1962

A Histochemical and Histological Study on the Effect of Cortisone on the Glycogen Content of the Upper Incisor in the Rat Fetus

Bernadette Eileen Ward
Loyola University Chicago

A HISTOCHEMICAL AND HISTOLOGICAL STUDY ON THE EFFECT OF CORTISONE ON THE GLYCOGEN CONTENT OF THE UPPER INCISOR IN THE RAT FETUS

by

Bernadette Eileen Ward

A Thesis Submitted to the Faculty of the Graduate School of Loyola University in Partial Fulfillment of the Requirements for the Degree of Master of Science

February

1962

LIFE

Bernadette E. Ward was born on April 20, 1938, in Plainfield, Illinois.

She was graduated from St. Francis Academy, Joliet, Illinois, June, 1955, and from The College of St. Francis, Joliet, Illinois, May, 1959, with the Degree of Bachelor of Science.

The author began her graduate studies in the Department of Anatomy, Stritch School of Medicine, Loyola University, Chicago, in September, 1959.

ACKNOWLEDGMENT

To Dr. Lincoln V. Domm, Professor of Anatomy, and Chairman, Department of Anatomy, under whose direction this problem was undertaken, I wish to express my sincere thanks for suggesting the problem and for his constructive criticism, unfailing patience and guidance.

I am also grateful to the other faculty members of the Department of Anatomy for the help they gave me.

Gratitude is given to Dr. Hannelore Loevy, Research Associate, and Mrs. Lucia Smelte, Technician, for their cooperation.

Appreciation is also extended to Merck and Company, Inc., Rahway, N. J. for their very generous gifts of cortisone (Cortone Acetate) supplied to Dr. L. V. Domm for use in these studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Many studies have now been reported on the effects of cortisone on the organs, tissues and cells of the body. Investigators have generally found that it has a pronounced effect on growth. The amount of certain enzymes in cells and tissues as well as the time of their appearance were also observed to be affected by cortisone. Reports in the literature on the influence of this steroid on the incisors and associated structures do not appear to be so numerous. It has been observed to accelerate eruption of the incisors and to have an effect on the alkaline phosphatase, ribonucleic acid and glycogen content of these teeth.

The purpose of this study was to investigate the effect of cortisone on the glycogen content of the maxillary incisor in the rat fetus and if possible to determine its role in the mechanism of eruption. The factors to be considered were determination of the period when glycogen first appears, its approximate quantity, its fate, and the layer or layers of the developing incisor in which it occurs.

Several theories have been advanced to explain the mechanism of tooth eruption and numerous papers have been written

concerning the action of cortisone on connective tissue and epithelial cells. In this investigation an attempt was made to use cortisone as a tool to further our information with reference to the factors responsible for eruption and also to advance our knowledge with respect to its action at the cellular level. It was felt that such a study should provide important information on the cellular effects of cortisone and that it might yield new information concerning the factors involved in the eruption of the rat incisor.

REVIEW OF LITERATURE

Several investigators have studied the effect of cortisone on the rate of eruption of the rat incisor. Leroy and Domm (1951) observed that growth of the incisor was modified in newborn, cortisone treated, albino rats. Domm and Marzano (1954) found that the relative growth rates of upper and lower incisors in adult male and female rats showed a constant relationship but that males showed a higher rate of growth of the lowers than the females. The growth rate of the incisors was greatly accelerated in both sexes following cortisone administration. These investigators also observed that hypophysectomized rats showed a deceleration in the incisor growth rate as well as altered relative growth rates and that cortisone administration in such rats, brought about

greatly accelerated rates. Cortisone injected newborn rats revealed a precocious eruption of upper and lower incisors. Garren (1954) also studied the effect of cortisone on the eruption rate in mature male rats. He reported that large doses of this hormone increased the eruption rate up to the end of the second week and that the rate then tapered off. He reasoned that the cause of this tapering off might be that the animals were beginning to suffer the toxic effects of the large doses and consequently the eruption rate did not respond optimally.

Goldsmith and Ross (1956) performed a histological and histochemical study on the effects of cortisone on the lower incisors of fetal rats when the mothers had been treated during pregnancy. They reported that the 18 day fetal rat revealed a precocious development of the lower incisors following such treatment. These investigators also noted that the amount of alkaline phosphatase and ribonucleic acid had increased in the ameloblasts and odontoblasts of the incisors in such fetuses. In addition, the amount of glycogen had increased in the stratum intermedium of the incisor. This condition was more pronounced in the 20 day than in the 18 day fetus. They also observed an increase in the mucopolysaccharide content of the odontoblasts on the labial side of the incisor.

Parmer, Katonah and Angrist (1951) reported studies on

effects of cortisone on the growth and development of newborn rats. They found that cortisone inhibited body growth but accelerated the rate of eruption of the incisors. Domm and Leroy (1955) observed that when cortisone was administered directly to pregnant rats, or to the fetus in utero, the eruption of incisors occurred earlier than in normals. Leroy and Domm (1955) in addition to the above also reported precocious eruption of incisors when cortisone was injected in newborn rats . The effects of cortisone administration in fetal and newborn rats were also studied by Domm and Mar-Yohana (1958). Histological studies on the upper incisor in the postnatally treated rat showed that the width of the ameloblast, enamel and dentine layers was slightly reduced. The pulp was conspicuously hyperemic and the incisors in some of the treated fetuses were larger than in the controls. Histological studies on postnatally treated rats revealed the oral epithelium to be reduced in thickness. Histochemical studies showed a decrease in the glycogen content of the epithelium in the area of the erupting incisors.

Domm and Wellband (1960) and (1961) observed that daily injections of cortisone in adrenalectomized rats produced an acceleration in the eruption rate of the maxillary incisors. The effect of adrenalectomy, and cortical extract or epinephrine administration, on the glycogen content of the rat liver and

lymphoid organs was reported by Strand and Gordon (1950). These authors observed that adrenalectomy depleted the glycogen content of the liver and lymphoid tissues and that it was restored by the administration of adrenal cortical extract or epinephrine.

Timiras and Koch (1952) studied the effect of cortisone on rabbit liver glycogen. They observed that cortisone increased the deposition of liver glycogen and produced cellular hypertrophy. When cortisone and DCA were administered simultaneously there was no increase in liver glycogen. Ross and Goldsmith (1955) investigated the effect of cortisone on the liver, intestine and kidney of fetal rats and observed an increase in the glycogen of the hepatic parenchyma on the 16th day.

Studies have also been carried out on the effect of cortisone on the glycogen content of the liver in adult rats and have shown that it will bring about an increase in the amount of glycogen in the liver. Williams, Davis and Lowe (1956) studied its effect, and that of epinephrine, on hepatic and myocardial glycogen in rats and mice. They found that large doses of cortisone increased the amount of glycogen in the cells of the hepatic parenchyma as well as in the myocardial fibers. The myocardium of control animals was faintly PAS positive while that of cortisone injected animals was intensely positive. Williams, Lowe and Thomas (1953) observed that when cortisone was injected into

rabbits there was an increase in the glycogen of the parenchymal cells of the liver. Nine days following the cessation of cortisone administration the liver cells had decreased in size and there was a decrease in the amount of glycogen in the parenchymal cells. Hess and Shaffran (1953) fasted rats for 24 hours after they had been injected with cortisone and observed an increase in the liver glycogen of such animals. After injecting a 5 mg dose the maximum deposition was observed between 31 to 48 hours later. The maximum deposition of glycogen following a 2.5 mg dose was lower and was reached earlier.

Horowitz (1942) made a histochemical study on the glycogen content of fetal rat heads. He reported that in the 17 day fetus glycogen was heaviest in the dental lamina and adjoining mouth epithelium and that at 20 days it was also found in the dental lamina and dental sac. He found no glycogen in the odontoblasts or dental papilla but observed heavy deposits along the outer edge of the ameloblasts especially on the labial side of the incisor.

Several investigators have made detailed studies on the structure and growth of the rat incisor. Addison and Appleton (1915) concluded that growth was due to a proliferation and growth of cells at the basal end of the enamel organ and at the basal end of the dental papillae. The anlage of the incisor was observed to appear on the 14th day of gestation. At 21 days the

enamel and dentine had begun to form and at 8 to 10 days postnatally the incisors erupted. The lower incisor was always longer than the upper and erupted earlier.

Gulat (1936) reported on the structure of the rat incisor at around 20 days prenatally paying particular attention to the enamel. He studied the fate of the ameloblasts on the surface of the incisor, where no enamel is formed, and the relation of the blood vessels to the anlage. He concluded that enamel formation does not begin until after the blood capillaries have penetrated the stellate reticulum of the incisor as far as the stratum intermedium. According to his observations this process takes place at birth.

MATERIALS AND METHODS

Albino rats* of the Sprague-Dawley strain were used in these experiments. The animals were kept in standard cages in an air-conditioned room where they were fed Dog Chow pellets and tap water ad libitum.

A total of 120 adult, mated, female rats were employed in our study of which 72 were found to be pregnant. Of the latter 36 were treated, and yielded 291 fetuses, while 36 served as controls and yielded 340 fetuses. (Tables I and II)

* Dated mated rats supplied according to our specifications by the Hormone Assay Laboratories, Inc., Chicago, Illinois.

The preparation of cortisone** containing 25 mg per cc was administered by means of a one-half cc capacity hypodermic syringe fitted with a number 21 needle. All injections were subcutaneous and alternated between the right and left flank. The injection site was always sterilized with 80 percent ethyl alcohol prior to injection.

Daily weighings were made from the 6th day of gestation, to and including the day on which the animals were to be sacrificed. Body weights were recorded each morning and the dosage for each day determined by the weight of the animal on that particular day. The total daily dose was divided in half and one part administered in the morning and the other in the afternoon. The dosages employed in our experiments were 50, 75, 100 and 150 mg of cortisone per kg of body weight per day. Controls daily received a comparable volume of the suspending vehicle of cortisone. All injections were begun on the 7th day of gestation.

The rats were sacrificed by ether anesthesia on the 16th, 18 and 20th days of gestation and the fetuses recovered and killed by decapitation. The heads were immediately placed

** Cortisone (Cortone Acetate) generously supplied to Dr. L. V. Domm by Merck, Sharp & Dohme, Division of Merck and Co., Inc., Philadelphia, Pa.

in Rossman's fluid for a fixation period of 18-24 hours. They were then dehydrated, imbedded in paraffin, and the left half of each head serially, sagittally, sectioned at 8 micra.

In order to determine the presence of glycogen in the incisor we employed the periodic acid-Schiff reaction (Lillie, 1954). A PAS control slide was prepared for each animal and this was treated with a 1% malt diastase solution at 37° C for one hour in order to remove all braces of glycogen. Following this procedure the slide was stained for glycogen by the same procedure as that employed for the experimental material. The tissues which contained glycogen stained a deep pink and in the photographs this layer is black.

Comparisons were then made between experimentals and experimental controls, for the 16th, 18th and 20th day fetal incisor, with reference to the amount of glycogen present, the layer containing it, the day on which it first appeared, and the length of time it persisted. (Table III)

EXPERIMENTAL RESULTS

Our investigation was designed to study the effect of various dosages of cortisone in the pregnant rat, om the deposition of glycogen in the maxillary incisor of the fetuses, at 16, 18 and 20 days. The approximate amount of glycogen, if present, in the dental papillae, the odontoblasts, ameloblasts, stratum

intermedium, stellate reticulum, and in the outer enamel epithelium of these incisors was observed and recorded. The stratum intermedium, stellate reticulum, and the outer enamel epithelium form the papillary layer in the 20 day incisor. The effect of cortisone on the size of the maxillary incisor in these fetuses was also observed. Control and treated animals for a given age and a given dosage were compared.

Cortisone treated pregnant rats gained weight at a slower rate than did controls injected with the suspending vehicle (Figures 1, 2 and 3) confirming the observations of Davis and Plotz (1954).

A. SIZE AND DEVELOPMENT OF MAXILLARY INCISOR

A study of the incisors of 16, 18 and 20 day fetuses, whose mothers had received daily injections of 50, 75, 100 and 150 mg of cortisone per kg of body weight, was made in order to determine the effect of cortisone on the size and development of these structures.

In the 16 day fetal incisor the 50 mg dosage of cortisone per day, for 9 days, did not appear to modify the gross size of the tooth, or the size of the cells in the inner and outer enamel epithelium, when compared with controls of the same age (Figures 5 and 6). No modifications were observed in the incisors of fetuses at this age, from mothers injected with 75, 100 or 150

mg of cortisone per kg of body weight, when compared with controls of the same age (Figures 7, 8, 9, 10, 11 and 12).

In 18 day fetuses whose mothers had received the 50 mg dosage of cortisone the labial process of upper incisors was longer and thinner than the lingual process, which was broader and shorter, a condition similar to that observed in controls (Figures 13 and 14). In the experimentals the gross size of the incisor, the size of the dental papillae, and the size of the ameloblasts, was similar to that observed in controls. The results were no different in fetuses where mothers had received the 75 or 100 mg dosages of cortisone (Figures 15, 16, 17 and 18). However, the maxillary incisors of 18 day fetuses, whose mothers had received the 150 mg dosage, appeared to be smaller than controls though the size of the ameloblasts and dental papillae was apparently not affected (Figures 19 and 20).

In 20 day fetuses the ameloblasts on the labial side and the odontoblasts on the labial and lingual sides of incisors were highly differentiated. The size of these cells and the size of the dental papillae were similar in experimental and control fetuses for each of the four dosages employed and the overall size of the upper incisors had not been modified (Compare figures 21, 22, 23, 24, 25, 26, 27 and 28).

B. DENTAL PAPILLAE

A comparison was made of the amount of glycogen deposited

in the dental papillae of the upper incisor in experimental and control fetuses of 16, 18 and 20 days.

Glycogen was not present in the dental papillae of 16 day fetuses from rats receiving the 50, 75, 100 or 150 mg dosages of cortisone nor was it present in any of the controls. It was also not observed in any of the experimentals, regardless of dosage, nor in any of the killed on the 18th or the 20th day of gestation.

C. ODONTOBLASTS

In the 16 day fetal incisor glycogen did not occur in the area of the future odontoblasts regardless of the dosage (50, 75, 100 or 150 mg). The same was also true for the 18 and 20 day experimentals, regardless of the dosage, nor was it observed in the odontoblasts of the controls at any of these ages.

D. AMELOBLASTS

Glycogen was not present in the inner enamel epithelium, the site at which the ameloblasts later develop, in 16, 18 or 20 day fetuses of rats having received the 50, 75, 100 or 150 mg dosage of cortisone. This finding in the experimentals was no different from that observed in the controls.

E. THE LAYERS CONTAINING GLYCOGEN

(OUTER ENAMEL EPITHELIUM, STELLATE RETICULUM, STRATUM INTERMEDIUM AND EPITHELIAL PAPILLAE)

In 16 day fetuses, whose mothers had received the 50 mg

dosage of cortisone for 9 days, the epithelial layer of the outer enamel of maxillary incisors contained a small amount of glycogen (Figures 5 and 6). The same condition was also observed in fetuses whose mothers had received the 75 (Figures 7 and 8), 100 (Figures 9 and 10), and 150 (Figures 11 and 12) mg dosages for the same period of time. The outer enamel epithelium of incisors of experimental fetuses at this age contained approximately the same amount of glycogen regardless of the dosage employed and did not differ significantly from controls.

In 18 day fetuses whose mothers had received the 50 mg dosage glycogen was deposited in the outer enamel epithelium and in the stellate reticulum of the incisor. A moderate amount of glycogen occurred in the outer enamel epithelium on the lingual and on the labial sides. The glycogen deposition was heaviest at the tips. The stellate reticulum contained a moderate amount of glycogen at the tips. All experimentals at this age and dosage revealed conspicuously heavier deposits than did the controls (Figures 13 and 14).

The 18 day fetuses whose mothers had received the 75 mg dosage of cortisone revealed glycogen in the outer enamel epithelium and stellate reticulum. The deposits began below the loop in the outer enamel epithelium on the lingual side and increased distally so that this epithelium contained the greatest

amount at the tips. The amount of glycogen in the outer enamel epithelium on the labial side was similar to that on the lingual side. The stellate reticulum contained a moderate amount of glycogen at the tips. The incisors of the experimentals at this age and dosage also contained more glycogen than did the controls (Figures 15 and 16).

In the 18 day experimentals whose mothers had received the 100 mg dosage of cortisone a heavy deposit of glycogen was present in the outer enamel epithelium at the tips of the incisors. The stellate reticulum also contained a heavy deposit at the tips. A smaller amount was present on the labial and lingual sides in the outer enamel epithelium than was present in the tips. This dosage at this age brought about the greatest deposition of glycogen observed in any of the incisors studied. The incisors of controls contained a noticeably smaller amount (Figures 17 and 18).

In 18 day fetuses whose mothers had received the 150 mg dosage of cortisone a small deposit of glycogen was observed in the stellate reticulum and a larger amount in the outer enamel epithelium around the tip of the incisor. The deposit in these areas was less intense in controls. The experimentals however contained smaller amounts on the labial and lingual sides than did the controls (Figures 19 and 20).

In the 20 day fetuses whose mothers had received the 50 mg dosage of cortisone glycogen was present on the lingual side in the papillary layer. It was also present in moderate amounts on the labial side in the stratum intermedium and around the tip of the incisor. The epithelial papillae contained heavy deposits. The controls were found to contain less glycogen in all of these areas (Figures 21 and 22).

In 20 day fetuses whose mothers had received the 75 mg dosage of cortisone a heavy deposit of glycogen was present in the papillary layer and in the epithelial papillae of the incisor. Small amounts were also present in the stratum intermedium around the tip and on the labial side. The incisors of experimental fetuses in this experiment definitely contained more glycogen than did the controls. (Figures 23 and 24).

In 20 day fetuses whose mothers had received the 100 mg dosage of cortisone glycogen was present, in the papillary layer on the lingual side and in the epithelial papillae, in smaller amounts than were observed in any of these areas in the controls. These observations are at variance with those of Goldsmith and Ross (1956) who noted an increase in the amount of glycogen in the stratum intermedium of lower incisors in the same type of experiment (Figures 25 and 26).

In 20 day fetuses whose mothers had received the 150 mg

dosage of cortisone glycogen was present in the papillary layer in noticeably larger amounts than were observed in these areas in any of the controls (Figures 27 and 28).

DISCUSSION

A decrease in the normal weight gain was observed in pregnant rats injected daily, beginning on the 7th day of gestation, with 50, 75, 100 and 150 mg of cortisone per kg of body weight. These results confirm those of Davis and Plotz (1954) who administered 3.0 mg of cortisone daily, for 16 to 19 days, in pregnant rats and observed an inhibitory effect on the normal weight gain. Novak (1956) recorded weights daily in pregnant rats receiving cortisone in dosages of 2.5, 3.5 and 5.0 mg per day. He also observed that cortisone brought about a decrease in weight gain as well as fluctuations in weight. He reasoned that the lower weight gain was caused by a loss of body fluids by the mother through some as yet unknown mechanism. Similar observations were also made by Perelmuter (1958) who injected pregnant rats daily with 3.5 to 6.5 mg of cortisone for the last 14 days of gestation. During the first week of treatment he observed a considerable loss of body weight which he also attributed to a loss of body fluids by the mother. Ingle and Thorn (1941) reported that cortisone and hydroxycorticosterone caused

an increase in the excretion of sodium chloride and potassium. This resulted in a loss of body weight, because excretion water was removed from the body.

In studying the development and structure of the maxillary incisor in fetal rats we found that the incisors of experimental fetuses did not differ in structural organization from those of controls. Addison and Appleton (1915) reported observations on the development and structure of normal, fetal rat, maxillary incisors for various days of development. They observed thickenings in the oral epithelium of the 14 day fetus in the region of the future incisor. These thickenings were more definite at 15 days and at 16 days they were described as broad masses of cells. In the upper jaw the differentiation of the enamel organ, from the epithelial ingrowth, was not clearly marked. We observed this condition in both control and experimental fetuses at 16 days. In the 18 day maxillary incisor these investigators observed a differentiation between the labial and lingual part of the organ. The labial process was broad and thin and extended more posteriorly than the lingual process. The latter was narrower and thicker. The inner layer of the enamel organ was composed of columnar elements which were similar on both the labial and lingual sides. Columnar odontoblasts were not present in the dental papillae at this age. We observed the

same degree of development in both 18 day experimental and control fetal incisors.

Addison and Appleton (Loc cit) observed that the 19 day maxillary incisor was crescentic in outline. Odontoblasts were differentiating into columnar cells on the labial side of the papillae. The enamel organ had three layers. Next to the inner layer there was a compact arrangement of two or three rows of cells which were destined to develop into the stratum intermedium. This layer was wider at the anterior end where the enamel-organ is continuous with the stalk which connects it to the oral epithelium. After 19 days the structures and layers became more definite and distinct. In our experimental and control incisors we observed the same structures and layers at 20 days as were present in the 19 day incisor but development was more advanced. Our study shows that cortisone did not modify the development, structure, or size of the cells in the maxillary incisor of the fetal rat, but the largest dosage of cortisone administered (150 mg per kg of body weight) did decrease its gross size in the 18 day fetus.

Goldsmith and Ross (1956) observed slight enhancement in the development of lower incisors in 18 day fetuses whose mothers had received daily injections of 75 or 100 mg of cortisone per kg of body weight and the condition was more pronounced in 20 day fetuses. In our experiments no enhancement in the develop-

ment of the upper incisor was observed in 18 or 20 day fetuses regardless of the dosage administered.

Mar-Yohana (1957) injected fetuses in utero on the 18th and 19th days of gestation with 500 gamma of cortisone per day and noted an increase in the length of the upper incisors. She also observed an increase in the size of the dental papillae in 18 day fetuses which had received a single injection of 500 gamma on the 17th day. All treated fetuses showed more prominent papillae than controls. No histological change was observed in the odontoblasts, dentine or enamel as a consequence of such treatment. In our histological study on the size of the maxillary incisor at 18 days we observed no increase in the length of these structures in our experimental fetuses. We did, however, observe a decrease in the size of the maxillary incisor in fetuses at 18 days whose mothers had received the 150 mg dosage of cortisone. In this case the area of the dental papillae was smaller than that of the controls. In all other incisors studied, regardless of the dosage employed, no difference was observed in the size of dental papillae between experimental and control fetuses. Our observations on the absence of histological changes in odontoblasts, dentine and enamel, following cortisone treatment, in general confirm those of Mar-Yohana (1957).

Domm and Mar-Yohana (1958) administered 1 and 2

injections of 500 gamma each to fetuses between the 17th and 20th days of gestation. They noted that the maxillary incisors of prenatals treated in this manner were larger in some of their animals while in our experiments cortisone did not appear to increase the size of these structures. Our technique, however, differed from that employed by these investigators in that they injected the fetus whereas we injected the pregnant rat.

Our observations confirm those of Horowitz (1942) who noted that glycogen was absent in the dental papillae and odontoblasts of 20 day normal fetuses. This investigator observed glycogen in the dental lamina and in the adjoining mouth epithelium. In our experiments we found glycogen to be present in these structures. Horowitz observed glycogen in a heavily stained band along the outer edge of the ameloblasts, particularly on the labial side of the tooth, in the 20 day fetus. In our study on 20 day experimental and control incisors we observed glycogen in the stratum intermedium layer which is along the outer boarder of the ameloblasts.

The results of our experiments differ somewhat from those of Goldsmith and Ross (1956), with respect to the amount of glycogen deposited in the incisor of the fetal rat, though it should be noted that their study was on mandibular whereas ours was on maxillary incisors. These investigators employed

three groups of rats in their experiments. One group was injected with 75 mg of cortisone per kg of body weight, a second with 100 mg of cortisone per kg of body weight and the third served as controls. Injections were begun on the 7th day of pregnancy. In one section of their paper they stated that 18 day control fetuses contained substantial amounts of PAS positive material in the stellate reticulum, particularly the stellate region over the developing tip of the incisor, which contained moderate deposits of glycogen, and in another that the 18 day control fetal lower incisor shows no glycogen in the stellate reticulum.

In our experiments on upper incisors we found that 18 day fetal controls contained a small amount of glycogen in the stellate reticulum in the area of the developing tip. We also observed glycogen in heavy amounts in the outer enamel epithelium of the normal. In the 18 day experimental fetus Goldsmith and Ross (1956) reported an increase in the amount of glycogen in the stratum intermedium of lowers. We did not observe glycogen in this layer of the uppers in any of our experimental 18 day fetuses regardless of the dosage employed. These investigators observed a heavy band of PAS positive material along the outer edge of the ameloblasts, on the labial side of lowers in 20 day control fetuses. Our observations on uppers confirm this finding.

They also reported small amounts of glycogen in the distal cytoplasm of the odontoblasts and in the stratum intermedium of the labial process of lowers whereas we found none in the odontoblasts but did observe small amounts in the stratum intermedium of the labial process and tip of uppers. In 20 day experimental fetuses Goldsmith and Ross observed glycogen in the same layers as in the 20 day controls but in greater quantity. We were able to confirm these observations in 20 day fetuses subjected to a 75 mg dosage. However, we found that fetuses whose mothers had been subjected to a 100 mg dosage revealed a noticeably smaller amount of glycogen than the controls or the fetuses subjected to the 75 mg dosage. Goldsmith and Ross (1956) apparently observed no significant difference in the amount of glycogen deposited in the layers of the lower incisor as a consequence of the difference in dosages administered. In our experiments the incisors of 18 day fetuses subjected to 100 mg of cortisone appeared to contain a heavier deposition of glycogen than those subjected to 75 mg.

Our observations would seem to indicate that cortisone or a metabolite of cortisone crossed the placental barrier to bring about the increase in the amount of glycogen observed in the 18 and 20 day fetal incisor. Similar observations have been made by other investigators. Jones, Lloyd and Whatt (1952)

administered cortisone to pregnant rats and noted a rise in fetal adrenal cholesterol. Fraser (1952) administered cortisone in the mouse, for periods of 4 days at different stages of pregnancy, and observed an increase in the incidence of fetal anomalies. Perelmuter (1958) also discussed this problem. After injecting pregnant rats with cortisone during the last 14 days of gestation he observed an increase in fetal mortality, a growth retarding effect on the fetal ovary and a reduction in fetal body weight on the 18th day of gestation.

Our study on the fetal rat incisor confirms the observations of others that cortisone will increase the amount of glycogen in certain tissues. Thus Timiras and Koch (1952) and Williams, Lowe and Thomas (1953) observed that glycogen had increased in the parenchymal cells of the rabbit liver following cortisone administration. Goldsmith and Ross (1955), Williams, Davis and Lowe (1956) and Hess and Shaffran (1953) rpported that cortisone increased the amount of glycogen in the liver of rats and Williams, Davis and Lowe (1956) that it increased the glycogen in the myocardial fibers of the rat.

Horowitz (1942) concluded that glycogen plays a role in the calcification mechanism since he observed it at the sites of calcification in the bones of fetal rat heads. In our study glycogen was observed in a heavy band along the papillary layer

and in the dental lamina but it was not found in the odontoblasts or the dental papillae. It is conceivable that glycogen may play a role in the calcification of the incisor but that it is broken down too rapidly to be observed, in the odontoblasts of the dental papillae, with the methods employed in our study.

Cortisone apparently had no effect on the size of the individual cells or on the gross size of the incisor at the dosages and under the conditions administered. Its effect on the rate of growth of the fetal incisor remains to be determined.

Glycogen is the form in which animals store carbohydrate. It occurs in various tissues of the body and is usually most abundant in the liver. The fact that liver glycogen is under endocrine regulation is now fairly well established. Epinephrine, a secretion of the adrenal medulla, decreases the amount of glycogen in the liver (Sutherland 1951). Cortisone, a product of the adrenal cortex increases liver glycogen (Timiras and Koch 1952).

Our experiments have shown that cortisone has an effect on the deposition of glycogen in the fetal incisor since moderate doses administered during pregnancy increased the amount observed in the epithelial papillae, the outer enamel epithelium, the stellate reticulum and in the stratum intermedium.

SUMMARY AND CONCLUSIONS

1. Pregnant rats received daily injections of 50, 75, 100 and 150 mg of cortisone per kg of body weight, beginning on day 7, for periods of 9, 17 and 19 days. Fetuses were recovered on the 16th, 18th and 20th days of gestation. A histological and histochemical study was made to determine the effect on the size and on the amount of glycogen in the various layers or tissues of the maxillary incisor.

2. In the 16 and 20 day fetus the development, structure and size of the maxillary incisor were not modified regardless of the amount of cortisone administered.

3. In the 18 day fetus dosages of 50, 75 and 100 mg did not modify the size of the incisor. However, a dosage of 150 mg resulted in incisors which were smaller than normals but the stage of development was no different from that of normals or of incisors from 18 day fetuses subjected to 50, 75 or 100 mg dosages.

4. Glycogen did not occur in the dental papillae regardless of the age or the amount of cortisone injected nor was it observed in the dental papillae of controls.

5. Neither the odontoblasts nor the ameloblasts of

experimental or control fetuses contained glycogen at any of the ages or dosages studied.

6. Experimental fetuses at 16 days revealed the same amount of glycogen in the outer enamel as did controls regardless of the dosage administered.

7. Fetuses at 18 days subjected to 50 or 75 mg dosages, contained heavier deposits in the outer enamel epithelium and stellate reticulum than did the controls while those subjected to 100 mg contained the heaviest deposits. However, those subjected to 150 mg contained less glycogen in these areas than did their respective controls or the fetuses subjected to any of the other dosages employed.

8. Heavy deposits occurred in the papillary layer and in the epithelial papillae of 20 day fetuses subjected to 50 and 75 mg dosages while these areas in those subjected to 150 mg contained less and those exposed to 100 mg the least. The amount of glycogen in the latter was less than that observed in controls whereas those exposed to the 50, 75 and 150 mg dosages contained more than their respective controls.

9. It is concluded that, glycogen plays a role in the calcification of the maxillary incisor of the rat fetus, since it has been shown by others to occur in other calcification centers

of the body, and that cortisone is concerned in the regulation of the amount deposited in this incisor.

LITERATURE CITED

Addison, W. H. F. and J. L. Appleton 1915 The structure and growth of the incisor teeth of the albino rat. J. Morph., 26: 43-95.

Davis, E. M. and E. J. Plotz 1954 The effects of cortisone acetate on intact and adrenalectomized rats during pregnancy. Endocrinol., 54: 384-395.

Domm, L. V. and R. Marzano 1954 Observations on the effect of certain hormones on the growth rate of the incisors of the albino rat. Anat. Rec., 118: (2): 383-384.

Domm, L. V. and P. Leroy 1955 Nouvelles observations sur l' action de la cortisone injectee a des rats pendant la gestation. Compt. rend. Acad. Sc., 241: 1514-1516.

Domm, L. V. and G. Mar-Yohana 1958 Histological and histochemical effects of cortisone on incisors and associated structures in the albino rat. XVth Internat. Cong. of Zool., Sect. VI, Paper 31.

Domm, L. V. and W. A. Wellband 1960 Effect of adrenalectomy and cortisone on eruption rate of incisors in young female albino rats. Proc. Soc. Exp. Biol. and Med., 104: 582-584.

_____ 1961 Effects of adrenalectomy, thyroidectomy, thyroadrenalectomy and cortisone on eruption rate of incisors in adult female rats. Proc. Soc. Exp. Biol. and Med., 107: 268-272.

Fraser, E. C. 1952 Experimental production of congenital anomalies with cortisone. J. Am. Med. Assoc., 148: 206. (Editorial)

Garren, L. F. 1954 The effect of hormones on the eruption rate of the rat incisor. Harvard Dent. Alumni Bull., 14: 3-6.

Goldsmith, E. D. and L. Ross 1956 A histochemical and histological study of the effects of cortisone on the lower incisors of fetal and post-natal rats. Acta. Endocrinol., 22 (1): 23-41.

Gulat, K. 1936 The development of the incisor teeth of rodents with special reference to the capillaries. Morph. Jahrb., 77: 382-398.

Hess, W. C. and I. P. Shaffran 1953 Influence of cortisone on rate of absorption and formation of liver glycogen by alanine. Proc. Soc. Exp. Biol. and Med., 86: 287-289.

Horowitz, N. H. 1942 Histochemical study of phosphatase and glycogen in fetal heads. J. Dent. Res., 21: 519-527.

Ingle, D. J. and G. W. Thorn 1941 A comparison of the effects of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone in partially depancreatized rats. Am. J. Physiol., 132: 670-678.

Jones, J. M., C. W. Lloyd and T. C. Whatt 1952 A study of the interrelationships of maternal and fetal adrenal glands of rats. Endocrinol., 53: 182-191.

Leroy, P. and L. V. Domm 1951 Observations on the effects of daily administration of cortisone in young white rats. J. Dent. Res., 30 (4): 488.

_____ 1955 Eruption precoce des incisiver superieures et inferieures des jeunes rats traites a la cortisone. Les Annales d'Endocrinol., 16: 773-784.

Lillie, R. D. 1954 Histopathologic technic and practical histochemistry. The Blakiston Company, Inc., New York, N. Y.

Mar-Yohana, G. 1957 Histological effects of cortisone on the maxillary incisors and associated tissues in the albino rat. Unpublished Master's thesis, Loyola University, Chicago, Illinois.

Novak, R., 1956 Some effects of cortisone on the mother and on the fetus when administered during pregnancy in the albino rat. Unpublished Master's thesis, Loyola University, Chicago, Illinois.

Parmer, G. L., F. Katonah and A. A. Angrist 1951 Comparative effects of ACTH, cortisone, corticosterone, desoxycorticosterone, pregnenolone on growth and development of infant rats. Proc. Soc. Exp. Biol. and Med., 77: 215-218.

Perelmuter, S. 1958 An experimental study on the influence of cortisone on pregnancy in the albino rat. Unpublished Master's thesis, Loyola University, Chicago, Illinois.

Ross, L. and E. D. Goldsmith 1955 Histochemical studies of effects of cortisone on fetal and newborn rats. Proc. Soc. Exp. Biol. and Med., 90: 50-55.

Strand, F. L. and H. S. Gordon 1950 A relation of the adrenal to glycogen content and respiration of lymphoid organs. Proc. Soc. Exp. Biol. and Med., 75: 555-559.

Sutherland, E. W. 1951 The effect of the hyperglycemic factor and epinephrine on enzyme systems of liver and muscle. Ann. N. Y. Acad. Sci., 54: 693-706.

Timiras, P. S. and P. Koch 1952 Morphological and chemical changes elicited in the liver of the rabbit by cortisone and desoxycorticosterone acetate. Anat. Rec., 113: 349-363.

Williams, W. L., C. U. Lowe and L. Thomas 1953 The effects of cortisone upon the parenchymal cells of the rabbit liver. Anat. Rec., 115: 247-263.

Williams, W. L., R. L. Davis and C. U. Lowe 1956 Effects of cortisone and epinephrine on hepatic and myocardial glycogen of mice and rats. Anat. Rec., 126: 43-59.

TABLE I

NUMBER OF ANIMALS IN EXPERIMENT

MATED	PREGNANT	NON-PREGNANT	DOSAGE* mg	NO. TREATED	NO. CONTROLS
120	72	48	50	5	5
			75	15	13
			100	13	14
			150	3	4
	TOTALS			36	36

* mg of cortisone per kg of body weight

TABLE II

NUMBER OF FETUSES RECOVERED

DOSAGE*	14 DAYS		18 DAYS		20 Days	
mg	No. Treated	No. Controls	No. Treated	No. Controls	No. Treated	No. Controls
50	16	16	9	14	10	11
75	27	43	36	24	64	45
100	32	30	24	65	43	46
150	10	12	9	11	11	23
TOTALS	85	101	78	114	128	125

* mg of cortisone per kg of body weight

TABLE III

GLYCOGEN CONTENT OF MAXILLARY INCISORS

AGE DAYS	LAYER	DOSAGE*							
		50mg		75mg		100mg		150mg	
		T	C	T	C	T	C	T	C
16	OEE	++	++	++	++	++	++	++	++
18	OEE (Tip)	++++	+++	++++	+++	+++++	+++	++++	+++
	OEE (Li.S.)	+++	++	+++	++	+++	++	+	++
	OEE (La.S.)	+++	++	+++	++	+++	++	+	++
	SR	+++	++	+++	++	++++	++	++	++
20	PL (Li.S.)	++++	+++	++++	+++	++	+++	++++	+++
	EP	++++	+++	++++	+++	++	+++	++++	+++
	SI (La.S. Tip)	+++	++	+++	++	+	++	+++	++

*mg of cortisone per kg of body weight

Abbreviations:

OEE - Outer Enamel Epithelium
OEE (Li.S.) - Outer Enamel Epithelium - Lingual Side
OEE (La.S.) - Outer Enamel Epithelium - Labial Side
SR - Stellate Reticulum
PL (Li.S.) - Papillary Layer - Lingual Side
EP - Epithelial Papillae
SI -(La.S., Tip) - Stratum Intermedium - Labial Side and Tip

Scale of Amounts:

+++++ Very heavy
++++ Heavy
+++ Moderate
++ Small
+ Traces

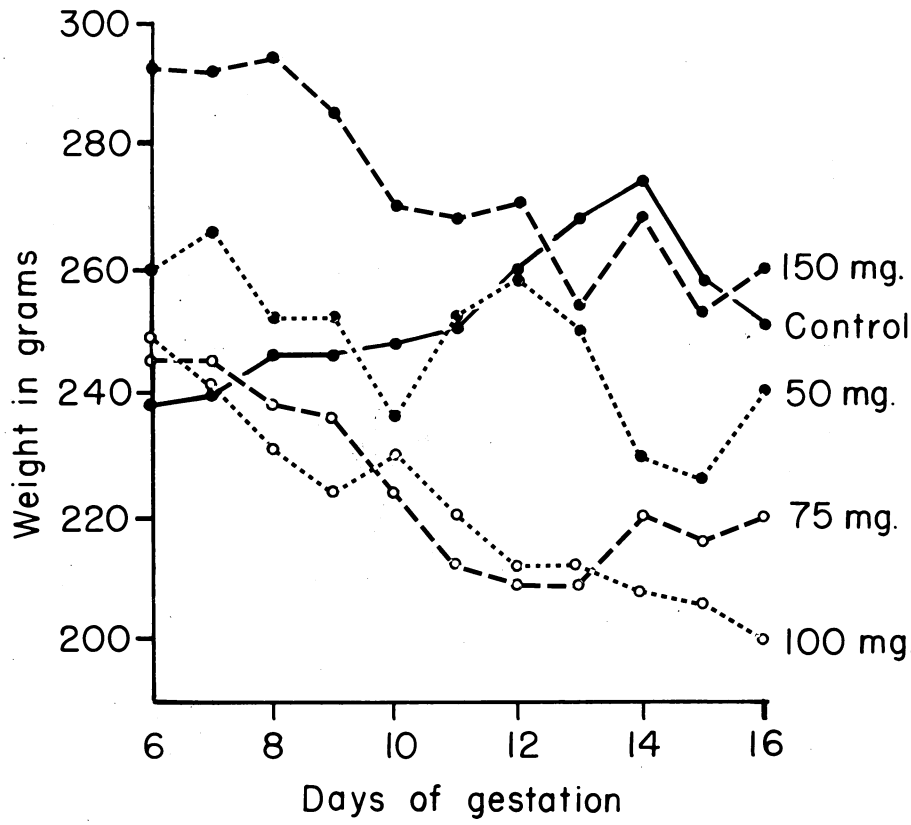
300
280
260
240
220
200
Weight in grams
6
8
10
12
14
16
Days of gestation
150 mg.
Control
50 mg.
75 mg.
100 mg.

FIGURE 1

Figure 2. Graph showing the subnormal weight gain observed in pregnant rats that had been injected daily with 50, 75, 100 and 150 mg of cortisone per kg of body weight during the last 17 days of gestation. Injections began on the 7th day of gestation.

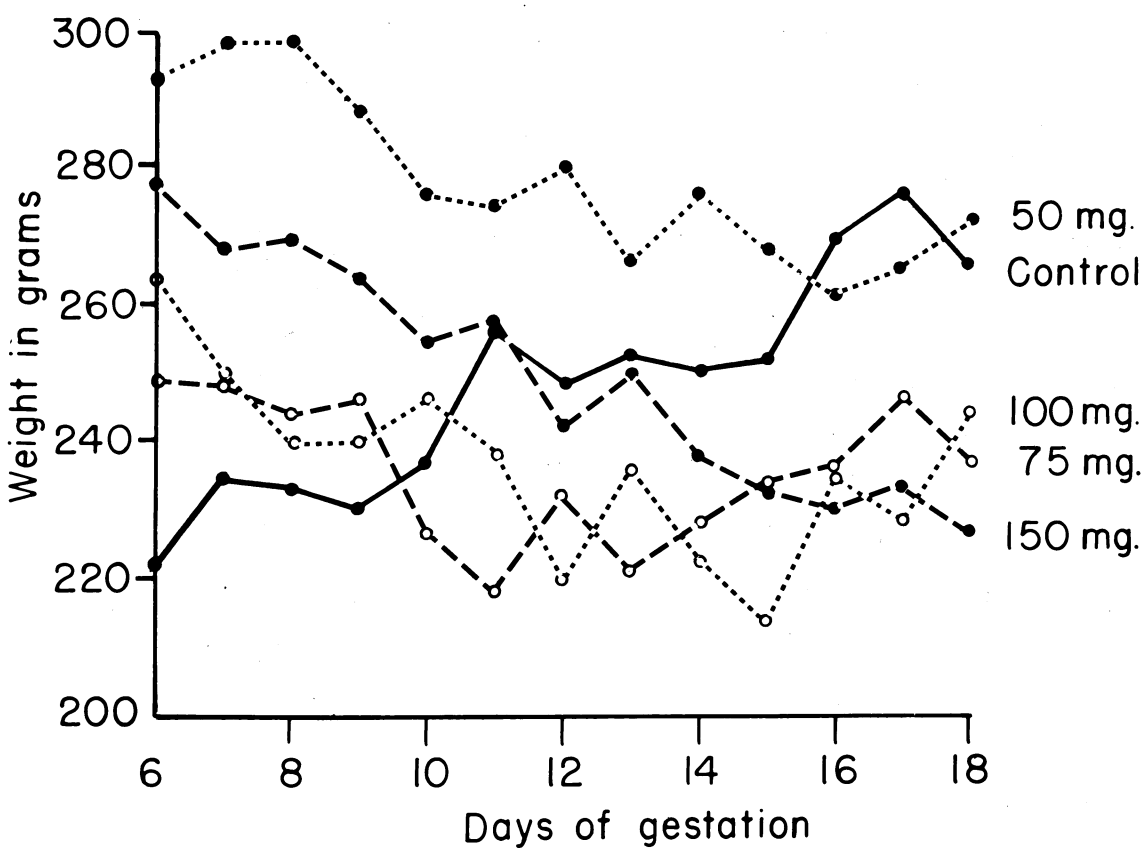
300
280
260
240
220
200
Weight in grams
6
8
10
12
14
16
18
Days of gestation
50 mg.
Control
100 mg.
75 mg.
150 mg.

FIGURE 2

Figure 3. Graph showing the subnormal weight gain observed in pregnant rats that had been injected daily with 50, 75, 100 and 150 mg of cortisone per kg of body weight during the last 19 days of gestation. Injections began on the 7th day of gestation.

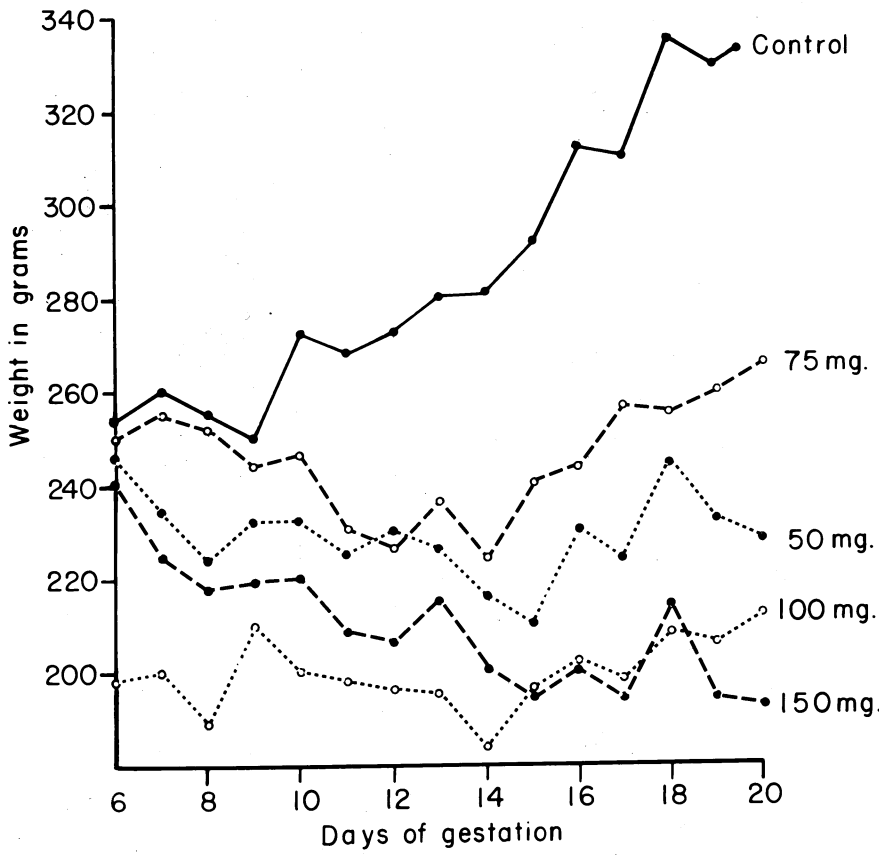
340
320
300
280
260
240
220
200
Weight in grams
Control
75 mg.
50 mg.
100 mg.
150 mg.
6
8
10
12
14
16
18
20
Days of gestation

FIGURE 3

PLATE I

Figure 4. A diagram to show the layers of the maxillary incisor in the 20 day fetal rat.

Abbreviations:

OEE - Outer Enamel Epithelium
SI - Stratum Intermedium
PL - Papillary Layer-composed of OEE, SR and SI
A - Ameloblasts
D - Dentine
O - Odontoblasts
P - Pulp
HES - Hertwig's Epithelial Sheath
SR - Stellate Reticulum-cells in the center of the enamel organ between the IEE and the OEE.
Epithelial Papillae-formed by the mesenchyme of the dental sac invaginating into the folds of the OEE.
Dental Lamina - the anlage of the enamel organ
Dental Papillae - the mesenchyme enclosed by the enamel organ.

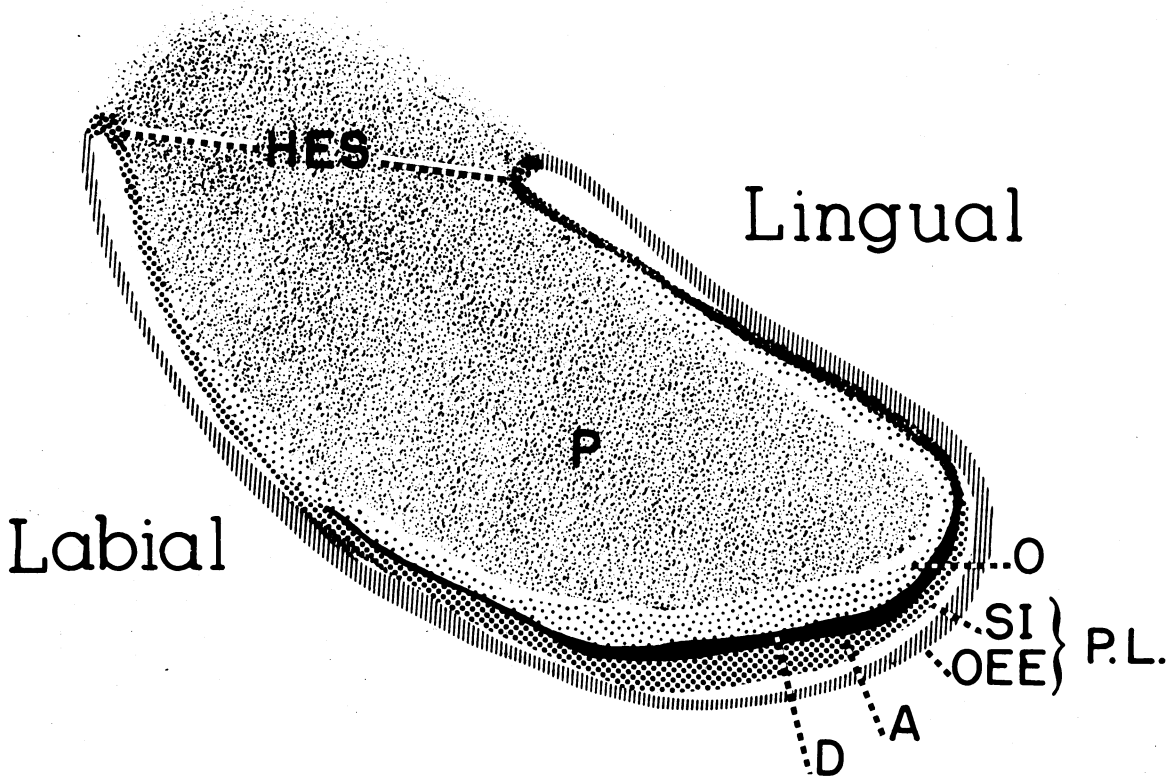

FIGURE 4

PLATE II

Figure 5. The maxillary incisor of a normal fetal rat age 16 days. (X 190).

Figure 6. The maxillary incisor of a fetal rat whose mother had received daily injections of 50 mg of cortisone per kg of body weight beginning on 7th and continued through 15th day of gestation. Age 16 days. Note glycogen in outer enamel epithelium. Compare figure 5. (X 190).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
G - Glycogen

FIGURE 5

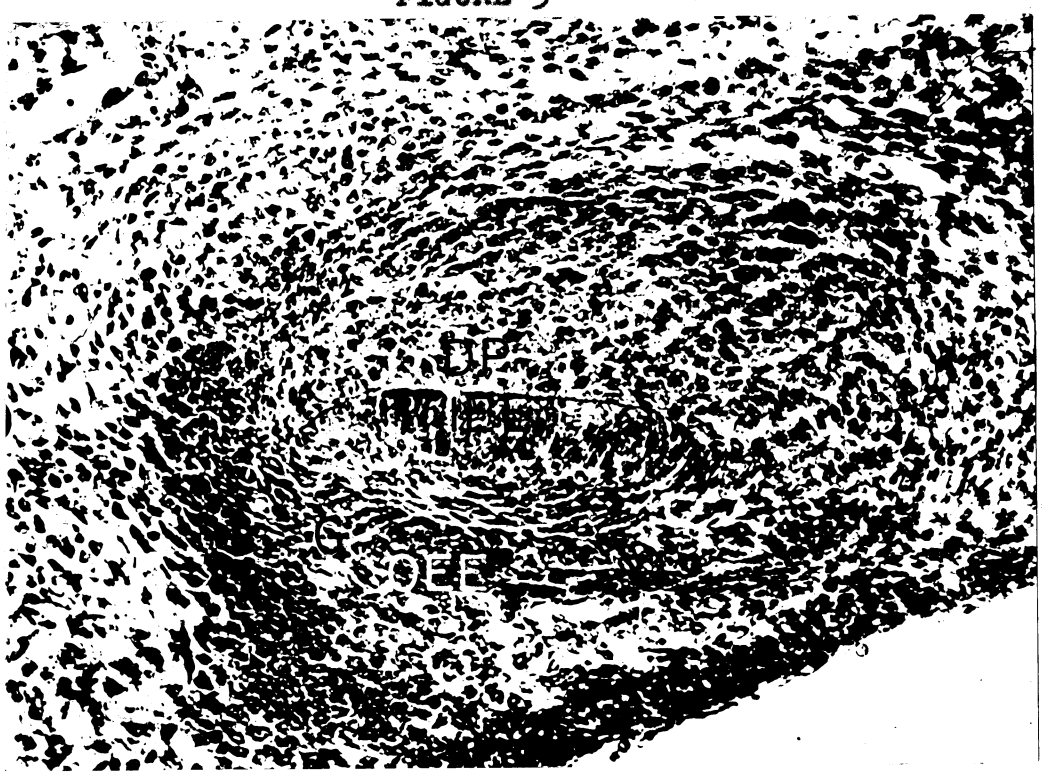

FIGURE 6

PLATE III

Figure 7. The maxillary incisor of a normal fetal rat age 16 days. (X 190).

Figure 8. The maxillary incisor of a fetal rat whose mother had received daily injections of 75 mg of cortisone per kg of body weight beginning on 7th and continued through 15th day of gestation. Age 16 days. Note glycogen in outer enamel epithelium. Compare figure 7. (X 190).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
G - Glycogen

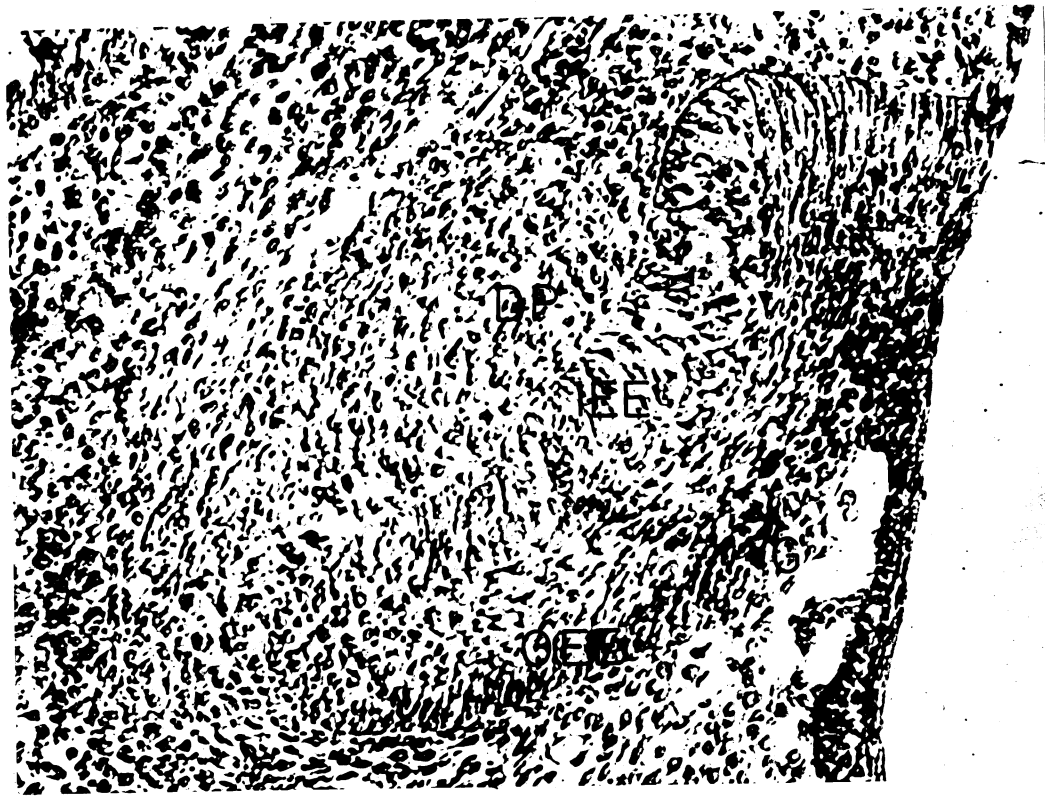

FIGURE 7

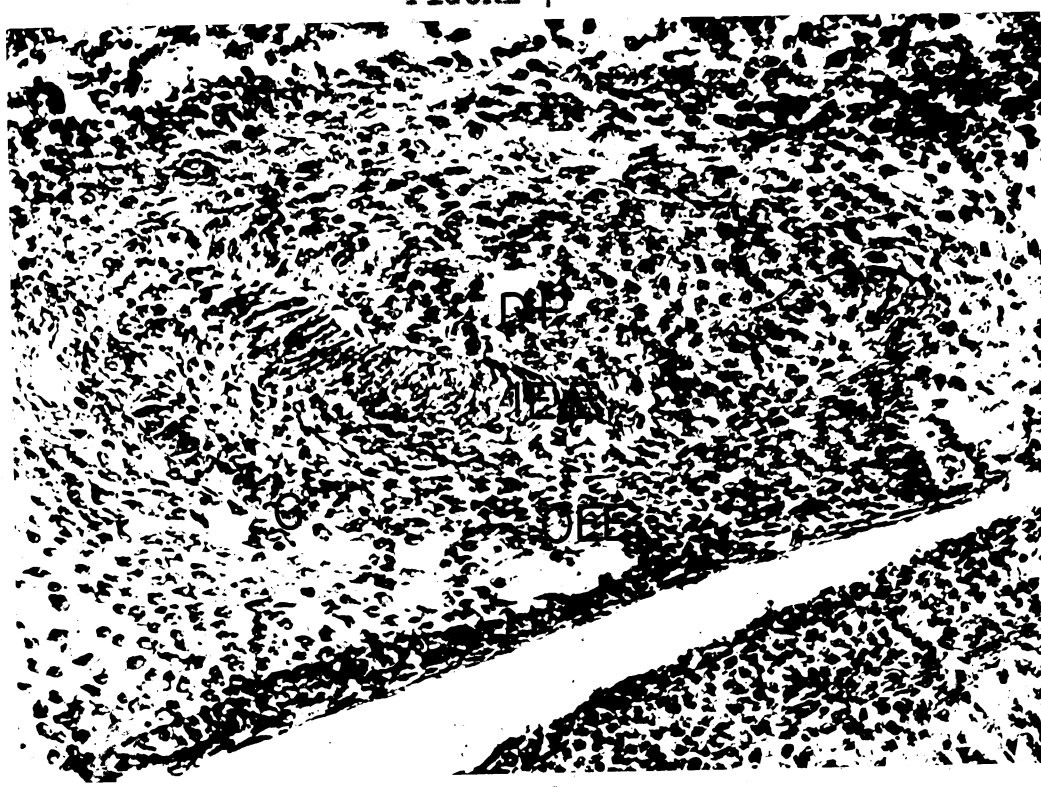

FIGURE 8

PLATE IV

Figure 9. The maxillary incisor of a normal fetal rat age 16 days. (X 190).

Figure 10. The maxillary incisor of a fetal rat whose mother had received daily injections of 100 mg of cortisone per kg of body weight beginning on 7th and continued through 15th day of gestation. Age 16 days. Note glycogen in outer enamel epithelium. Compare figure 9. (X 190).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
G - Glycogen

FIGURE 9

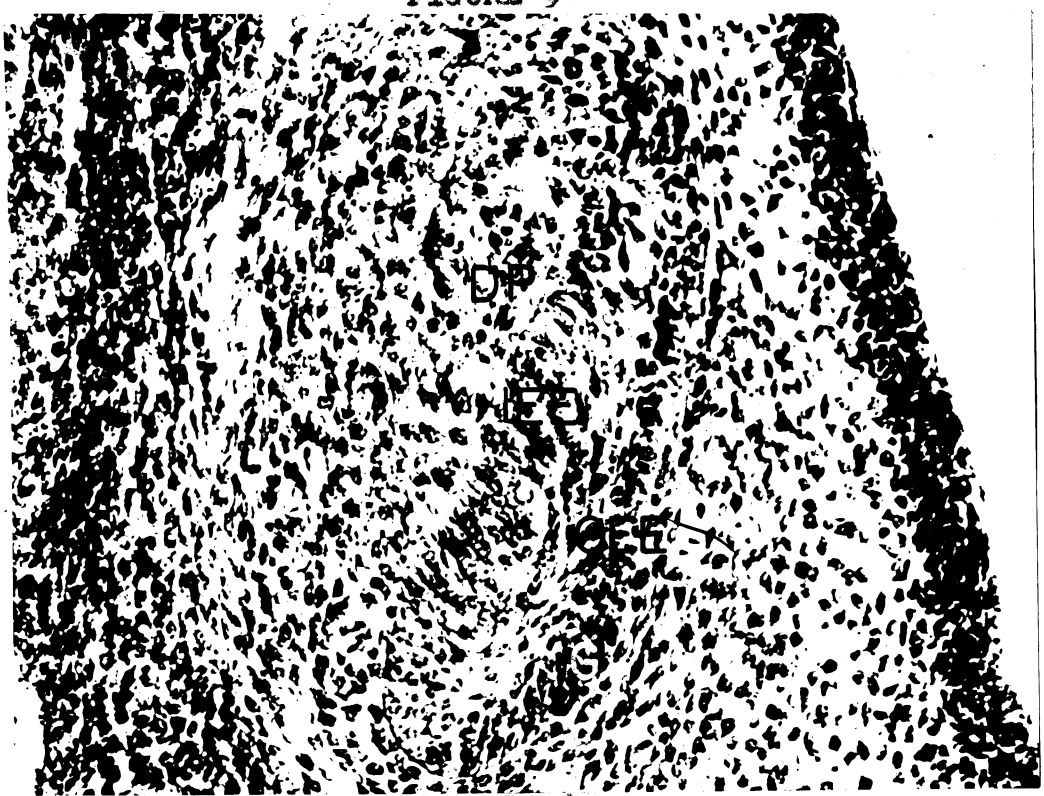

PLATE V

Figure 11. The maxillary incisor of a normal fetal rat age 16 days. (X 190).

Figure 12. The maxillary incisor of a fetal rat whose mother had received daily injections of 150 mg of cortisone per kg of body weight beginning on 7th and continued through 15th day of gestation. Age 16 days. Note glycogen in outer enamel epithelium. Compare figure 11. (X 190).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
G - Glycogen

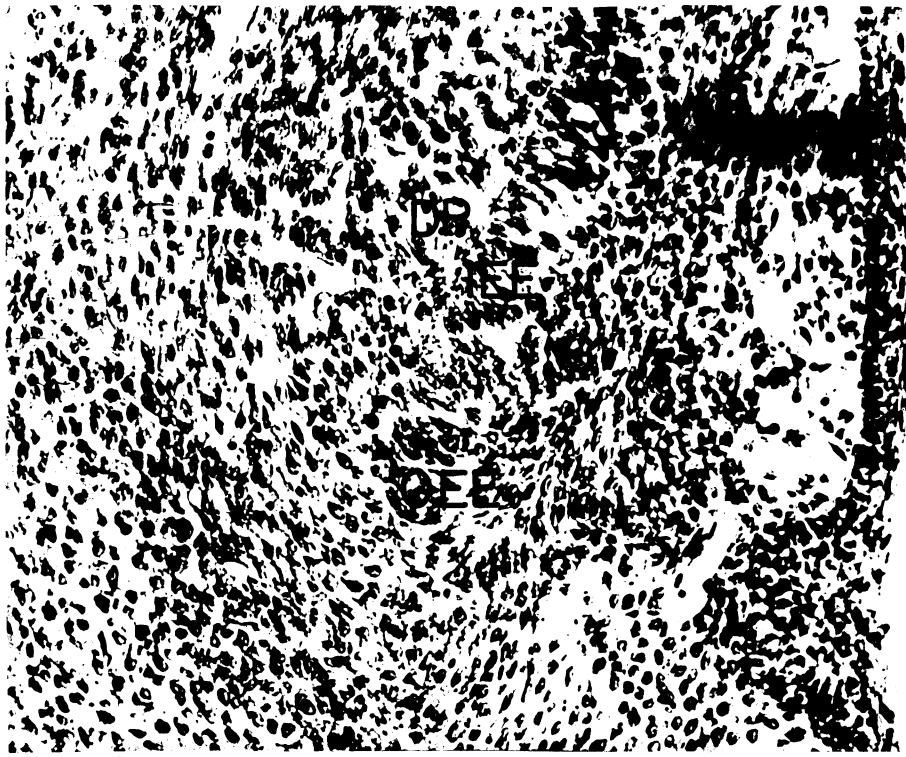

FIGURE 11

FIGURE 12

PLATE VI

Figure 13. The maxillary incisor of a normal fetal rat age 18 days. Glycogen occurs in outer enamel epithelium and in stellate reticulum. (X 115).

Figure 14. The maxillary incisor of a fetal rat whose mother had received daily injections of 50 mg of cortisone per kg of body weight beginning on 7th and continued through 17th day of gestation. Age 18 days. Note glycogen in outer enamel epithelium and in stellate reticulum. Compare figure 13. (X 115).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
SI - Stratum Intermedium
SR - Stellate Reticulum
G - Glycogen

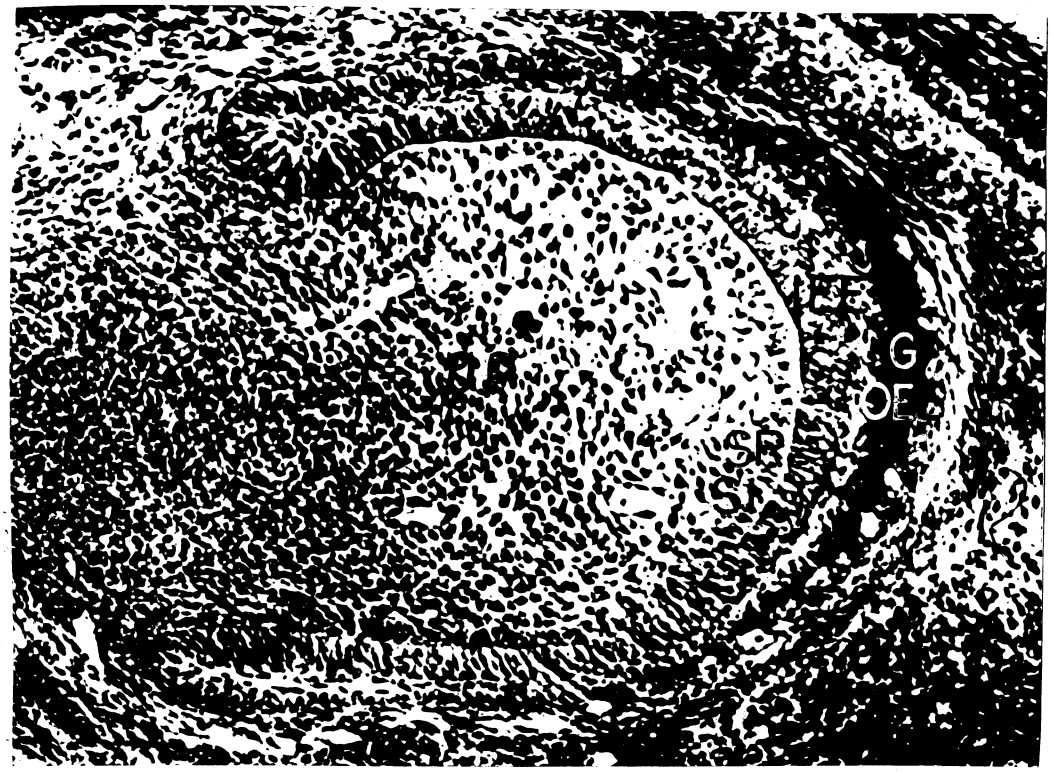

FIGURE 13

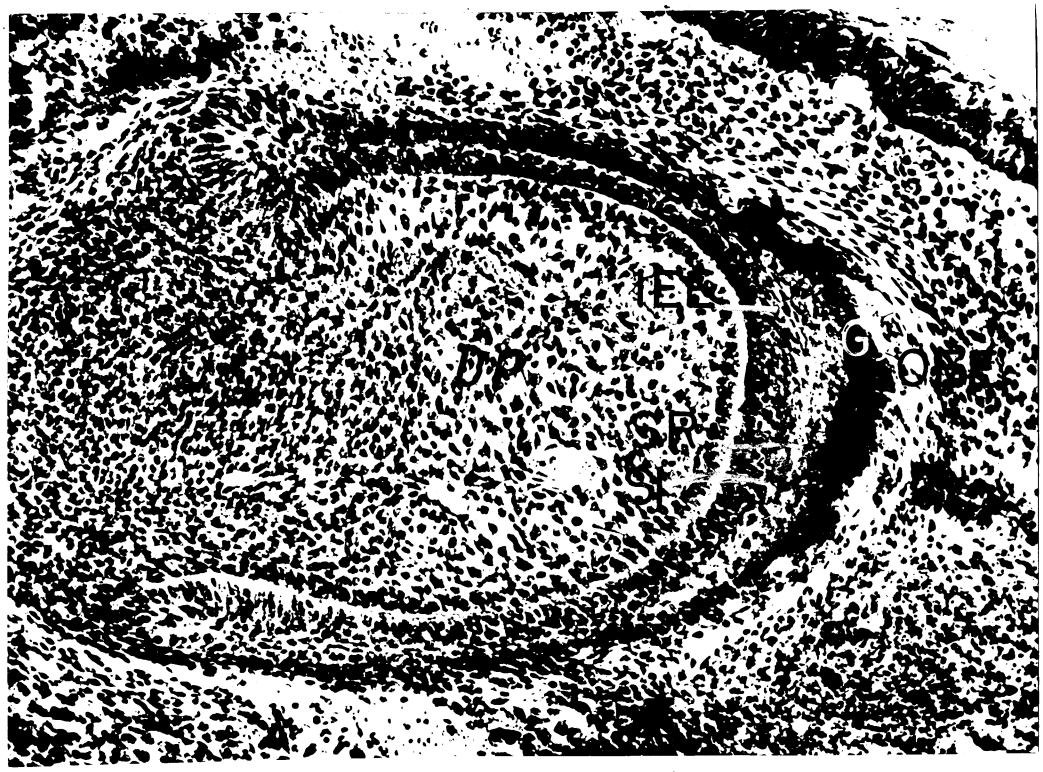

FIGURE 14

PLATE VII

Figure 15. The maxillary incisor of a normal fetal rat age 18 days. (X 115).

Figure 16. The maxillary incisor of a fetal rat whose mother had received daily injections of 75 mg of cortisone per kg of body weight beginning on 7th and continued through 17th day of gestation. Age 18 days. Note greater amount of glycogen in outer enamel epithelium and stellate reticulum than in control. Compare figure 15. (X 115).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
SI - Stratum Intermedium
SR - Stellate Reticulum
G - Glycogen

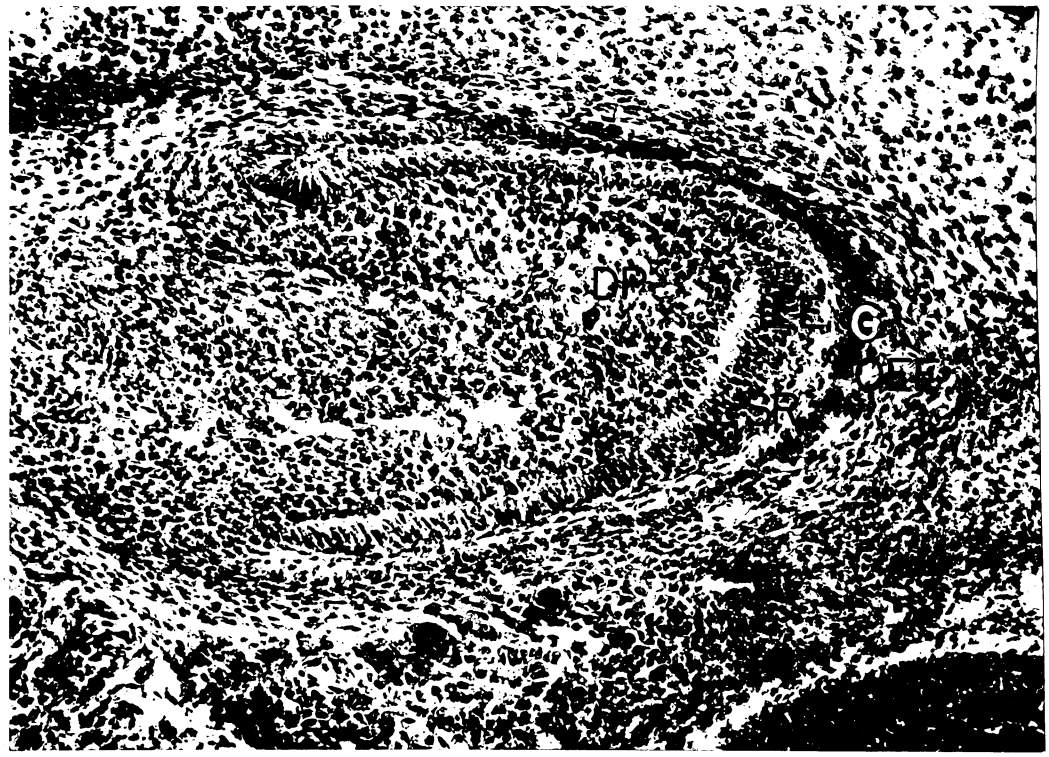

FIGURE 15

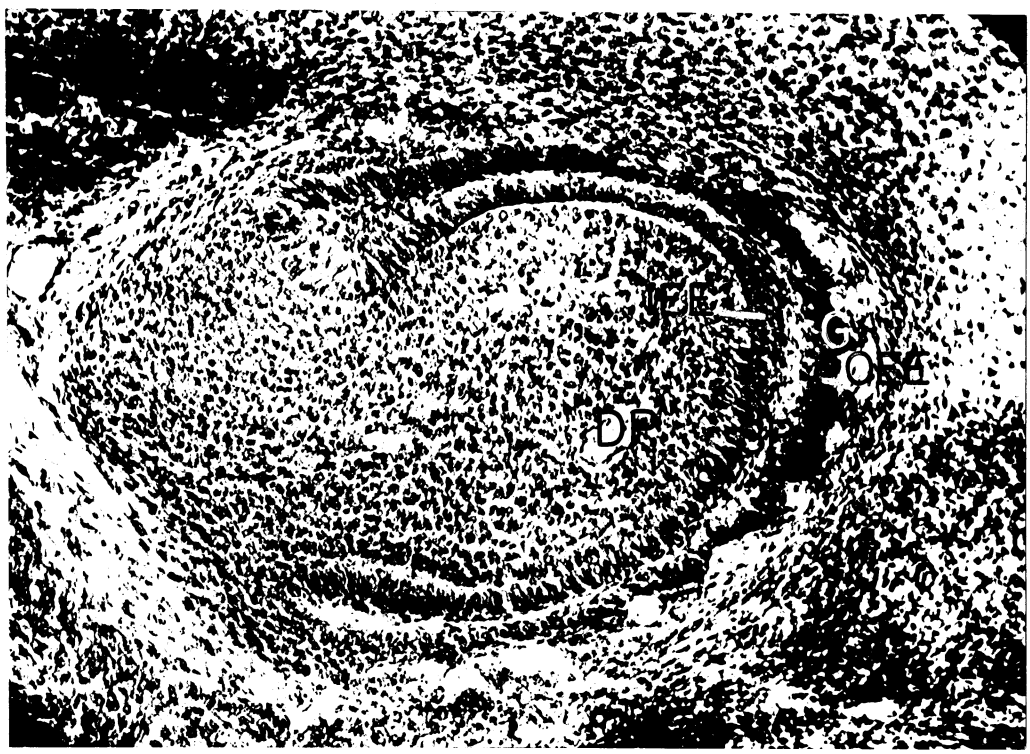

FIGURE 16

PLATE VIII

Figure 17. The maxillary incisor of a normal fetal rat age 18 days. (X 115).

Figure 18. The maxillary incisor of a fetal rat whose mother had received daily injections of 100 mg of cortisone per kg of body weight beginning on 7th and continued through 17th day of gestation. Age 18 days. Note heavier deposition of glycogen in outer enamel epithelium and stellate reticulum than in control. Compare figure 17. (X 115).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
SI - Stratum Intermedium
SR - Stellate Reticulum
G - Glycogen

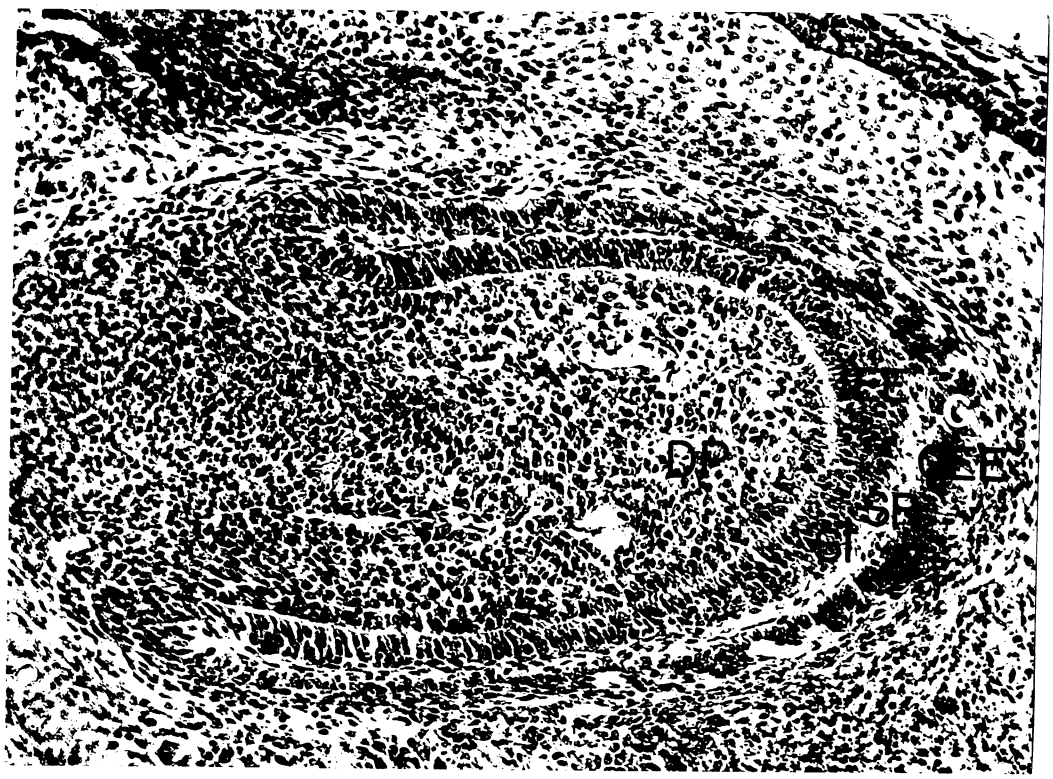

FIGURE 17

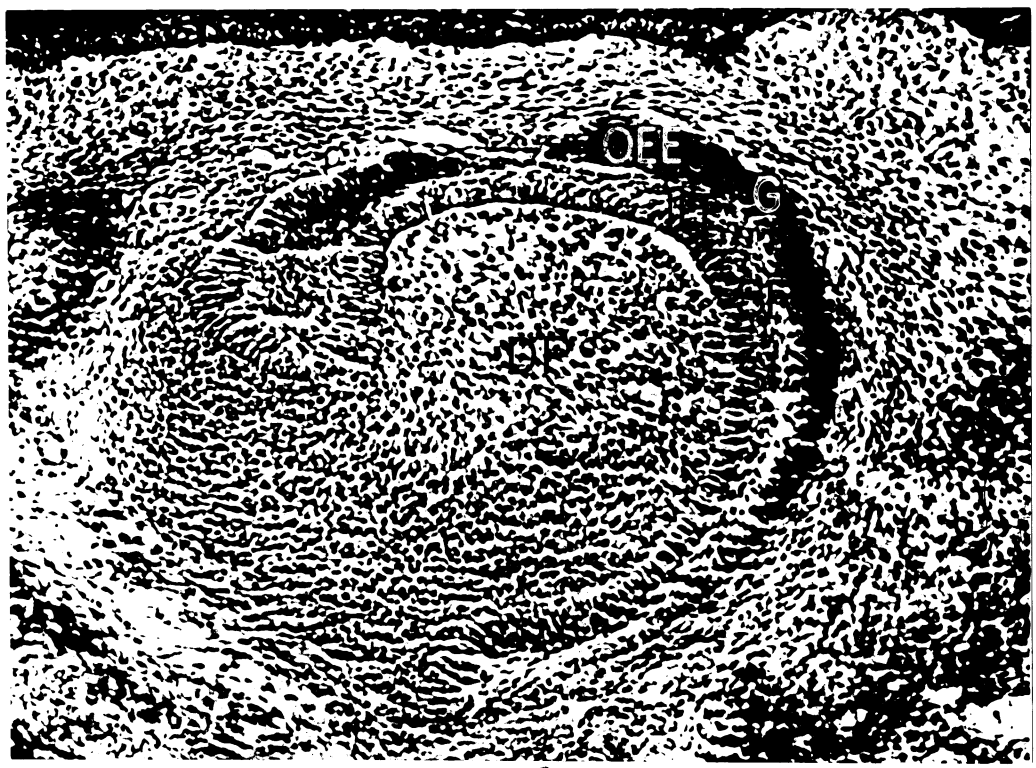

FIGURE 18

PLATE IX

Figure 19. The maxillary incisor of a normal fetal rat age 18 days. (X 115).

Figure 20. The maxillary incisor of a fetal rat whose mother had received daily injections of 150 mg of cortisone per kg of body weight beginning on 7th and continued through 17th day of gestation. Age 18 days. Note glycogen in outer enamel epithelium and in stellate reticulum. Compare figure 19. (X 115).

Abbreviations:

DP - Dental Papillae
IEE - Inner Enamel Epithelium
OEE - Outer Enamel Epithelium
SI - Stratum Intermedium
SR - Stellate REticulum
G - Glycogen

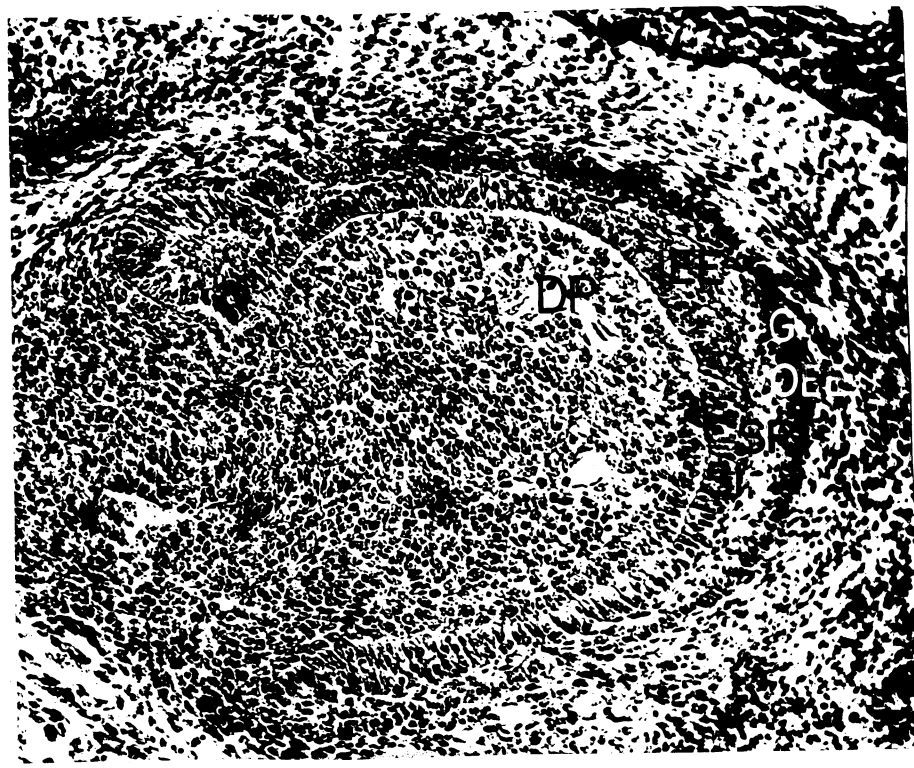

FIGURE 19

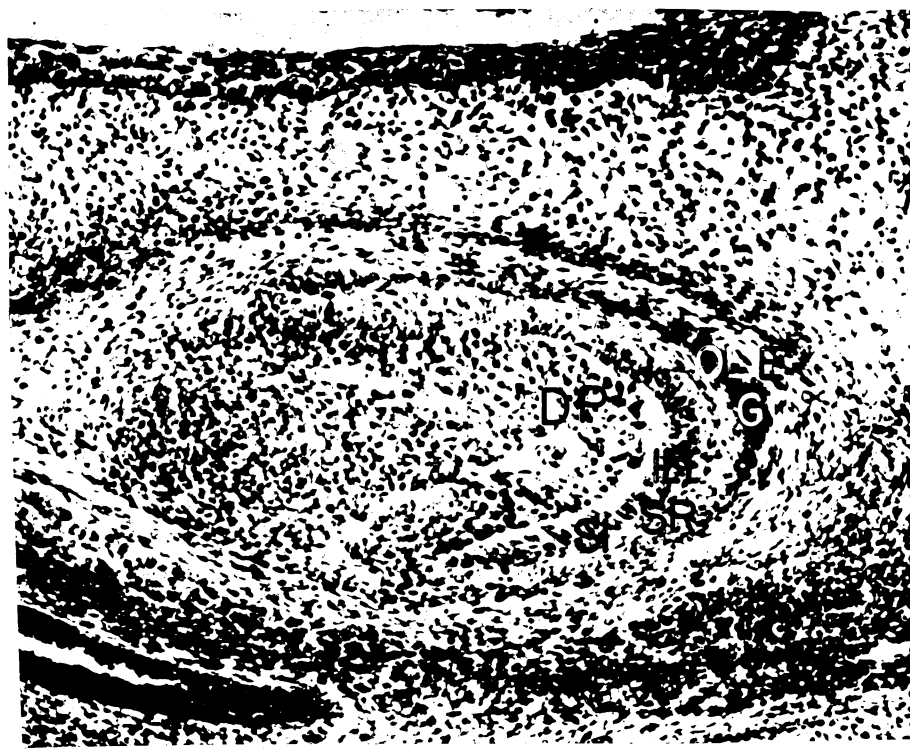

FIGURE 20

PLATE X

Figure 21. The maxillary incisor of a normal fetal rat age 20 days. Note glycogen in papillary layer. (X 65).

Figure 22. The maxillary incisor of a fetal rat whose mother had received daily injections of 50 mg of cortisone per kg of body weight beginning on 7th and continued through 19th day of gestation. Age 20 days. Note heavy deposit of glycogen in papillary layer, particularly in epithelial papillae. Compare figure 21. (X 65).

Abbreviations:

P - Pulp
O - Odontoblasts
HES - Hertwig's Epithelial Sheath
D - Dentine
A - Ameloblasts
PL - Papillary Layer
EP - Epithelial Papillae
G - Glycogen

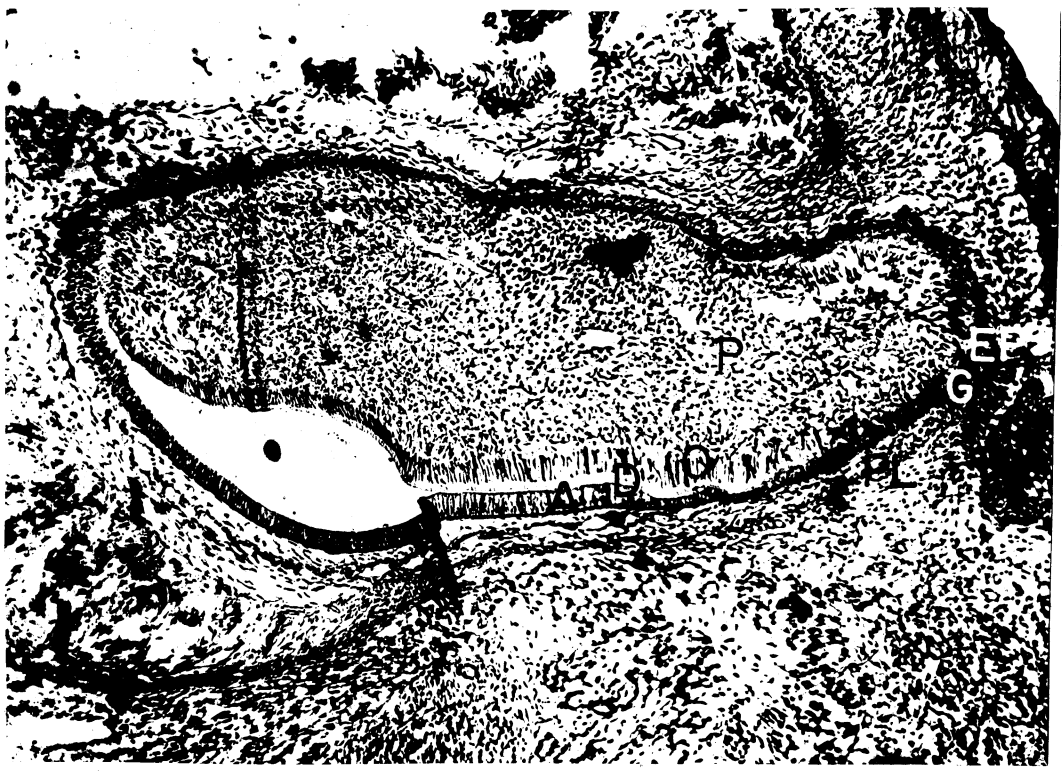

FIGURE 21

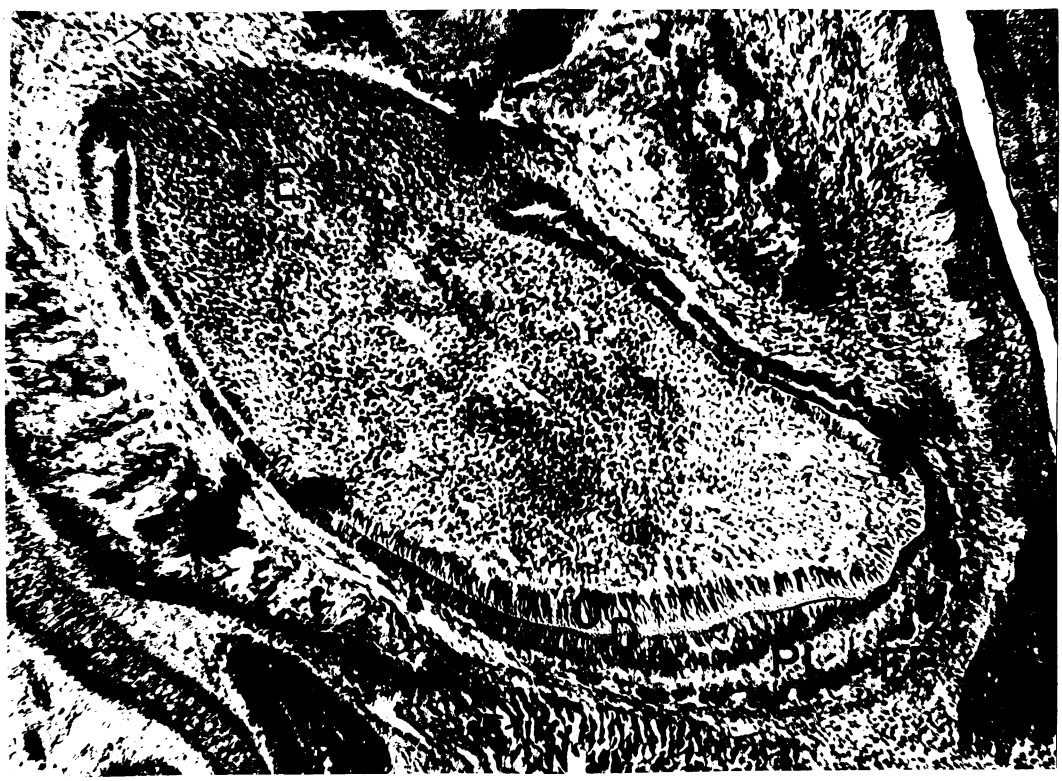

FIGURE 22

PLATE XI

Figure 23. The maxillary incisor of a normal fetal rat age 20 days. (X 60).

Figure 24. The maxillary incisor of a fetal rat whose mother had received daily injections of 75 mg of cortisone per kg of body weight beginning on 7th and continued through 19th day of gestation. Age 20 days. Note glycogen in papillary layer. Compare figure 23. (X 60).

Abbreviations:

P - Pulp
O - Odontoblasts
HES - Hertwig's Epithelial Sheath
D - Dentine
A - Ameloblasts
PL - Papillary Layer
EP - Epithelial Papillae
G - Glycogen

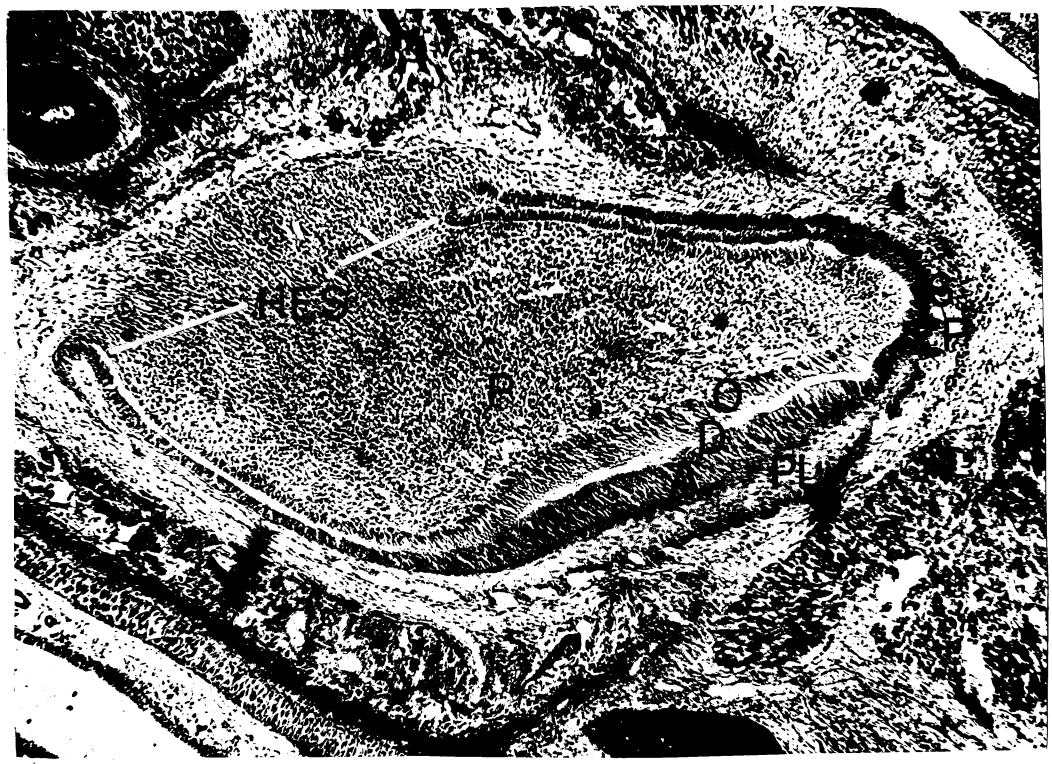

FIGURE 23

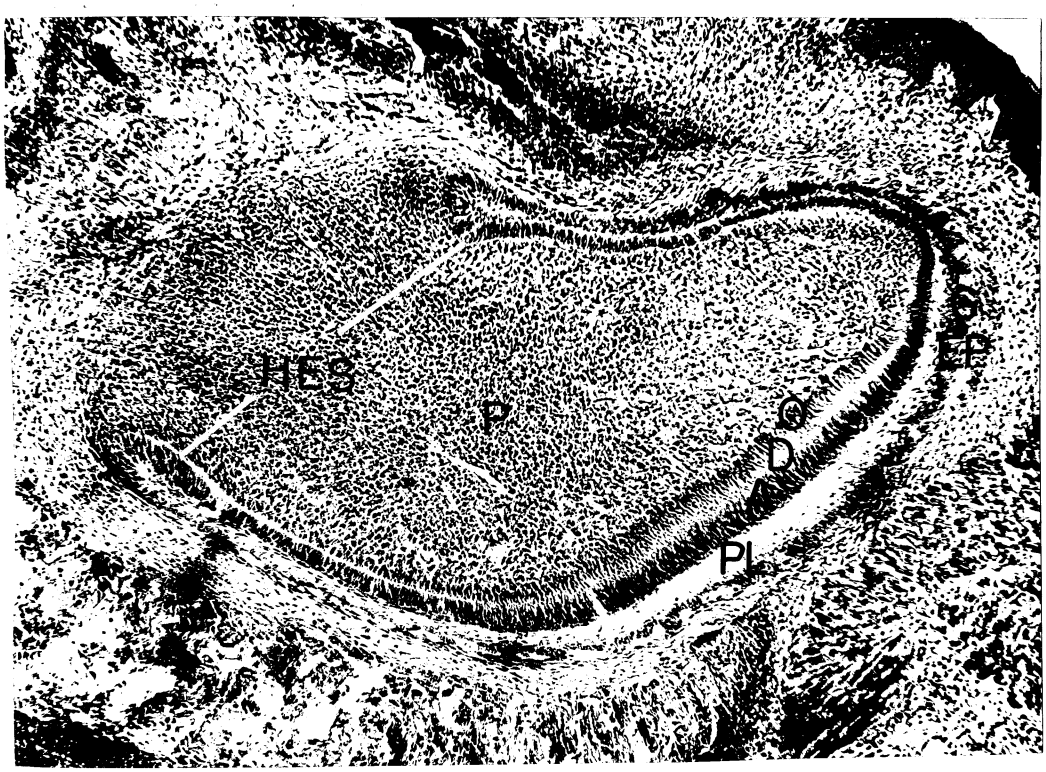

FIGURE 24

PLATE XII

Figure 25. The maxillary incisor of a normal fetal rat age 20 days. (X 60).

Figure 26. The maxillary incisor of a fetal rat whose mother had received daily injections of 100 mg of cortisone per kg of body weight beginning on 7th and continued through 19th day of gestation. Age 20 days. Note small amount of glycogen in papillary layer. Compare figure 25. (X 65).

Abbreviations:

P - Pulp
O - Odontoblasts
HES - Hertwig's Epithelial Sheath
D - Dentine
A - Ameloblasts
PL - Papillary Layer
EP - Epithelial Papillae
G - Glycogen

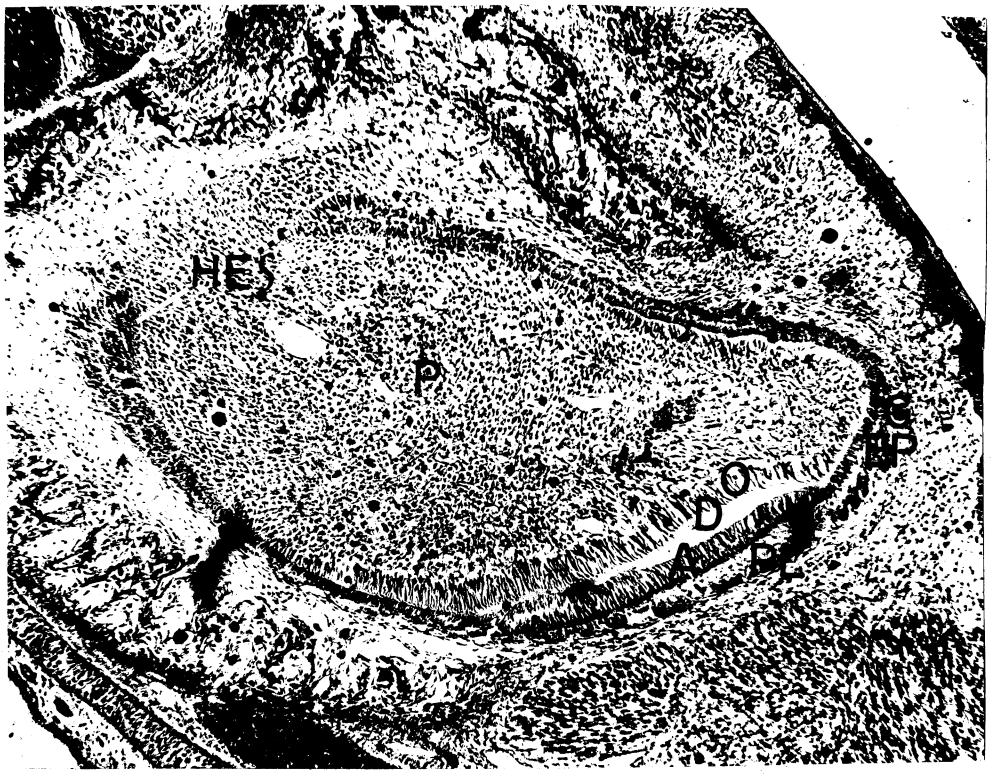

FIGURE 25

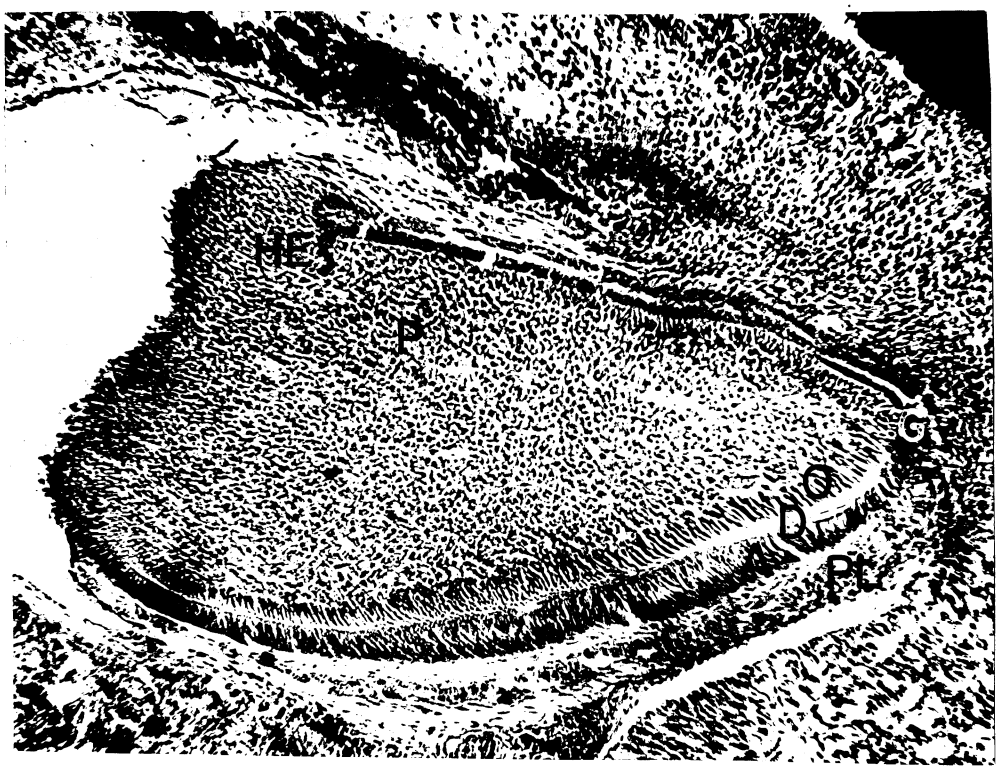

FIGURE 26

PLATE XIII

Figure 27. The maxillary incisor of a normal fetal rat age 20 days. (X 65).

Figure 28. The maxillary incisor of a fetal rat whose mother had received daily injections of 150 mg of cortisone per kg of body weight beginning on 7th and continued through 19th day of gestation. Age 20 day. Note glycogen in papillary layer. Compare figure 27. (X 65).

Abbreviations:

P - Pulp
O - Odontoblasts
HES - Hertwig's Epithelial Sheath
D - Dentine
A - Ameloblasts
PL - Papillary Layer
EP - Epithelial Papillae
G - Glycogen

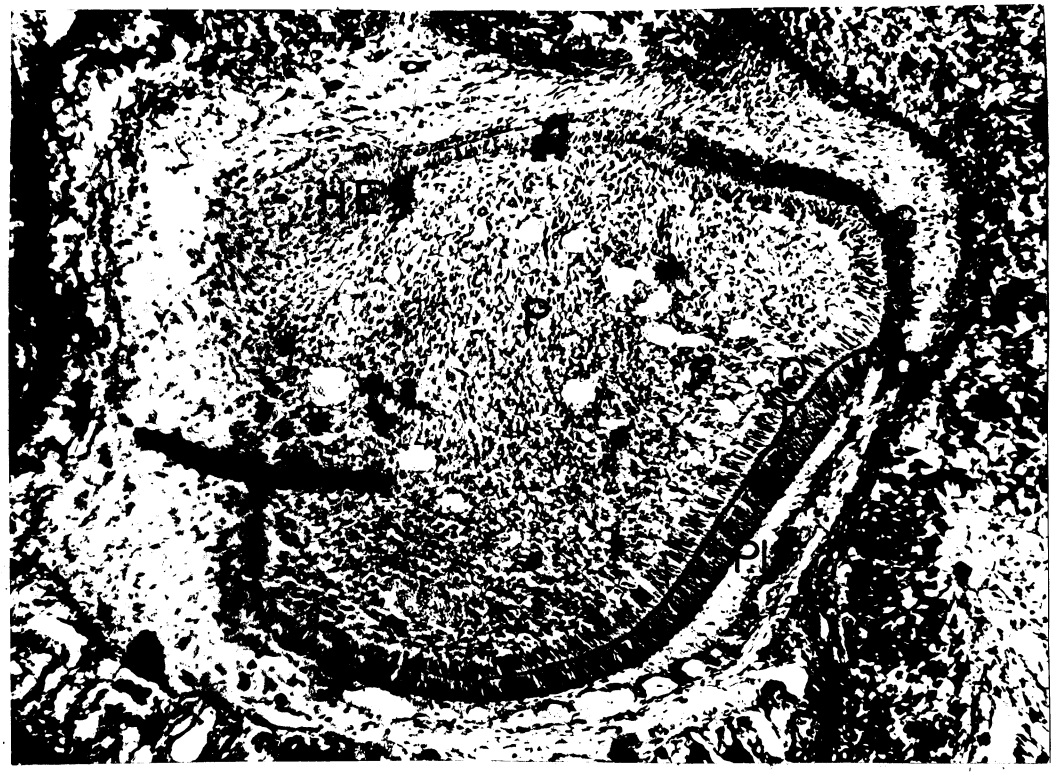

FIGURE 27

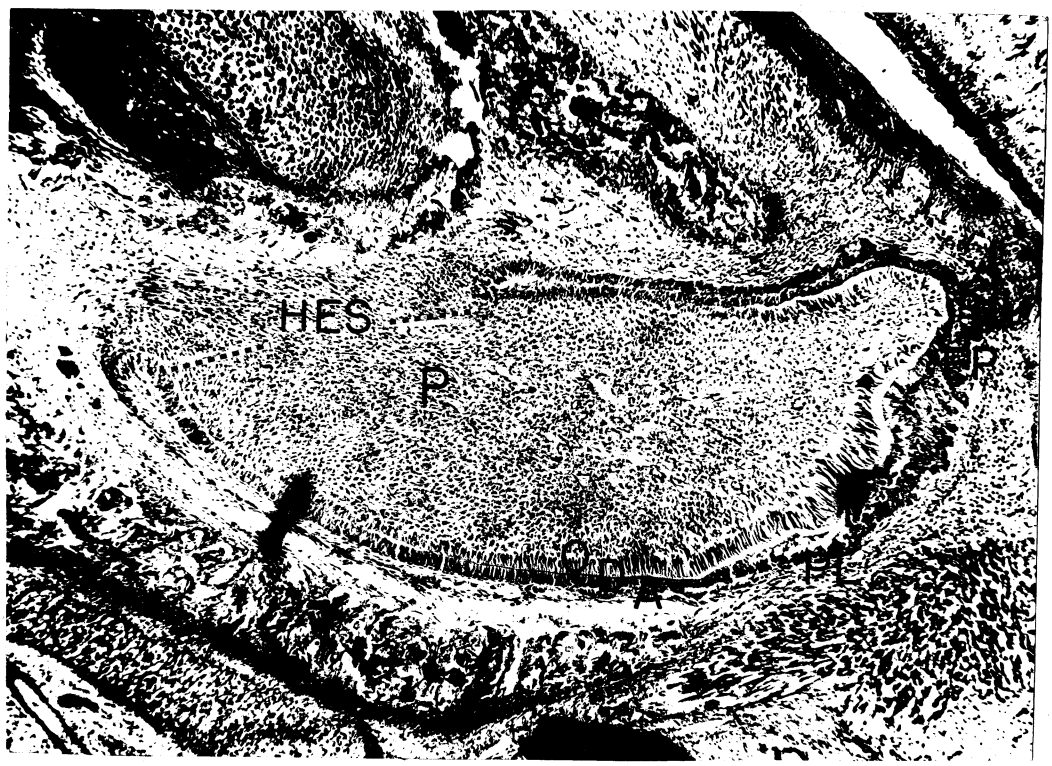

FIGURE 28

APPROVAL SHEET

The thesis submitted by Bernadette E. Ward has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

1-24-62
Date

Lincoln E. Homan
Signature of Adviser