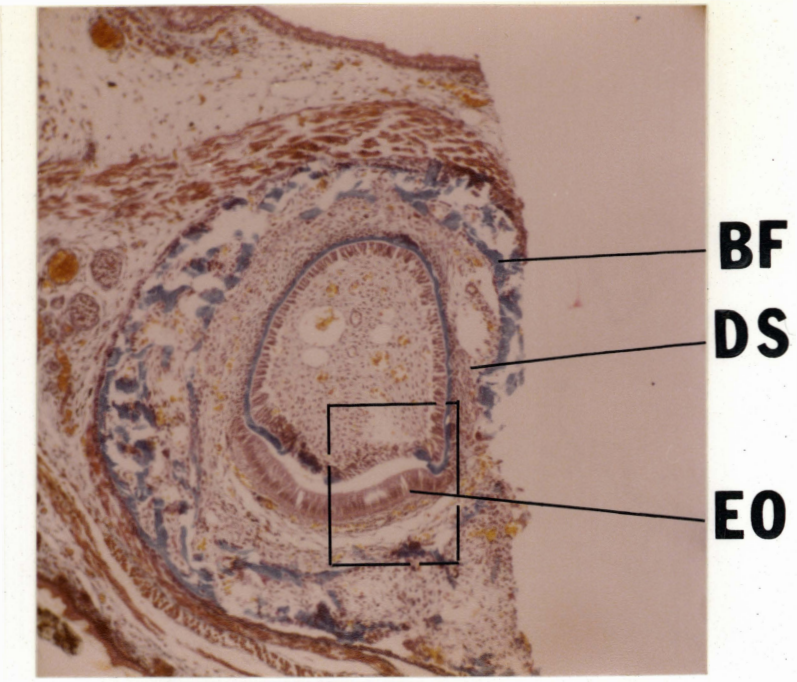

Fig. 29

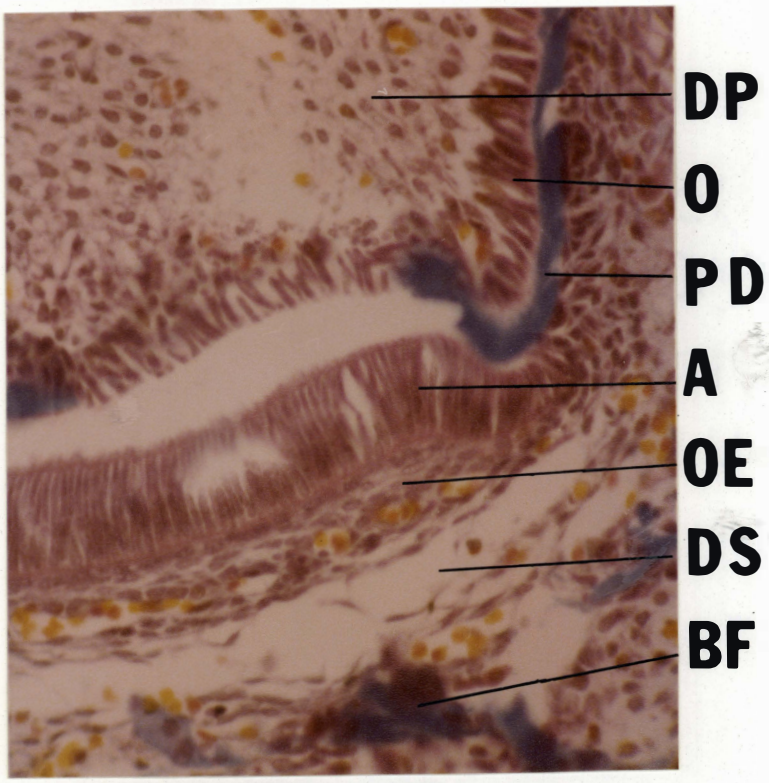

Fig. 30

Figs. 31-46 are sections of Chinese hamster molars stained with one of the following methods: pyronine-methyl green (P/MG), periodic acid-Schiff (PAS), or eosin-hematoxylin.

Fig. 31 - Sagittal section through the jaw of a 14 day Chinese hamster embryo stained with the P/MG method. Proliferation of the oral epithelium denotes the beginning of the dental lamina. DL, dental lamina; OE, oral epithelium.

Fig. 32 - Sagittal section through the jaw of a 14 day Chinese hamster embryo stained with the eosin-hematoxylin method. DL, dental lamina; OE, oral epithelium.

Fig. 33 - Transverse section through the upper left jaw of a 16 day Chinese hamster embryo stained with the P/MG method. DL, dental lamina; EO, enamel organ.

Fig. 34 - Transverse section through the upper right jaw of a 16 day Chinese hamster embryo stained with the PAS method. DL, dental lamina; EO, enamel organ.

Fig. 35 - Transverse section through the upper right jaw of a 16 day Chinese hamster embryo stained with the eosin-hematoxylin method. DL, dental lamina; EO, enamel organ.

Fig. 36 - Transverse section through the upper left jaw of a 17 day Chinese hamster embryo stained with the P/MG method. DL, dental lamina; DP, dental papilla; EE, enamel epithelium.

Fig. 37 - Transverse section through the right jaw of a 17 day Chinese hamster embryo stained with the PAS method. EO, enamel organ.

Fig. 38 - Transverse section through the lower left jaw of an 18 day Chinese hamster embryo stained with the PAS method. EO, enamel organ; MC, Meckel's cartilage; DS, dental sac; BF, bone formation.

Fig. 39 - Transverse section through the right jaw of an 18 day Chinese hamster embryo stained with the eosin-hematoxylin method. EOU, enamel organ (upper); EOL, enamel organ (lower); OC, oral cavity.

Fig. 40 - Transverse section through the right jaw of a 19 day Chinese hamster embryo stained with the PAS method. EOU, enamel organ (upper); EOL, enamel organ (lower); OC, oral cavity; MC, Meckel's cartilage.

Fig. 41 - Sagittal section through the left jaw of a 19 day Chinese hamster embryo stained with the eosin-hematoxylin method. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; SR, stellate reticulum; DL, dental lamina; OC, oral cavity.

Fig. 42 - Transverse section through the lower right jaw of a 20 day Chinese hamster embryo stained with the P/MG method. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; SR, stellate reticulum; DS, dental sac; OC, oral cavity.

Fig. 43 - Magnification of area indicated in Fig. 42. DP, dental papilla; O, odontoblasts; A, ameloblasts.

Fig. 44 - Transverse section of the lower left jaw of a 20 day Chinese hamster embryo stained with the PAS method. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; SR, stellate reticulum; MC, Meckel's cartilage; OC, oral cavity.

Fig. 45 - Transverse section of the lower right jaw of a 21 day Chinese hamster embryo stained with the P/MG method. DP, dental papilla; IE, inner enamel epithelium; SR, stellate reticulum; DS, dental sac; OC, oral cavity.

Fig. 46 - Magnification of area indicated in Fig. 45. DP, dental papilla; O, odontoblasts; A, ameloblasts.

Fig. 47 is a section of mouse molar stained with the Lehman's polychrome method.

Fig. 47 - Transverse section through the upper right jaw of an 18 day mouse embryo stained with the Lehman's polychrome method. DP, dental papilla; IE, inner enamel epithelium; OE, outer enamel epithelium; SR, stellate reticulum; OC, oral cavity; T, tongue; BF, bone formation.

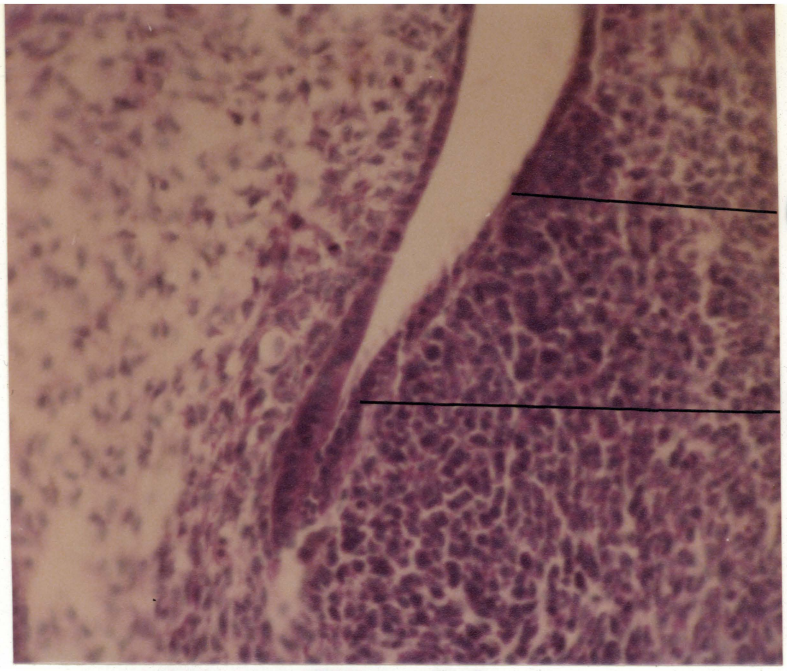

Fig. 31

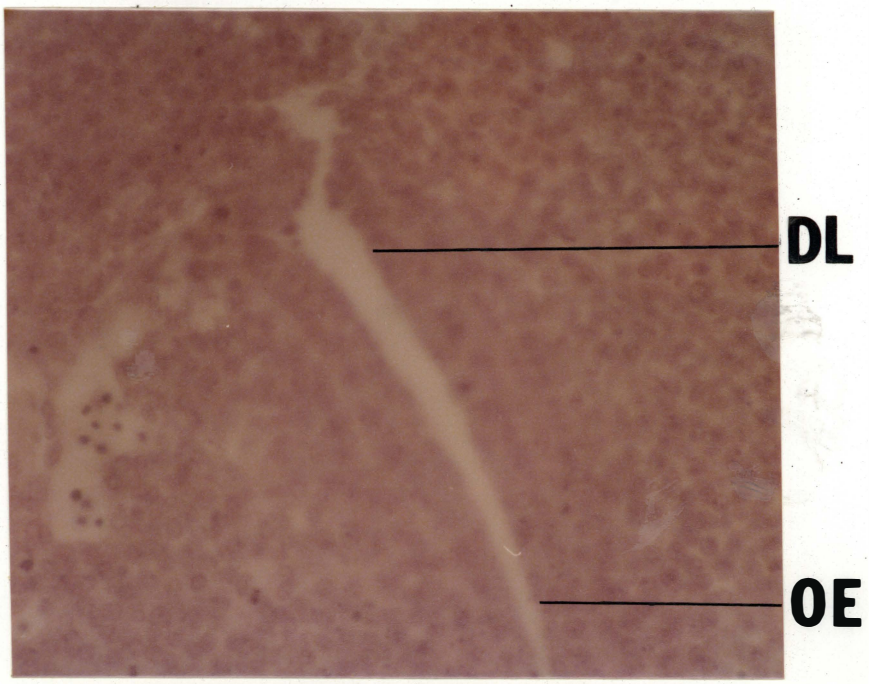

Fig. 32

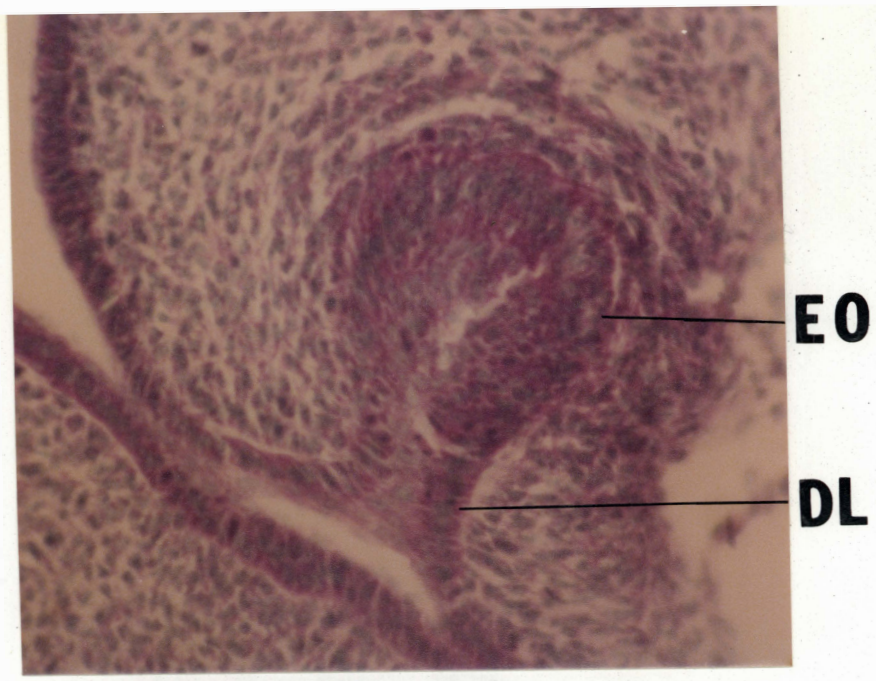

Fig. 33

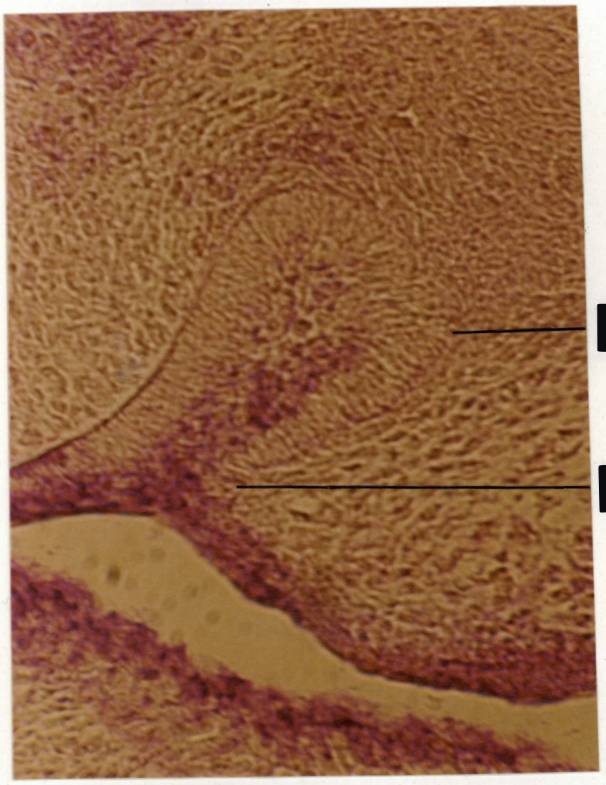

Fig. 34

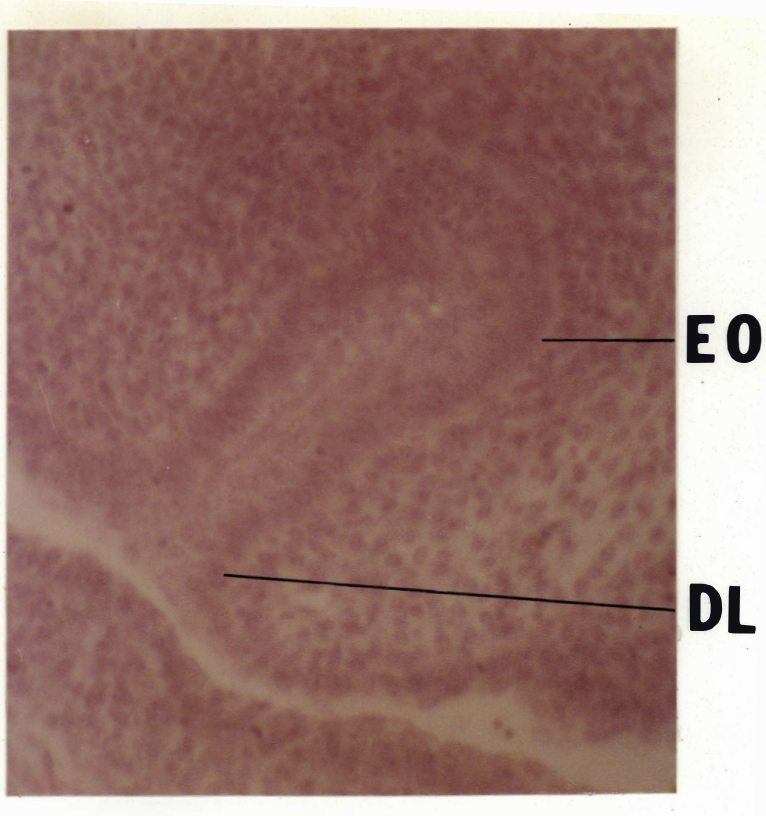

Fig. 35

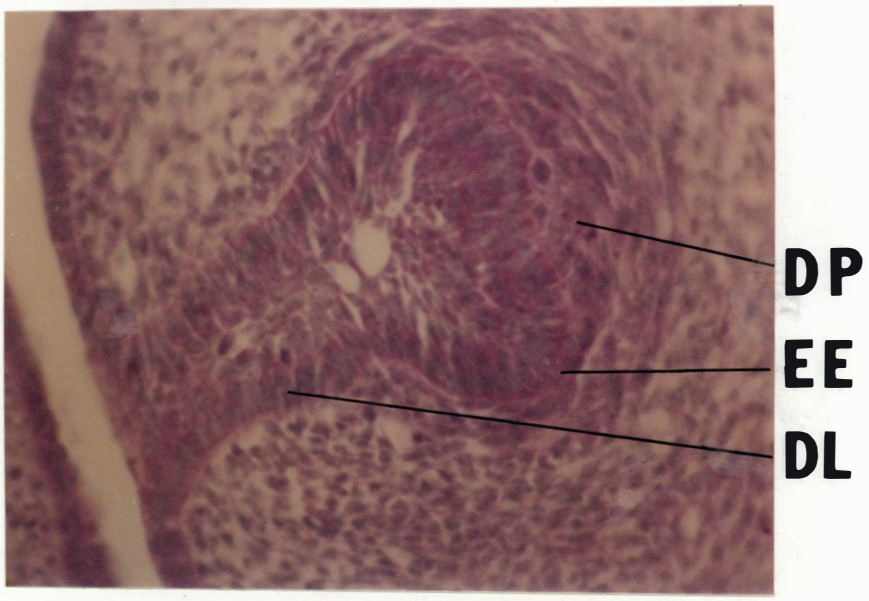

Fig. 36

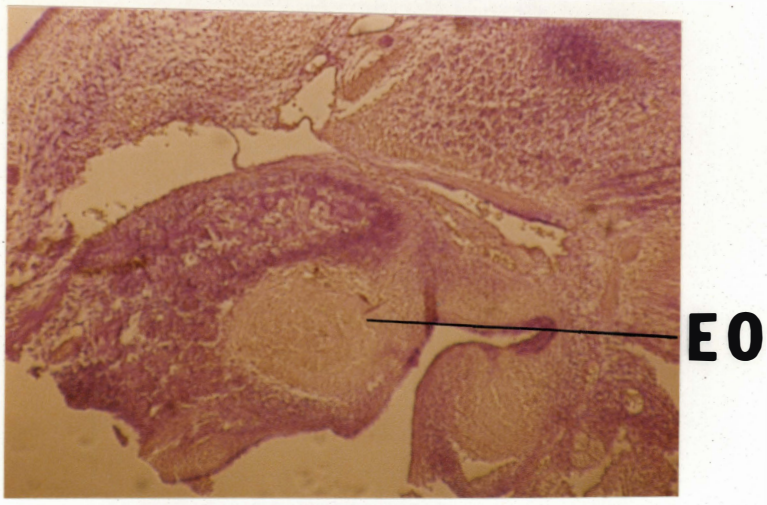

Fig. 37

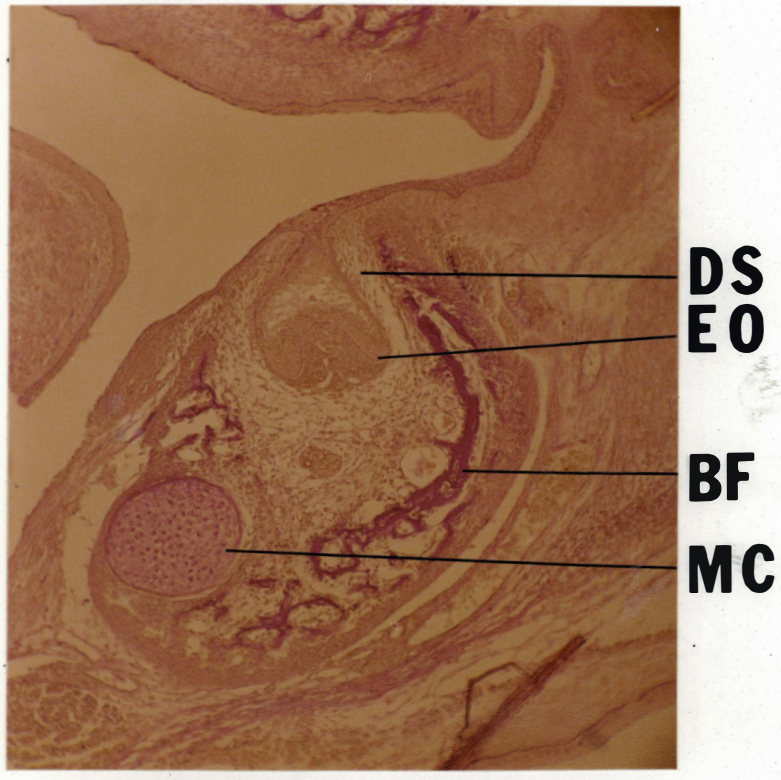

Fig. 38

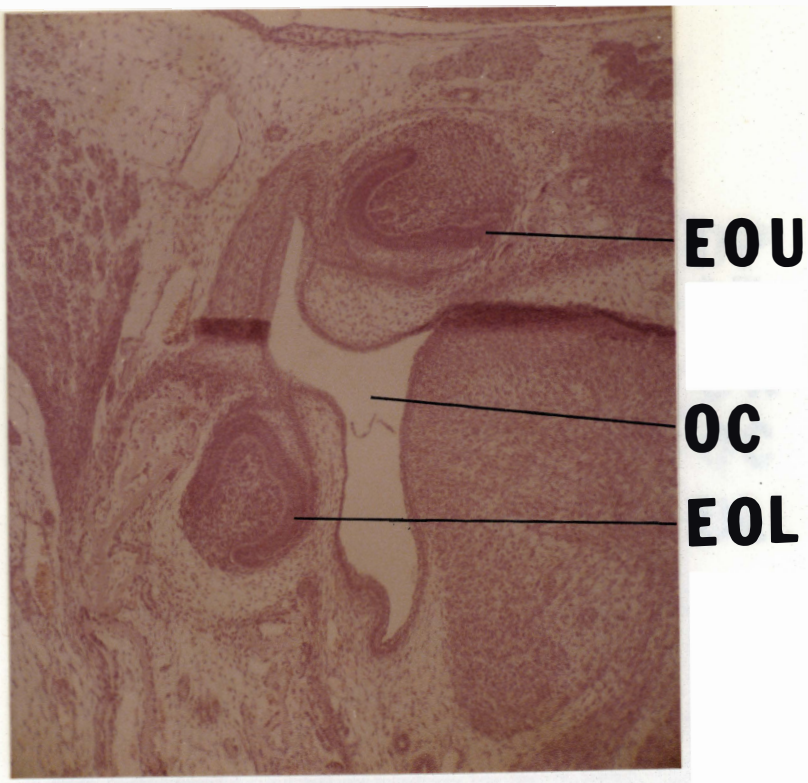

Fig. 39

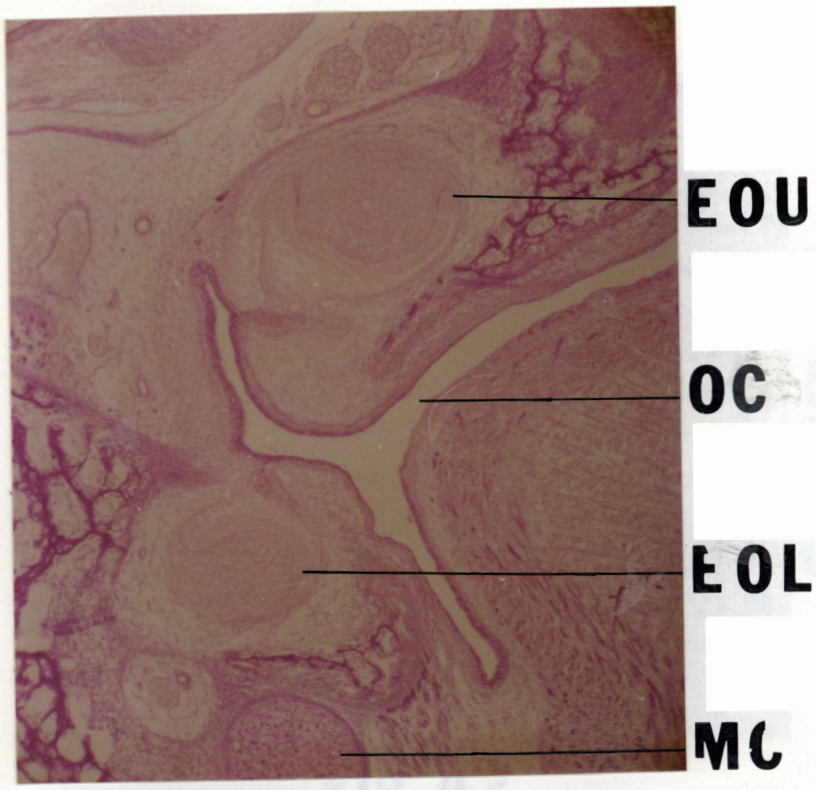

Fig. 40

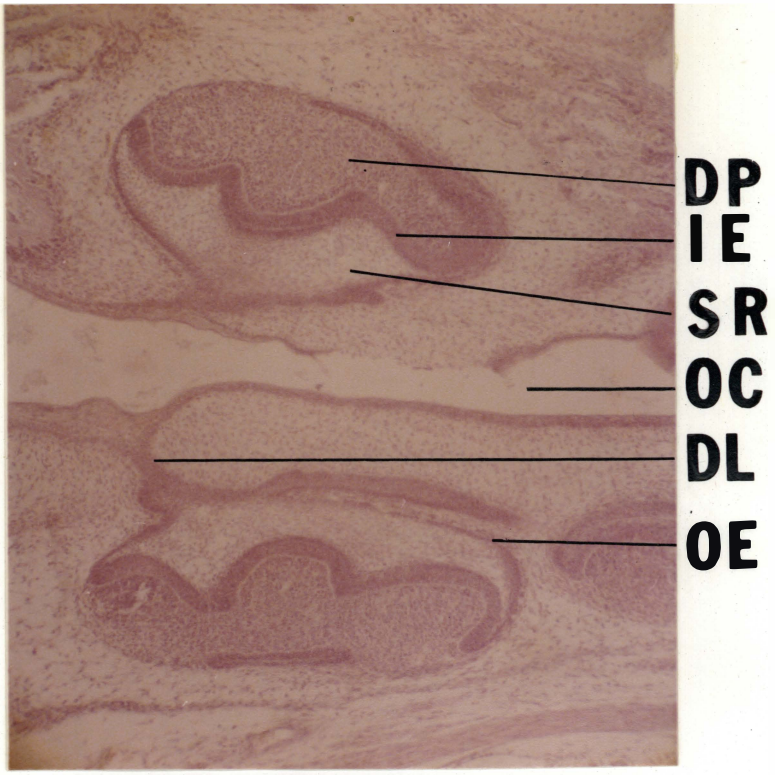

Fig. 41

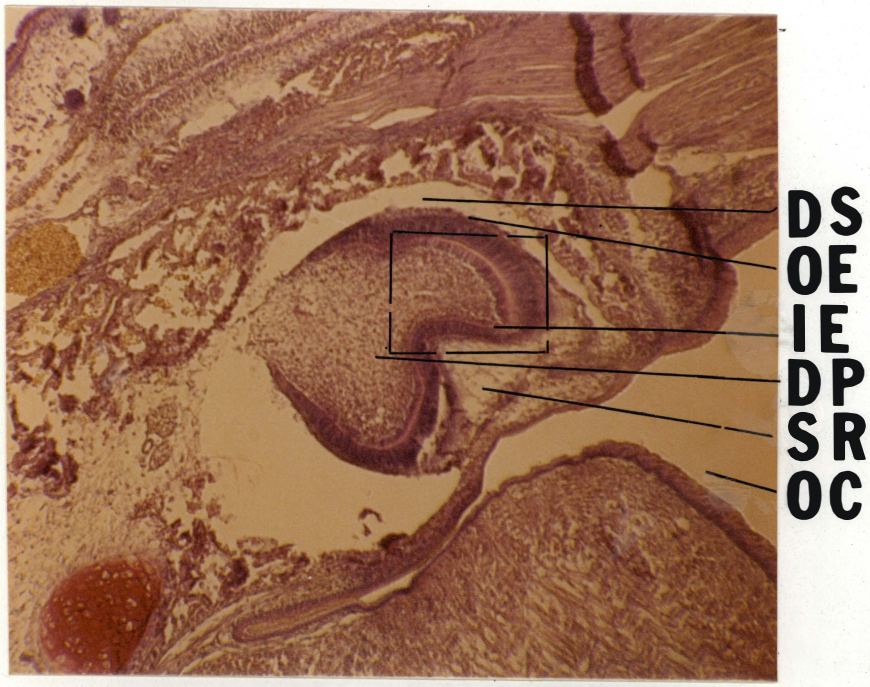

Fig. 42

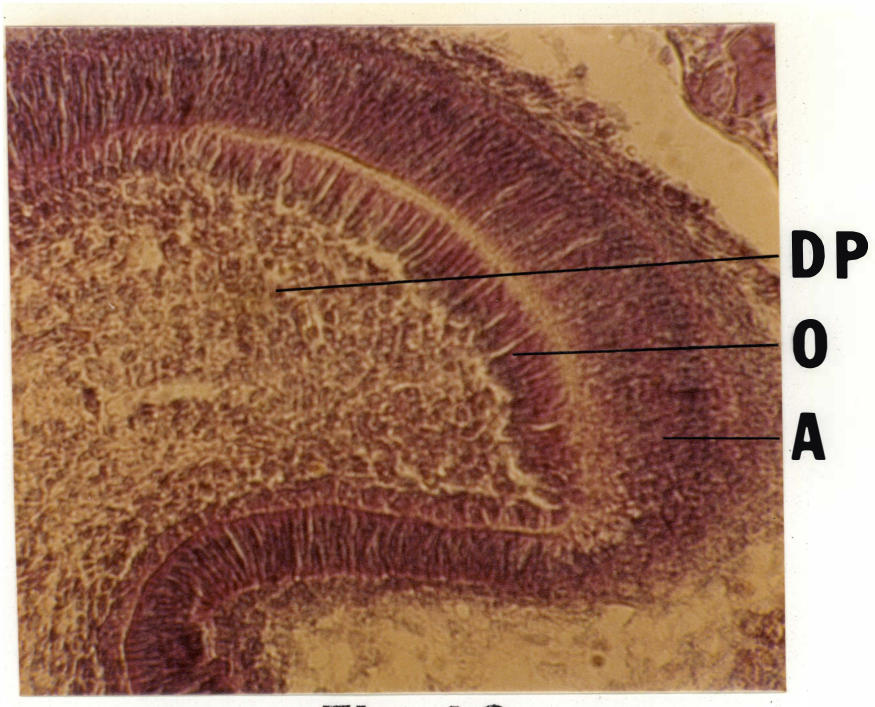

Fig. 43

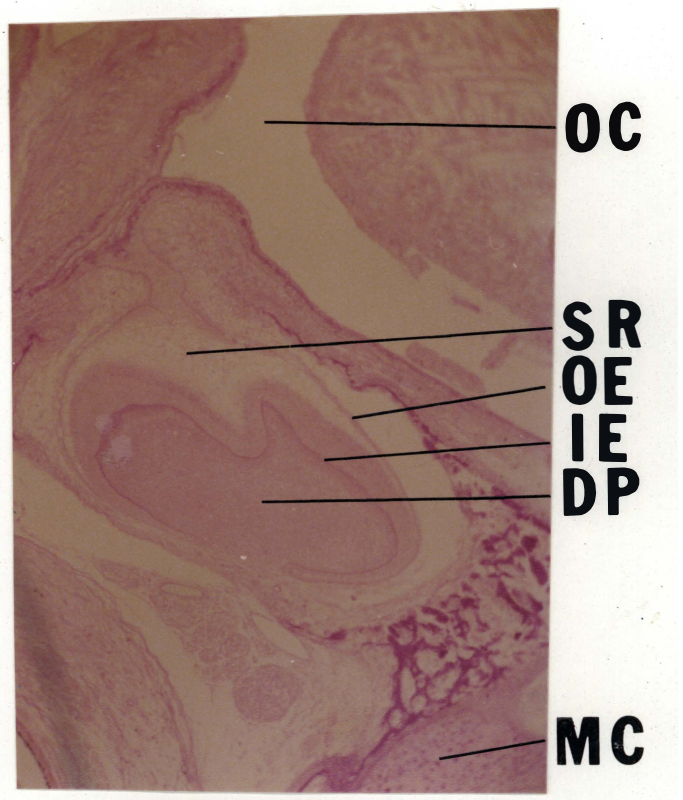

Fig. 44

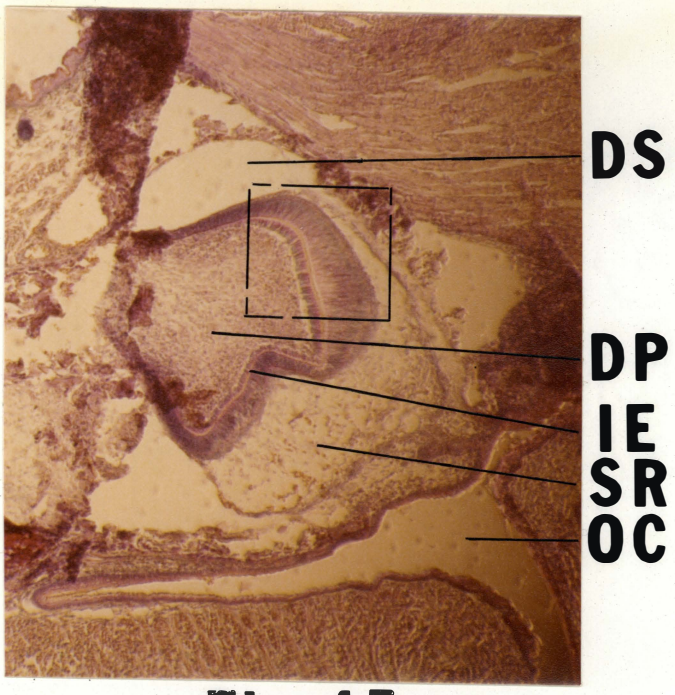

Fig. 45

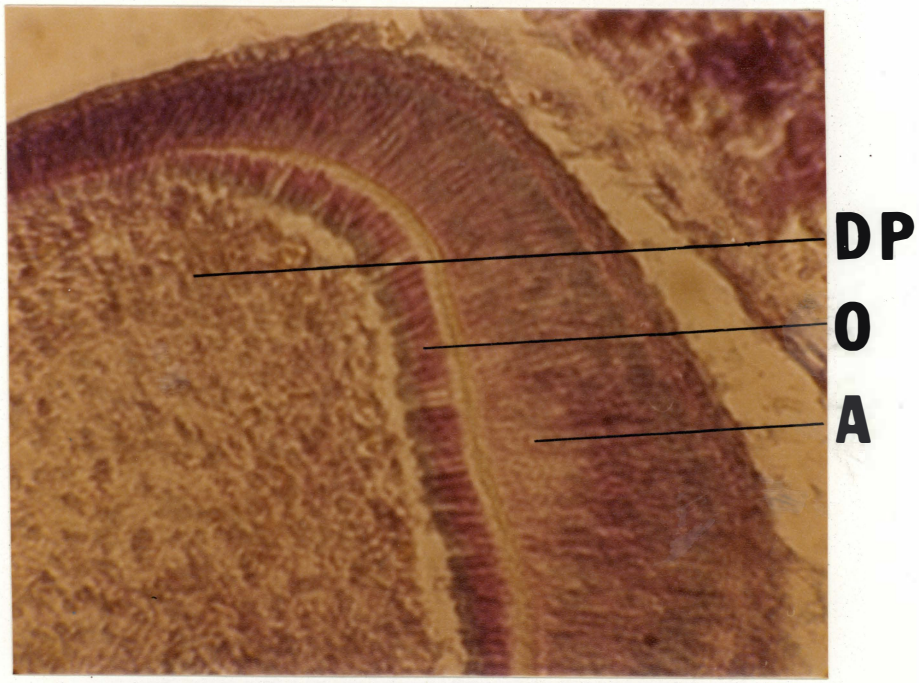

Fig. 46

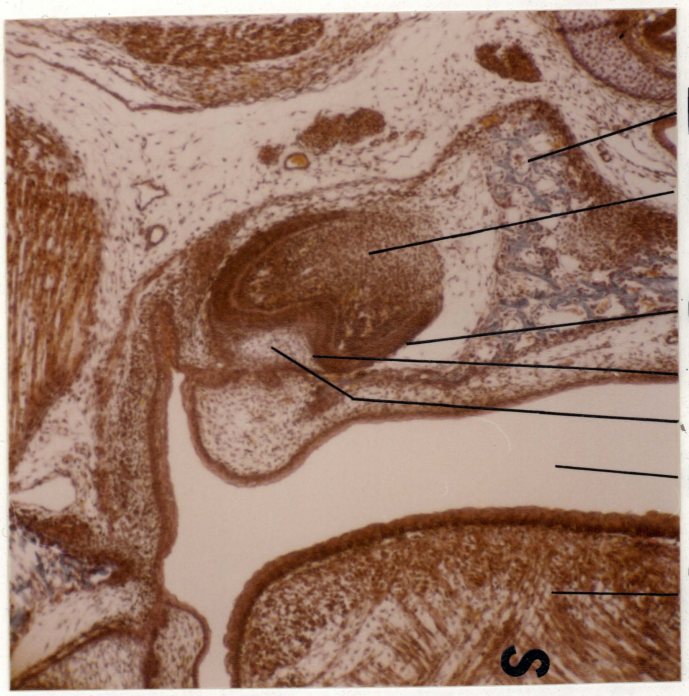

Fig. 47