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Histological Effects of Cortisone on the Maxillary Incisors and Associated Tissues in the Albino Rat

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HISTOLOGICAL EFFECTS OF CORTISONE ON THE MAXILLARY INCISORS AND ASSOCIATED TISSUES IN THE ALBINO RAT

by

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LIFE

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	1
MATERIALS AND METHODS	4
OBSERVATIONS	7
DISCUSSION	14
SUMMARY AND CONCLUSIONS	21
LITERATURE CITED	25
TABLES	28
FIGURES AND PLATES	33

LIST OF TABLES

Table		Page
I.	EXPERIMENTS ON PRENATAL CORTISONE TREATED RATS	28
II.	EXPERIMENTS ON POSTNATAL CORTISONE TREATED RATS . . .	29
III.	MEASUREMENTS ON MAXILLARY INCISORS OF POSTNATAL RATS .	30
IV.	MEASUREMENTS ON SECTIONS OF MAXILLARY INCISORS AND ALVEOLAR BONE IN POSTNATAL RATS	31
V.	MEASUREMENTS ON SECTIONS OF MAXILLARY INCISOR AND ALVEOLAR BONE IN POSTNATAL RATS	32

LIST OF FIGURES

Figure		Page
1.	A DIAGRAM SHOWING THE POINTS AT WHICH MEASUREMENTS ON THE UPPER INCISOR WERE MADE	33
2.	THE FREE END OF THE INCISAL SOCKET OF A CONTROL POSTNATAL RAT. AGE 6 DAYS	34
3.	THE FREE END OF THE INCISAL SOCKET OF A POSTNATAL RAT WHICH HAD RECEIVED 5 DAILY 1 MILLIGRAM INJECTIONS OF CORTISONE STARTING ON THE DAY OF BIRTH. AGE 6 DAYS	35
4.	THE ORAL EPITHELIUM OF A CONTROL FETUS AGE 20 DAYS	36
5.	THE ORAL EPITHELIUM OF A FETUS WHICH HAD RECEIVED 2 INJECTIONS OF CORTISONE, 500 GAMMA PER INJECTION ON THE 17th AND 19th DAYS OF GESTATION. AGE 20 DAYS	37
6.	THE ORAL EPITHELIUM OF A CONTROL POSTNATAL RAT AGE 6 DAYS	38
7.	THE ORAL EPITHELIUM OF A POSTNATAL RAT WHICH HAD RECEIVED DAILY INJECTIONS OF 1 MILLIGRAM OF CORTISONE FOR 5 DAYS STARTING ON THE DAY OF BIRTH. AGE 6 DAYS	39
8.	THE UPPER INCISOR OF A CONTROL FETUS AGE 20 DAYS	40
9.	THE UPPER INCISOR OF A FETUS WHICH HAD RECEIVED 2 INJECTIONS OF CORTISONE, 500 GAMMA PER INJECTION, ON 18th AND 19th DAYS OF GESTATION. AGE 20 DAYS	41
10.	THE DENTAL PAPILLA AND ENAMEL ORGAN OF A CONTROL FETUS. AGE 18 DAYS	42
11.	THE DENTAL PAPILLA OF A FETUS WHICH HAD RECEIVED 500 GAMMA OF CORTISONE ON THE 17th DAY OF GESTATION. AGE 18 DAYS	43
12.	THE BLOOD VESSELS IN THE PULP OF AN INCISOR OF A CONTROL POSTNATAL RAT AGE 11 DAYS	44
13.	THE BLOOD VESSELS IN THE PULP OF AN INCISOR OF A POSTNATAL RAT WHICH HAD RECEIVED DAILY INJECTIONS OF 1 MILLIGRAM OF CORTISONE FOR 4 DAYS BEGINNING ON THE 7th DAY. AGE 11 DAYS	45

INTRODUCTION

Very early in the work with cortisone it became apparent that it had a powerful inhibitory effect on the growth of laboratory animals. This fact has since been repeatedly confirmed by many investigators. Contrary to this general observation some investigators noted an apparent developmental acceleration in certain structures of the body such as the incisors of the albino rat which erupted earlier in cortisone treated than in normal animals. It was also noted that there was an acceleration in the separation of the lower lips from the gingiva and in the opening of the eyes of such animals. Few investigators have observed and studied these phenomena. Thus little information on the probable mechanism underlying these conditions particularly the eruption of incisors is at present available.

We were interested in the factor or factors underlying the observed acceleration in the eruption of the incisors in cortisone treated rats hence decided to make a comparative study of the histology of the incisor and associated structures in young cortisone treated and normal albino rats.

Studying the biological effects of cortisone in albino rats Parmer et al (1951) observed that the continuously growing incisors appeared earlier in the oral cavity of treated than of control animals which would seem to indicate that there was an acceleration in the eruption process. Leroy and Domm (1951) reported modifications in the growth of incisors of cortisone treated rats. Domm

and Marzano (1954) working with mature rats of both sexes measured the rate of growth of the incisors and observed an acceleration in the normal rate of eruption of the continuously growing incisors following cortisone administration. Garren (1954) comparing the rate of eruption in mature cortisone treated male rats with that of normals made observations similar to those of Domm and Marzano. He noted that the rate of eruption in cortisone treated rats was reduced after a period of 3 to 4 weeks so that it approached that of controls. This condition was also observed by Domm and Marzano (unpublished data) but it appeared earlier in the experiments of Garren where larger daily dosages were administered.

Goldsmith and Ross (1954) trying to explain the reason for precocious eruption of mandibular incisors in cortisone treated newborn albino rats suggested that it was due to an acceleration in growth as evidenced by acceleration of odontogenesis and amelogenesis. Domm and Leroy (1955) working with newborn rats confirmed the observations of Parmer et al (1951) on the early eruption of incisors in cortisone treated newborn rats and extended this finding to include young rats born of cortisone treated mothers as well as young that had been injected in utero. These investigators observed that cortisone causes an irregularity in the growth of facial bones and that it also has an effect on epithelium and connective tissue causing a noticeable shrinkage which may in turn be responsible for the observed precocious eruption of incisors.

Addison and Appleton (1915) observed and described the gross and histological changes associated with the normal eruption of teeth in the albino rat (Mus Norvegicus albinus). However, despite a very considerable amount of work

by these authors and others there is still no unanimity of opinion with respect to the mechanism underlying this process. Many theories have been advanced but none seems to offer an entirely satisfactory explanation. One of the oldest and simplest theories to account for tooth eruption was that it is the result of root elongation first advanced by Hunter, (1771). This and the theory that eruption is due to pulpal constriction, first proposed by Zuckerkandl (1891) was refuted by Tomes (1914) who among others observed that fully formed teeth and also rootless teeth are known to erupt. Delabarre (1855) believed that the tooth is pushed or squeezed from the alveolus into the oral cavity by the growth of the alveolar bone. This theory fails to explain the eruption of multirooted teeth (Massler and Schour, 1941). Berten (1900) cited by Demolis, (1926) suggested that tooth eruption is the result of the action of the muscles of the cheeks and lips on the alveolar process, which serves to squeeze the crown of the tooth into the oral cavity. However, it has been observed that teeth will erupt in cases of facial paralysis (Massler and Schour, 1941). Constant (1900) as a result of anatomical studies on domesticated animals suggested that blood pressure could be the impelling force by which the teeth are caused to erupt. There is circumstantial as well as experimental evidence in support of this theory (Massler and Schour, 1941).

This was the status of the problem at the start of our investigation and since no one had made a careful histological study of the tissues involved in the precocious eruption of cortisone treated rats it was felt that such a study might help to clarify the problem.

MATERIALS AND METHODS

Albino rats of the Sprague-Dawley strain were used in our investigation. The animals were reared in an air-conditioned room where they were fed Purina Fox Chow and given tap water ad libitum.

Our study deals primarily with two age groups. In Group II ninety-four litter mate rats ranging in age between 1 and 11 days were utilized. From a total of 94 rats, 58 were treated and 36 were kept as controls. Group I consisted of 57 fetuses injected in utero whose gestation age at the time of injection ranged from 17 to 20 days. Fetuses received 1, 2 or 3 injections and were sacrificed 24 hours following the last injection.

The cortisone used in our experiments was Cortone acetate generously supplied to Dr. L. V. Domm by Merck and Company, Rahway, New Jersey. This was a suspension containing 25 mg per cc of the synthetic compound. The injections in both groups were performed in the same manner. A quarter cc capacity hypodermic syringe was employed. A number 24 needle was used for injections of prenatal rats and a number 26 needle for postnatal rats. All injections were subcutaneous. The fetuses were treated in utero by exposure of uterine horns through a mid-abdominal incision of pregnant female under ether anesthesia, according to the method of Domm and Leroy (1955). Since albino rats are fairly resistant to infection we observed only ordinary aseptic precautions. The use of 80% alcohol to sterilize the necessary surgical instruments and to sterilize the abdominal region were the only precautions observed. The recovery after surgery was excellent. For purposes of identification the controls in Group I (prenatal

rats) were injected with Evans blue (T1824). Such fetuses were pale blue at the time of recovery by cesarean section and in those not sacrificed the dye had usually not completely disappeared until the animals were about two weeks old.

The dose of cortisone injected in Group II was 1.0 mg per injection. The number of injections varied from 1 to 5 in different experiments. Also the age at which the first injection was given varied from the first to the seventh day after birth. The pertinent data are given in Table II. Five hundred gamma was the dose per injection administered in Group I. In two females one injection was given to each fetus, in three others each fetus received two injections and in a 6th female the fetuses received two injections of 500 gamma each and a third injection of 250 gamma (Table I).

Daily weight records were kept of all experimental animals beginning on the first day of treatment. Controls and treated were litter mates in all experiments, they were chosen at random but an effort was made to have a fairly even weight distribution between experimental and control groups when experiments began.

All animals were weighed and killed by decapitation twenty-four hours after the last injection. The heads were immediately placed in a calcium formal solution and transferred to a refrigerator the minimum period of fixation being approximately 7 days.

Since the specimens were fixed in a calcium-formal solution decalcification was done separately. The apparatus used was a standard Ionic Bone Decalcifyer manufactured by the Martin Sweet Company. This is a cylindrical pyrex jar containing a solution composed of 10 ml of formic acid, 8 ml of hydrochloric

acid, and 82 ml of distilled water. The material to be decalcified was placed in a small electrode cup which contained a silver wire constituting the positive pole. The negative pole of this apparatus is a carbon rod placed in the pyrex container. The heads were kept in the apparatus for from one to five hours after the electric current was established following which they were washed in tap water. Following decalcification and washing in tap water, the prenatal heads were embedded in paraffin and the postnatal in celloidin. The great majority of the heads were sectioned sagittaly. Those embedded in paraffin were cut at 10 micra and those in celloidin at 14 micra. The entire head was serially sectioned in each case. The sections of fetal heads were stained with Delafield's Hematoxylin and Eosine Y and those of postnatal heads with Hematoxylin and "Triosine" which is composed of Eosine, Erythrosine, and Orange G. This latter mentioned stain is superior for the differentiation of blood vessels.

With the aid of a Tri-Simplex Microprojector manufactured by the Bausch and Lomb Company sections were projected onto a white paper and serial drawings prepared. The distance at which projection was made and the magnification were kept constant in all measurements relating to a particular experiment. From these serial sections comparable sections from treated and controls were chosen and appropriate measurements made. The length and width of incisors were measured with the aid of the Tri-Simplex Microprojector while finer structures such as the odontogenic, enamel and dentine layers were measured with the aid of a compound binocular microscope. Both instruments were equipped with an ocular micrometer.

A statement with reference to the method employed in preparing the

materials that were measured will be helpful. In all the experiments an attempt was made to compare identical parasagittal sections between treated and controls. Our landmark in choosing identical sections was for the most part the appearance of the incisal socket. In order to procure exact parasagittal sections heads were marked with red india ink by a line passing along the axis dividing them into bilateral halves. The red line could be seen through the embedding material and the knife blade could thus be arranged parallel to this line. This arrangement proved fairly satisfactory and was followed for all the material. However, variations in the angle of the knife blade modified the angle of the sections and this in some cases caused variations in the size of the structures being measured especially in the measurements on the size of the upper incisors. In order to obtain a further check on the size of incisors a series of identical measurements was made on the dissected incisors of rats from several postnatal experiments. The incisors were dissected under a binocular dissecting microscope and measured with the aid of an ocular micrometer (Table III).

It should be noted that our objective, where measurements were made in this investigation, was to demonstrate relative rather than absolute changes in size thus values are presented simply in terms of micrometer scale divisions.

OBSERVATIONS

Our observations were made chiefly on structures suggested by different authors to be factors in the process of eruption. A brief discussion of current theories of eruption was given above and readers interested in more information

are referred to Massler and Schour (1941) for a more detailed discussion.

Measurements were made of: (1) Maximum length and width of incisors; size was determined from the study of serial sections. (2) Width of alveolar bone at both its midlingual, midlabial and anterior labial sides. (3) Size of odontogenic, enamel and dentine layers. The ameloblast and odontoblast layers were measured under high power magnification on the labial side at a point within the middle anterior-posterior third of the incisor. (See Figure 1). The dentine and enamel were measured under low power magnification near the apical end in a section through the greatest length and width of the incisors. The anterior end was chosen for several reasons: First, because the width of these layers is normally greatest and some of the error resulting from measuring a narrow layer under low magnification could thus be avoided. Secondly, due to the increase in the height of the dentine and enamel layers as the anterior end of the incisor is approached a constant point for measuring these layers could not be found. Hence, the occlusal end of the pulp chamber was chosen as the most constant available landmark and measurements made adjacent to it on the labial side. (See Figure 1)

The appearance of blood vessels in the pulp and in the supporting structures of incisors, the labial alveolar periosteum of incisors, the periosteum covering the labial side of labial alveolar process, especially at its free end, and the oral epithelium were studied for possible effects of cortisone.

Body weight

Daily weight records were kept in all our experiments on postnatal rats, and in all cases the cortisone treated rats failed to show weight gains comparable to those of controls. Table III gives the weights of experimental and control animals at the beginning and the end of some of our experiments.

Effects on the incisal socket

In our prenatal rats where the minimum dose of cortisone was 500 gamma and the maximum 1 mg given in utero in two daily injections, no apparent change in the incisal socket could be observed.

In postnatal rats as shown from measurements on the labial side a reduction is observed. Measurements included the thickness of both the covering periosteum and the bone. The effect is less noticeable on the lingual alveolar process. However, in this case measurements were confined to the bone only while its covering periosteum was not included. This latter structure also revealed a reduction in size. After the first injection of 1 mg the effect although slight was apparent though not convincing. However, following the 4th and 5th injections a definite decrease in size was noted (Table IV and V).

Our histological studies on the free end of the incisal socket on the labial side show a noticeable diminution in the osteogenic layer of the periosteum. This was especially noticeable in a group of rats which had received injections of cortisone started on the day of birth and continued to the 5th day (Figure 2 and 3). The number of trabeculae in the middle plate of the free end of the alveolar process are reduced and are replaced by a wide intertrabec-

ular space which contains numerous blood cells (Figure 2 and 3). This abundance of blood cells could be attributed to an inhibitory effect on bone formation and the absence of an effect on blood vessel formation thus giving the crowded appearance observed. Another suggestion might be the appearance of hemopoietic bone marrow which seems plausible considering the hemopoietic effect of cortisone observed by Finch, Crockett, Ross and Bayless (1951). The possibility that hyperemia was produced in our experiments is also suggested.

Labial alveolar periosteum

In the 18 day fetus tooth anlage, a periodontal membrane as such can not be recognized, but in 20 and 21 day old fetuses collagenous fibers and fibroblasts are seen to intercede between the tooth and its bony surroundings. These structures are not very numerous in either treated or control but they are none-the-less present in both.

In postnatal rats treated with 1, 2 or 3 mg of cortisone no definite conclusion could be reached with respect to an effect on this structure. A reduction in its thickness is seen in some rats treated with a total of 4 or 5 mg of cortisone (Figure 2 and 3). In some of the treated rats collagen fibers seem to be fewer in number and in others the cells of the labial alveolar periosteum also show a reduction in number. The blood vessels which become prominent with consecutive injections in some of the treated animals make it increasingly difficult to determine the precise character of this structure.

Oral epithelium

The albino rat is among those animals in which keratinization of the

oral epithelium is normally observed. The effect of cortisone is seen in this tissue following the injection of 1mg in both postnatal and prenatal rats. The nucleoli appear prominent in the treated of both groups. In prenatal rats a phenomenon which was observed in the case of fetuses given an injection of 500 gamma on the 17th and 19th day was a shrinkage of the nuclei (Figure 4 and 5). This condition was observed only in this experiment and may be a result of the technic employed. In those rats treated on the 18th and 19th day and killed on the 20th day of gestation and those treated on the 19th and 20th this shrinkage was not observed.

Following later injections an increase in the number of granules is observed in the epithelial cells of postnatal rats. These granules are refractile and variable in size (Figure 6 and 7). The above phenomenon is associated with a decrease in the thickness of the oral epithelium and an increase in the thickness of the keratinised layer which covers the outer surface of the oral epithelium (Figure 6 and 7). Whether this increase in thickness of the cornified layer is due to a decrease in the rate of desquamation or an increase in the rate of formation of keratinized cells was not determined.

Size of the maxillary incisors

The determination of the length and width of the upper incisors from histological sections as described above was not as ideal as one would want to have it. However, the following observations are supported by data from histological sections and from dissected incisors (Tables III-V).

In prenatal rats given 2 injections of 500 gamma each on the 18th and

19th days of gestation an increase in the length of the upper incisors was observed (Figure 8 and 9). In 18 day fetuses which had received a single injection of 500 gamma on the 17th day the dental papillae also show an increase in size (Figure 10 and 11). In general all treated prenatal rats showed more prominent papillae than controls. This would seem to indicate a stimulation of the growth of maxillary incisors under the influence of cortisone in prenatal rats. However, in prenatal rats which were injected on the 17th and 19th days of gestation such an increase was not observed.

In postnatal rats we observed considerable variation in the size of the upper incisors following the injection of 1 mg of cortisone as indicated from measurements of sections. In Table III measurements of the size of dissected incisors are given from which it must be concluded that the size decreased slightly when 1 mg of cortisone was injected on the day of birth but not so when 1 mg was administered on the 3rd day postnatally.

The inhibitory action of cortisone on the upper incisors of postnatal rats became more apparent with successive injections. Rats having received 4 or 5 mg of cortisone generally had smaller incisors than their litter mate controls (Table IV and V). This was particularly evident in one experiment where dissected incisors of rats given a total of 4 and 5 mg in daily doses of 1 mg beginning on the day of birth were measured (Table III).

The odontogenic cells, enamel and dentine

The odontoblasts which differentiate from the mesenchymal tissue of dental papillae generally did not seem to be affected by cortisone treatment

during the prenatal period. However, in a few prenatal rats of 20 days having received a total of 1 mg in utero a decrease in the number of these cells was observed but because of the infrequent occurrence of this condition in treated rats further observations will be required before definite conclusions can be drawn.

The ameloblasts, which secrete enamel and are of ectodermal derivation, showed a slight but constant decrease in size in postnatal rats having received more than one injection. This decrease in size of ameloblasts was also observed in treated prenatal rats in which the maxillary incisors had undergone an increase in growth as a consequence of cortisone treatment (Figure 9).

Thus with consecutive injections a slight decrease in the width of the ameloblast layer and in the width of the dentine and enamel layers was observed (Table IV and V). Whether this applies also to the odontoblast layer remains to be seen. This is in accord with the decrease observed in the size of the incisors in postnatal rats receiving more than one injection. The general histological appearance of these layers is however similar to that of controls.

Blood vessels.

The appearance of the blood vessels in the pulp of incisors and in the supporting structures presents an interesting picture. In prenatal rats of 18 days the blood vessels of dental papillae have not yet formed. However, blood spaces are present which are surrounded by primitive endothelial cells.

In fetuses of 20 and 21 days the dental papillae show blood vessels with walls similar to those found in older animals, the endothelial cells are

slender and their nuclei elongated. In comparing the endothelium of treated and control fetuses, age 20 days, one finds that the treated approach more nearly the mature state than do the controls. However, by the end of the first day postnatally the appearance of the blood vessels approaches that normally present in the pulp of older rats.

Following 1 injection of cortisone, (500 gamma in prenatal and 1 mg in postnatal), we observed a dilation of the blood vessels in the pulp. Since the pulp of the continually growing incisor has a rich blood supply the hyperemia is not always easily recognized however, its presence in some treated rats could be established without any doubt. In this connection it should be indicated that all postnatal rats were killed by decapitation and immediately following cessation of bleeding the specimens were put into the fixative. Our method of killing the animals may therefore be the reason for the empty blood vessels sometimes observed in our preparations.

A dilation of blood vessels and hyperemia was also observed in rats that had received several consecutive daily injections. There was some variation here also but the condition was demonstrable in approximately half of the treated animals (Figure 12 and 13). However, in dissected incisors the majority of those from treated rats showed a pinkish color which was more intense in the treated than in the controls.

DISCUSSION

Our observations confirm the work of Glickman, Stone and Chawla (1953)

who, experimenting with mice 4 to 6 weeks old receiving daily injections of 0.5 mg of cortisone for 43 days, concluded that a decrease in the interdental septa of the alveolar bone occurred. We have also observed a decrease in the incisal socket of newborn rats having received a total of 2 to 5 mg of cortisone. The observations of Goldsmith (1953) on the alveolar bone of mature rats having received daily injections of 3.0 mg of cortisone for a period of 1 year do not agree with ours. He reports an increase in the amount of alveolar bone and a narrowing of the marrow spaces of the mandible and attributes this result to a retardation in endosteal resorption. This apparent contradiction in results we believe to be due to the fact that Goldsmith's injections were started at an age when growth of the facial bones was already completed hence no inhibitory action on growth would be evident in his material whereas there might be a disturbance in the process of bone resorption. The smaller size of the alveolar process in our experimentals is attributed to impairment of growth of bone which is at its peak at this time.

In studying the morphological effects of cortisone on the growing skull of rats, Moss (1955) observed a decrease in the facial vault. He also noted that the sutural lines were acellular, avascular and relatively fibrous. This observation is in accord with that of Massler and Schour (1951) who in experimenting with alizarine red "S" on rats reported that the increase in length and width of facial bones occurred at the internasal and frontonasal sutures. Thus the effects observed in our experiments can be interpreted as being due to a combined inhibition of growth at the sutural lines and in the periosteum of

the alveolar process both of which are principally composed of connective tissues. Other observations on the effects of cortisone on connective tissues confirm this point. In this connection the work of Ragan, Howes, Plotz, Meyer and Blunt (1949) who found a decreased amount of granulation tissue in wounds of cortisone treated rabbits and Castor and Baker (1950) who observed a reduction in the number of fibroblasts in dermis, associated with fused collagenous fibers, in rats given topical applications of cortisone is relevant. Asboe-Hansen (1952) demonstrated that the mast cells of connective tissue also become degranulated under the influence of cortisone.

The above results support the observations of Domm and Leroy (1955) who in their study on cortisone injected rats concluded that a shortening of the maxillary bones occurred in treated animals. These authors also observed a shrinkage of the gingival tissues in young cortisone treated rats. Gingival tissue being primarily composed of connective tissue and epithelium did undergo regression in our experiments. Our studies on oral epithelium in cortisone treated rats revealed an increase in keratinization which reduced the thickness of the oral epithelium. The consequent decrease in depth of tissues through which the tooth must pass in order to erupt into the oral cavity is suggested by Domm and Leroy (1955) as one of the factors responsible for the precocious eruption of incisors in cortisone treated rats.

While the shrinkage of the oral epithelium may be a factor in the precocious eruption of the incisors in newborn cortisone treated rats it does not explain the accelerated growth and attrition observed by Domm and Marzano (1954)

and Garren (1954) in mature cortisone treated rats whose teeth had erupted long before treatment began.

Our observations on the effects of cortisone on the growth of the continuously growing upper incisors of the rat present an interesting picture. The occurrence of effects and their intensity depended on the duration of treatment and the age at which injections were begun. In most of our prenatal rats injected in utero an increase in the size of the upper incisors was observed. In postnatal rats a single injection did not reveal any very noticeable effects, however additional injections apparently had an inhibitory effect resulting in a decrease in the size of the upper incisors. A decrease in the size of incisors following injection of cortisone in young rats was also reported by Domm and Leroy (1955).

An initial increase in the size of transplanted molars was observed by Fleming (1953). This author transplanted the molars of 20 day old guinea pig fetuses into the eye chambers of mature guinea pigs. Some of the hosts were treated daily for 8 days with 2.5 mg of cortisone while others served as controls. Following the initial injection there was an augmentation in the size of transplants but with additional injections a regression ensued. In explaining his observations this author suggested that the abundance of plasma due to the increased dilation of blood vessels in the iris and the early vascularization of transplants resulted in an initial stimulatory effect on the growth processes. The initial stimulation was followed by a later inhibition in the growth of transplants accompanied by a decrease in the dilation of blood spaces.

and Ross
Goldsmith/(1954) studying the mandibular incisors in newborn rats given 0.1 mg daily injections of cortisone reported having observed an accelerated odontogenesis and amelogenesis. This would seem to imply a direct effect on the growth of dental tissues. However, from the work of Fleming (1953) one could conclude that an indirect effect on growth occurred being secondary to an early dilated vascularization of transplanted molars and a consequent increased availability of plasma. In our study on prenatal rats an increase in length of incisors was observed in rats also having well formed blood vessel walls. We also observed that dilation of blood vessels in postnatal rats is associated with a decrease in length of incisors under conditions in which precocious eruption would nevertheless have occurred. In other words it would appear that we may simultaneously have an increase in rate of eruption and a decrease in rate of growth the latter indicated by the observed decrease in the size of the incisors. Herzberg and Schour (1941) after removing the basal growing end of the continuously growing incisor reported a continuation of normal eruption thus supporting the concept that eruption may occur without growth and also that growth and eruption are two independent processes.

The effects on amelogenic and odontogenic cells, enamel and dentine were also studied. These layers show little difference between treated and controls. The slight decrease in the length of ameloblasts and the slight decrease in the width of the dentine and enamel layers may be regarded as evidence of an inhibitory action of cortisone on these structures.

Since experimental evidence seems to exclude growth as the cause of eruption attention was directed to other effects associated with the adminis-

tration of cortisone in the rat. A seemingly significant finding in our study was the presence of dilated blood vessels in the pulp of the incisors and frequently also in the surrounding tissues following single and multiple injections. This gave a hyperemic aspect to the structures being studied. Fleming (1953) reported the occurrence of dilated blood spaces around the transplants of molars in guinea pigs having received injections of cortisone. Similar observations were made by Applebaum and Seelig (1955) in studying the pulp of molars in rats treated with cortisone. An increase in the volume of blood cells was reported by Finch, Crockett, Ross and Bayles (1951) in anemic arthritic patients who had received cortisone. Dilation of blood vessels and hyperemia could conceivably be indirect factors in causing an increase in the size of incisors by virtue of the fact that they may provide an increased amount of nutrient material to the tissues involved. However, it should be noted that cortical extracts have been observed to decrease the permeability of capillaries (Menkin, 1940). Opsahl (1949) observed a decrease in the spreading reaction of india ink after treatment with cortical extracts so that although hyperemia at first might mean an increase in the availability of nutrient materials it would ultimately result in a decrease of this material owing to the decrease in permeability and spreading. Consequently, the hyperemia will only result in increased pressure in the tissue which in turn may result in decreased activity of odontogenic cells and thus inhibition of growth. Inhibition of growth was observed in the incisors in our experiments where hyperemia occurred.

The presence of blood in increased quantity in the dental structures

under consideration raises other questions namely, how this condition is brought about and what its effects are. The answer may have a bearing on the process of eruption. Constant (1900) advanced the idea that blood pressure was an important factor in the process of eruption.

The observations of Ashton and Cook (1952) in in vivo studies using the rabbit ear chamber method demonstrated a direct effect of cortisone on blood vessel walls. They reported that the injection of cortisone caused constriction of the walls of arteries and veins. This observation was confirmed by Wymen, Fulton, and Schulman (1953) in the Golden Hamster cheek pouch in in vivo studies. As stated above we have observed dilation of blood vessels in the pulp of incisors in our cortisone treated rats and in some instances also in the supporting structures. This is in apparent contradiction with the observations by Ashton and Cook (1952) and of Wymen et al (1953). However, it should be noted that the veins in the pulp of incisors have walls in which smooth muscle is not seen. Whether this is a factor in the observed dilation cannot be determined from our experiments. However, the presence of hyperemia itself is our chief concern here. The constriction of systemic blood vessels could very well bring about an increase in blood pressure. As a matter of fact a hypertensive condition following cortisone treatment is reported by several authors notably Knowlton, Loeb, Stoerk and Seegal (1949), and Applebaum and Seelig (1955).

The possible relationship between blood pressure and eruption was suggested by Constant (1900) who based his contention on anatomical studies carried out in lambs and pigs. This author called attention to the relationship

between the tooth and the alveolar bone, namely that the tooth is not in direct contact with the surrounding bone during the process of eruption but instead a gelatinous tissue with extensive vascularization is placed between them. Thus, as the tooth grows it pushes against this highly vascular gelatinous tissue which probably acts as an erectile tissue. It is the opposition of this tissue to the pressure of growth which this investigator believed to be responsible for the occlusal movement of the tooth. This concept might allow one to suggest a relationship between hypertension, hyperemia and the increased rate of eruption in cortisone treated rats. In this connection King (1936) observed an increased rate of eruption in the rabbit following removal of the inferior dental nerve as well as following the direct removal of the sympathetic ganglia supplying the head region. Hyperemia of the pulp after elimination of the sympathetic discharge was also noticed in his histological studies. This author attributes the increased rate of eruption to an increase in the growth rate caused by hyperemia. Thus, the exact role of hyperemia can not be established in accelerated eruption. But that it may be one of several factors seems indicated from both anatomical studies and experimental data.

SUMMARY AND CONCLUSIONS

1. In these experiments rats of two age groups were used. Fifty-seven prenatal rats in which 500 gamma of cortisone per injection was administered once or twice in utero, and 94 postnatal rats ranging from 1 to 11 days in age and given 1 to 5 mg of cortisone in daily 1 mg doses.

2. The effects of cortisone depended on the administered dose and the age at which injections were given. Individual variations occurred.

3. The incisal socket in prenatal rats did not show any apparent change in treated animals however, where several injections had been administered in postnatal rats the alveolar process was smaller in treated than in controls. The inner or osteogenic layer of the periosteum covering the labial alveolar process was thinner, the difference in size being particularly evident at its free end. In the middle plate of the free end of the alveolar process the number of trabeculae was reduced and replaced by a space containing blood cells.

4. The labial alveolar periosteum revealed modifications in postnatal rats after 4 or 5 injections. It showed a decrease in width and in some cases its cells and collagenous fibers were reduced in number.

5. Cortisone administration produced rapid changes in the oral epithelium. In postnatal rats the nucleoli of epithelial cells appeared prominent and the granules had increased in number where several injections had been administered. This was accompanied by an increase in thickness of the keratinized layer and a decrease in the thickness of the non-karatinized portion of the oral epithelium.

6. The size of the upper incisors had increased in 20 day prenatal rats which had received injections on the 18th and the 19th days of gestation.

An increase in the size of dental papillae could also be demonstrated in 18 day old prenatal rats which had received an injection on the 17th day of gestation. However, there was a tendency for the occurrence of longer incisors in 21 day prenatal rats having received injections on the 19th and the 20th days and in 20 day prenatal rats having received an injection on the 19th day of gestation. A decrease in the size of dissected incisors was observed in postnatal rats following single and repeated injections. The experiments show that cortisone, in the dosages and at the ages administered, had a stimulatory effect on upper incisors in prenatal and an inhibitory effect in postnatal rats.

7. Histologically there was no apparent change in odontogenic cells, enamel or dentine. However, a slight decrease in the width of these layers was observed following repeated injections in postnatal rats which coincides with the observed decrease in the size of upper incisors. In prenatal rats in which an increase in the size of incisors was observed, a decrease in the length of ameloblasts was also manifested, the explanation for which is not evident.

8. A dilation of the blood vessels in the pulp of the incisors and in the supporting structures was observed in pre and postnatal rats after one injection of cortisone, presenting a hyperemic appearance. The hyperemia however was less evident in postnatal rats though it could be readily demonstrated following several injections.

9. The attempt was made to establish a relationship between the effects observed in these experiments and the occurrence of precocious eruption in cortisone treated rats. The size of the incisors and of the alveolar bone, which were smaller in treated rats, would seem to indicate that precocious eruption cannot be attributed to an acceleration in the growth of these structures. It is suggested that the shrinkage of tissues and the resultant decrease in depth of tissues, through which the tooth must pass in order to erupt, as well as the induced hyperemia, are factors which must be taken into account in any consideration of the problem.

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TABLE I

PRENATAL CORTISONE TREATED RATS

GROUP I

	Age at 1st inj. (days)	Control Fetuses	Treated Fetuses	Dose per inj. Gamma	Total Dose (mg)	No. of injs.	No. Recovered Alive C	T	Dead C	T
Female No. 1	1st 17	2	9	500	0.5	1	2	5	0	2
Female No. 2	1st 17 2nd 19	2	9	500 500	1.0	2	1	5	1	1
Female No. 3	1st 18 2nd 19	4	10	500 500	1.0	2	3	6	1	1
Female No. 4	1st 19 2nd 20	3	5	500 500	1.0	2	3	5	0	0
Female* No. 5	1st 18 2nd 19 3rd 20	4	14	500 500 250	1.25	3	0	0	4	11
Female No. 6	1st 19	4	8	500	0.5	1	4	8	0	0
Total		19	55				13	29	6	15

* Since all the fetuses of this female were dead on the morning following last injection histological studies could not be made.

A total of 74 fetuses were injected in utero. Of these 63 were recovered. We could not account for 11 fetuses.

TABLE II

POSTNATAL RATS TREATED WITH CORTISONE

GROUP II

Expts.	Age at 1st inj. (Days)	No. of Tr'd.	No. of Contr.	Dosage per inj. (mg)	No. of inj.	Total Dose (mg)	Duration of treatment (Days)
2-a	0*	4	2	1	1	1	1
2-b	0	4	2	1	2	2	2
3	0	5	4	1	3	3	3
3-a	0	3	2	1	4	4	4
3-b	0	4	3	1	5	5	5
5-a	3	4	2	1	1	1	1
5-b	3	4	2	1	2	2	2
6-a	3	4	3	1	3	3	3
6-b	3	4	2	1	4	4	4
4-I	5	4	1	1	1	1	1
4-II	5	4	2	1	2	2	2
4-a	5	3	2	1	3	3	3
4-b	5	2	2	1	4	4	4
7-a	7	2	1	1	1	1	1
7-b	7	2	2	1	2	2	2
8-a	7	3	2	1	3	3	3
8-b	7	2	2	1	4	4	4
Totals		58	36				

* Day of birth

TABLE III

MEASUREMENTS ON DISSECTED MAXILLARY INCISORS IN POSTNATAL RATS

Expts.	Age at 1st inj. (days)	Dose per inj. (mg)	Total Dose (mg)	Weight in grams				Incisor Length				Incisor Width			
				Control		Treated		Control		Treated		Control		Treated	
				Beg.	End	Beg.	End	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
								60	58	54	52	19	17	16	17
II-1	0	1	1					65	60	57	60	15	17	18	18
								65	62	64	60	18	18	17	18
										60	60			19	19
Aver.				5.6	5.7	6.0	5.5	63.3	60.0	58.8	58.0	17.3	17.3	17.8	18.0
S. D.								±4.1	±2.8	±6.0	±6.9	±3.0	±1.0	±1.7	±1.4
								89	87	75	75	23	22	22	20
II-4	0	1	4					88	87	76	80	23	25	23	20
										75	75			23	22
Aver.				6.1	11.5	6.1	6.9	88.5	87.0	75.3	76.6	23.0	23.5	22.7	20.7
S. D.								±1.0	±0.0	±2.0	±4.1	±0.0	±2.2	±1.0	±1.7
								89	95	78	78				
III-5	0	1	5					94	93	77	77				
								85	85	81	78				
										81	79				
Aver.				4.8	8.9	5.1	4.9	89.3	90.1	79.2	78.0				
S. D.								±6.4	±7.4	±3.6	±1.4				
								85	80	85	80	23	23	26	25
II-2	3	1	1					88	86	82	80	24	24	23	24
Aver.				8.1	10.0	8.6	8.7	86.5	83.0	83.5	80.0	23.5	23.5	24.5	24.5
S. D.								±2.2	±4.2	±2.2	±0.0	±1.0	±1.0	±2.2	±1.0
								100	97	103	101	27	30	26	26
II-3	5	1	1					100	102	100	101	29	27	25	24
								101	99	95	94	27	27	24	27
Aver.				14.2	15.7	12.3	11.9	100.3	99.3	99.3	98.6	27.8	28.2	25.0	25.7
S. D.								±1.0	±3.6	±5.7	±5.0	±1.4	±2.4	±1.4	±2.4

TABLE IV

MEASUREMENTS ON SECTIONS OF MAXILLARY INCISORS AND ALVEOLAR BONE IN POSTNATAL RATS*

Rats	Incisors		Alveolar Width			Width of			
			Labial		Lingual	Amel.	Odont.	Enamel	Dentine
	W	L	Ant.	Mid.	Mid.	Layer	Layer	Layer	Layer
Contr.	45.0	100.0	17.0	4.0	5.0	28.0	35.0	4.0	12.0
Contr.	45.0.	148.0	19.0	5.5	5.0	30.0	35.0	3.0	11.0
Aver.	45.0	124.0	18.0	4.7	5.0	29.0	35.0	3.5	11.5
S. D. ±	0	33	1.0	1.0	0	1.4	0	1	1
Tr'ed.	36.5	111.0	15.0	2.0	4.0	26.0	30.0	3.0	10.5
Tr'ed.	40.0	113.0	14.0	2.0	6.0	25.0	35.0	4.0	11.0
Tr'ed.	44.0	105.0	15.5	1.5	6.0	25.0	28.0	3.0	9.5
Tr'ed.	44.0	90.5	14.0	2.0	6.0	27.0	30.0	3.5	10.5
Aver.	40.1	106.3	14.6	1.8	5.5	25.7	30.7	3.3	10.2
S. D. ±	7.2	17	1.4	2.9	1.7	1.4	5.1	1	1

* Dosage 1 mg per day for 4 days beginning on the third day.

Amel. - Ameloblast

Odont. - Odontoblast

TABLE V

MEASUREMENTS ON SECTIONS OF MAXILLARY INCISORS AND ALVEOLAR BONE IN POSTNATAL RATS*

Rats	Incisors		Alveolar Width			Width of			
			Labial		Lingual	Amel.	Odont.	Enamel	Dentine
	W	L	Ant.	Mid.	Mid.	Layer	Layer	Layer	Layer
Contr.	42.5	89.0	19.0	6.0	6.0	30.0	33.0	5.0	11.0
Contr.	41.0	83.5	20.0	5.0	4.0	32.0	34.0	5.0	11.0
Aver.	41.7	86.2	19.5	5.5	5.5	31.0	33.5	5.0	11.0
S. D. ±	1	3.8	0	1	1.8	1.6	1	0	0
Tr'ed.	43.0	37.0	12.5	3.0	3.0	30.0	32.0	4.0	10.0
Tr'ed.	39.5	76.5	10.0	2.0	5.0	28.0	35.0	4.0	10.0
Tr'ed.	38.0	54.0	12.0	2.0	5.5	27.0	35.0	4.0	10.0
Aver.	40.1	55.6	11.5	2.3	4.5	28.3	34.0	4.0	10.0
S. D. ±	16	28	2	1.2	1.7	2.2	2.4	0	0

* Dosage 1 mg per day for 5 days beginning on day of birth.

Amel. - Ameloblast

Odonto.- Odontoblast

PLATE I

Figure 1. Diagram showing the points at which measurements on the upper incisor were made.

Abbreviations: A - Ameloblasts
AB - Alveolar bone
D - Dentine
E - Enamel
O - Odontoblasts

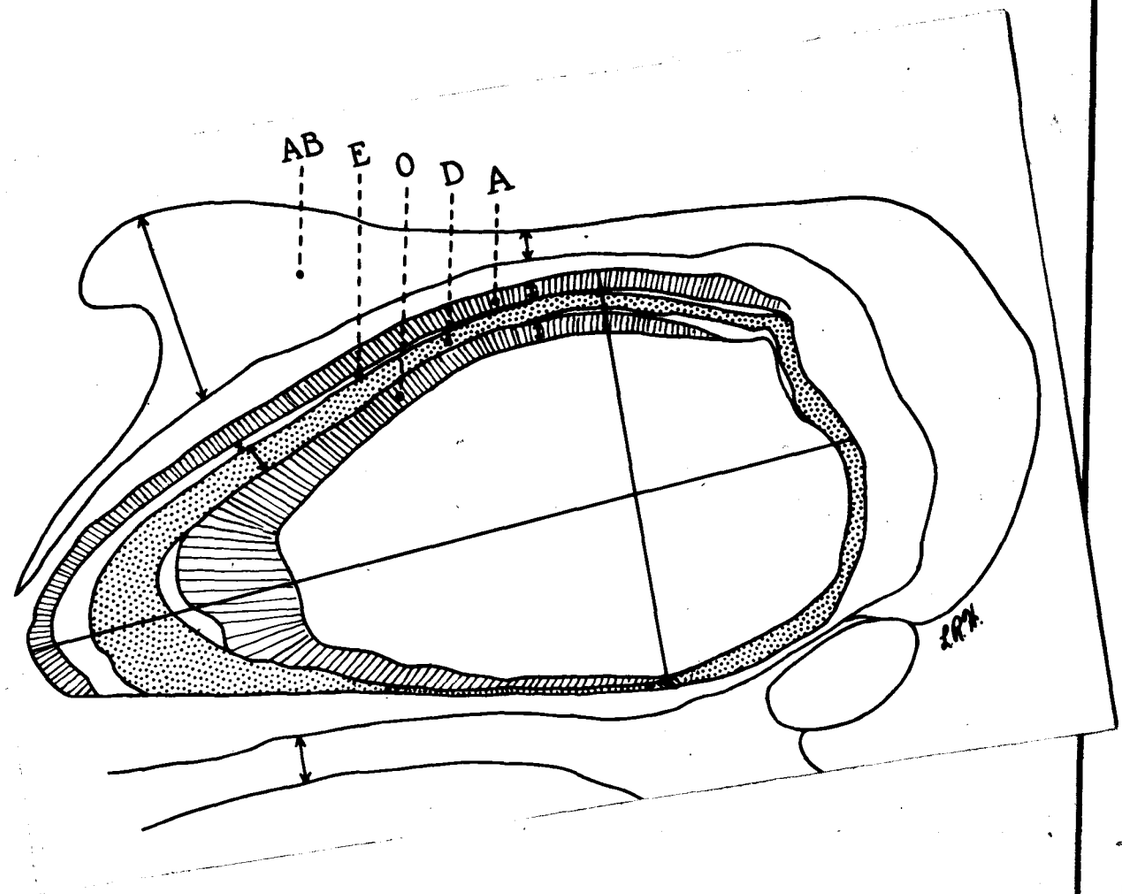

FIGURE 1

PLATE II

Figure 2. The free end of the incisal socket of a control postnatal rat age 6 days. (x90).

Abbreviations:
A - Ameloblasts
AB - Alveolar bone
D - Dentine
E - Enamel
O - Odontoblasts
OsL - Osteogenic layer
PO - Periosteum
POM - Periodontal membrane

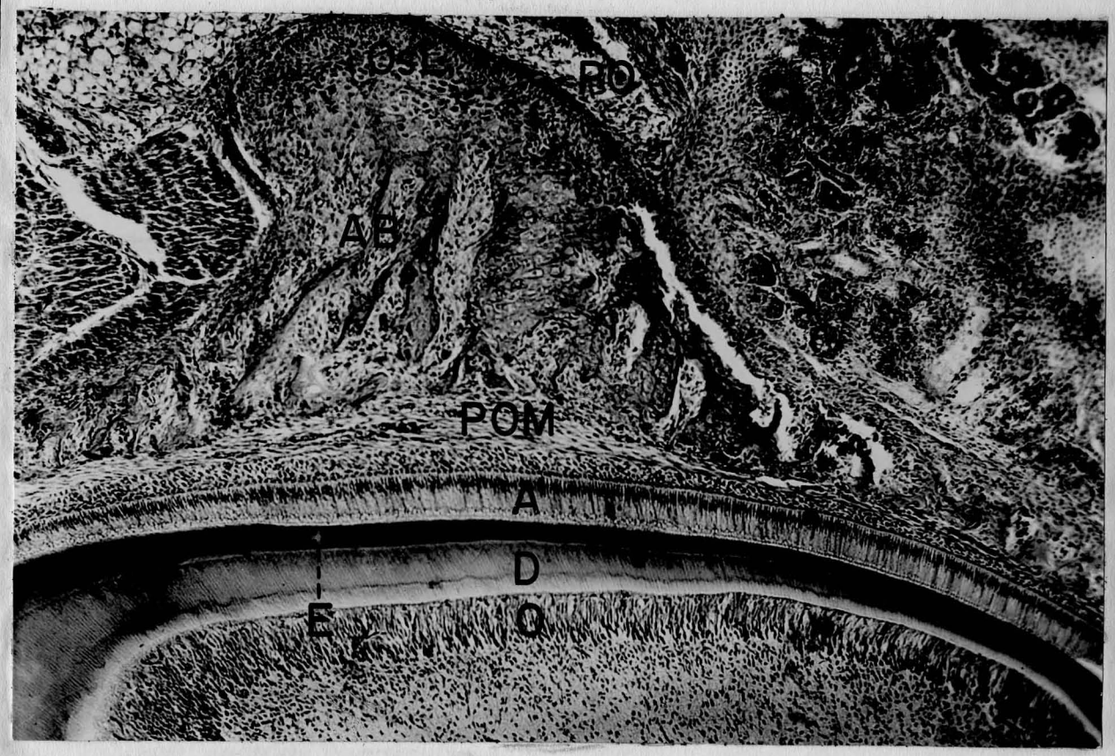

FIGURE 2

PLATE III

Figure 3. The free end of the incisal socket of a postnatal rat which had received 5 daily 1 mg injections of cortisone starting on the day of birth. Age 6 days. (x90).

Abbreviations:
A - Ameloblasts
AB - Alveolar bone
D - Dentine
E - Enamel
O - Odontoblasts
OsL - Osteogenic layer
PO - Periosteum
POM - Periodontal membrane

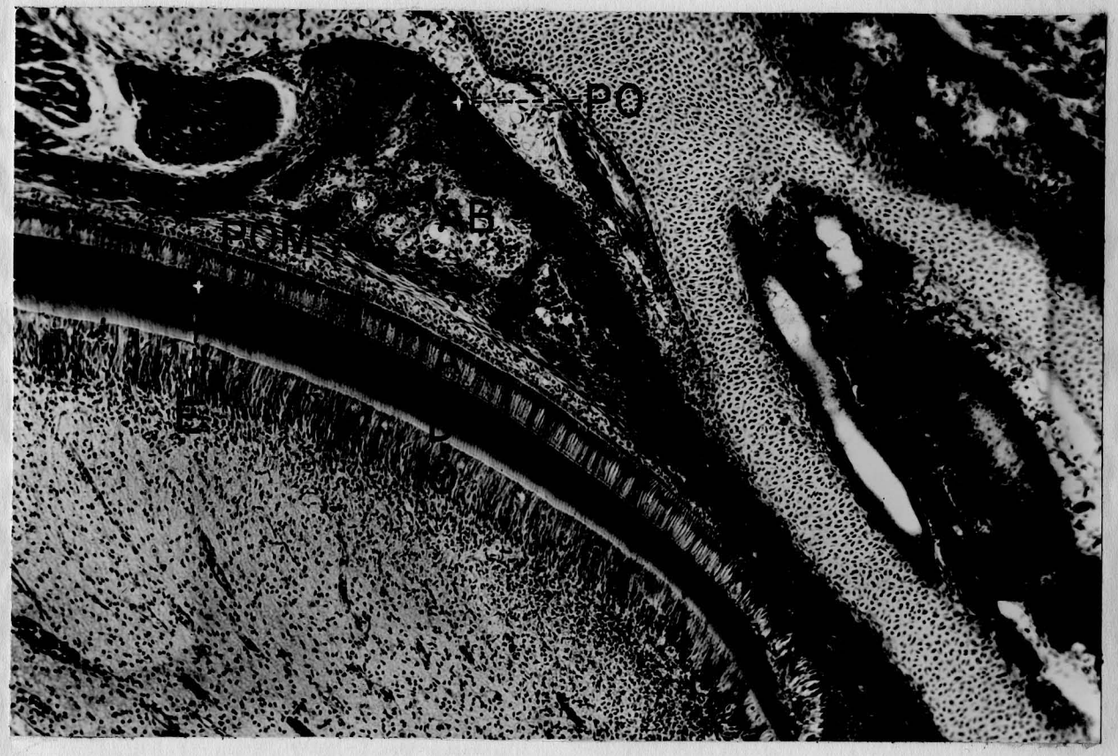

FIGURE 3

PLATE IV

Figure 4. The oral epithelium of a control fetus age 20 days. (x390).

Abbreviations: EC - Epithelial cells
OC - Oral cavity
OE - Oral epithelium

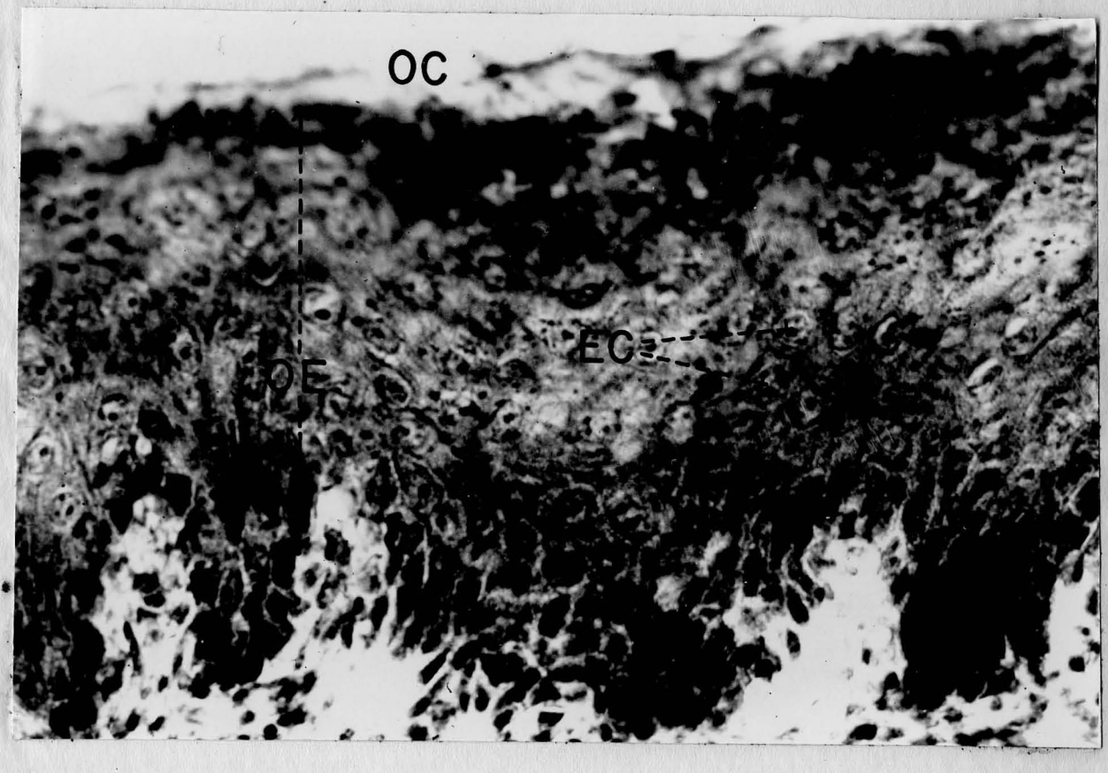

FIGURE 4

PLATE V

Figure 5. The oral epithelium of a fetus which had received 2 injections of cortisone, 500 gamma per injection on the 17th and 19th days of gestation. Age 20 days. (x390).

Abbreviations: EC - Epithelial cells
OC - Oral cavity
OE - Oral epithelium

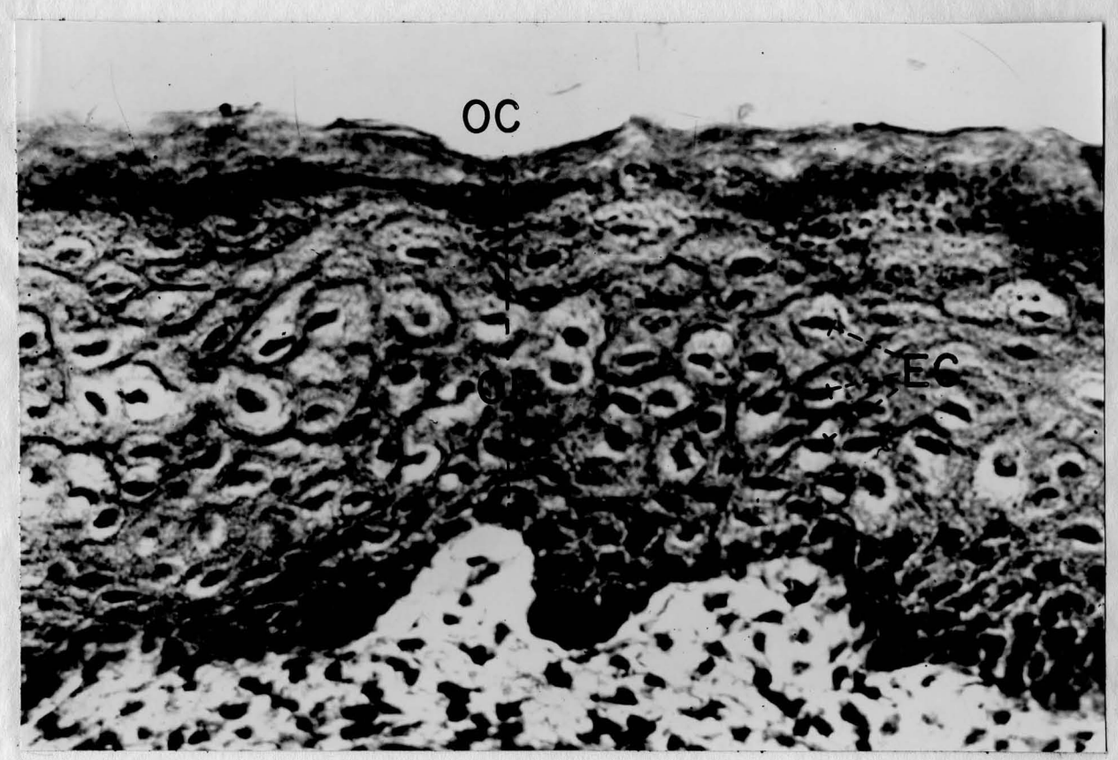

FIGURE 5

PLATE VI

Figure 6. The oral epithelium of a control postnatal rat age 6 days. (x390).

Abbreviations: CL - Cornified layer
G - Granules
OC - Oral cavity
OE - Oral epithelium

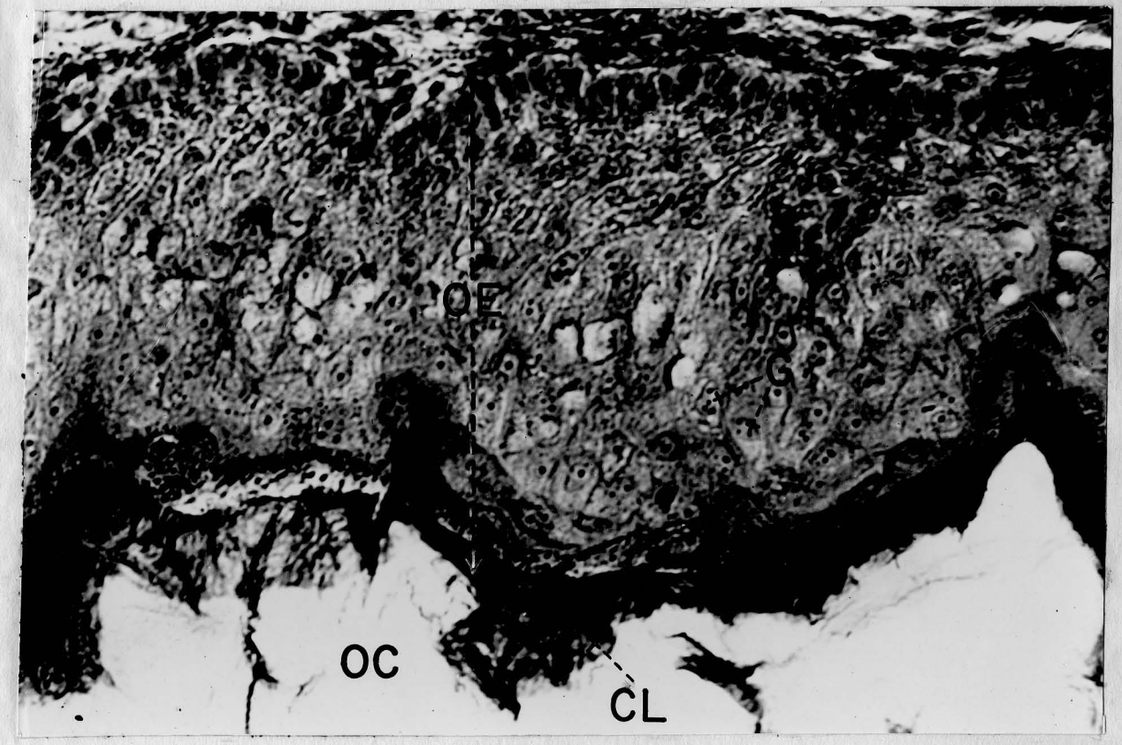

FIGURE 6

PLATE VII

Figure 7: The oral epithelium of a postnatal rat which had received daily injections of 1 mg of cortisone for 5 days starting on the day of birth. Age 6 days. (x390).

Abbreviations:
CL - Cornified layer
G - Granules
OC - Oral cavity
OE - Oral epithelium

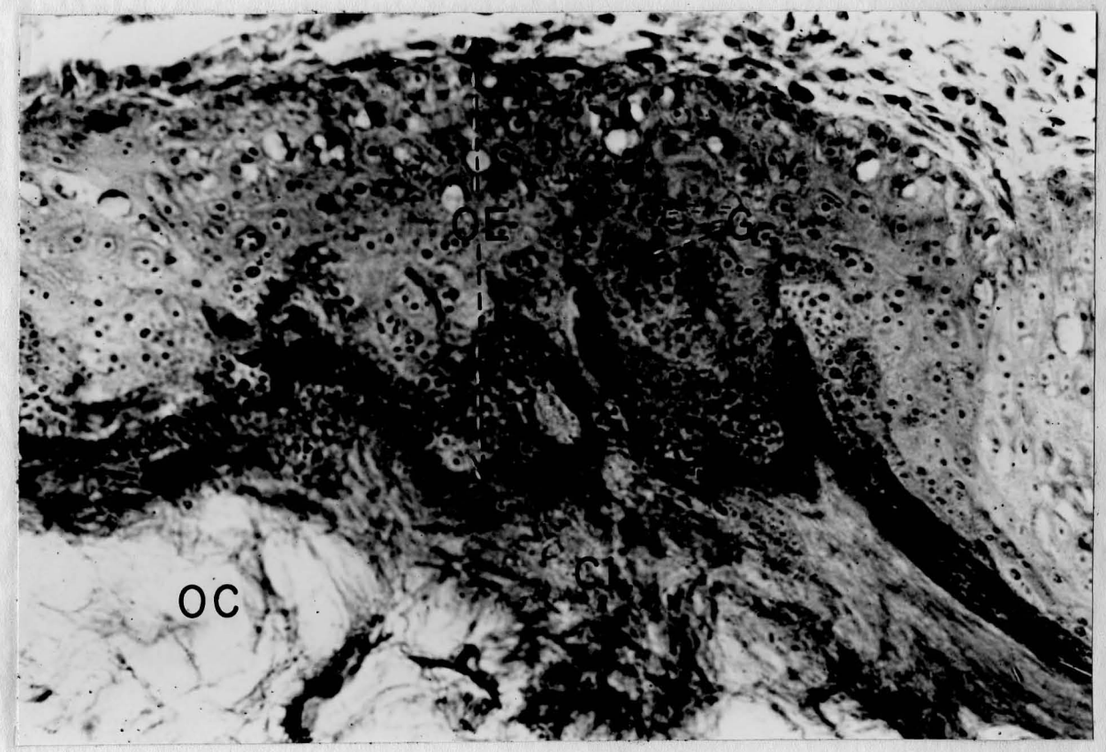

FIGURE 7

PLATE VIII

Figure 8. The upper incisor of a control fetus age 20 days. (x75).

Abbreviations:
A - Ameloblasts
AB - Alveolar bone
D - Dentine
HE - Hertwig's epithelial sheath
O - Odontoblasts
P - Pulp
POM - Periodontal membrane

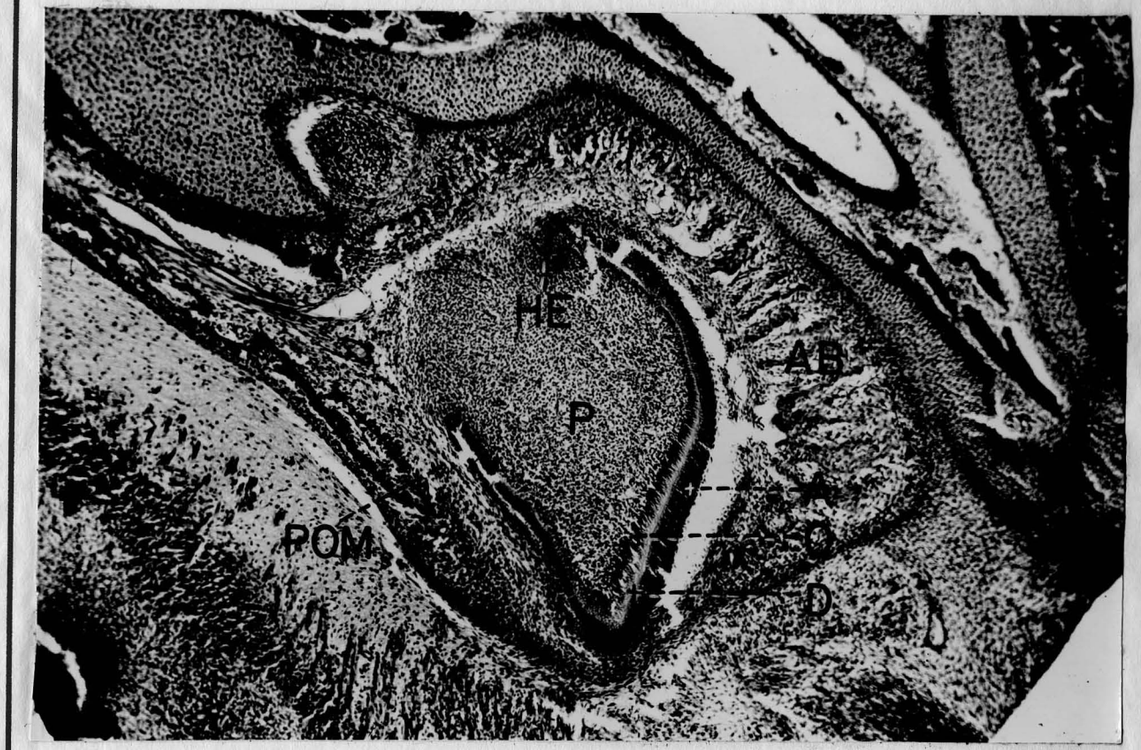

FIGURE 8

PLATE IX

Figure 9. The upper incisor of a fetus which had received two injections of cortisone, 500 gamma per injection on the 18th and 19th days of gestation. Age 20 days. (x75).

Abbreviations:
A - Ameloblasts
AB - Alveolar bone
D - Dentine
HE - Hertwig's epithelial sheath
O - Odontoblasts
P - Pulp
POM - Periodontal membrane

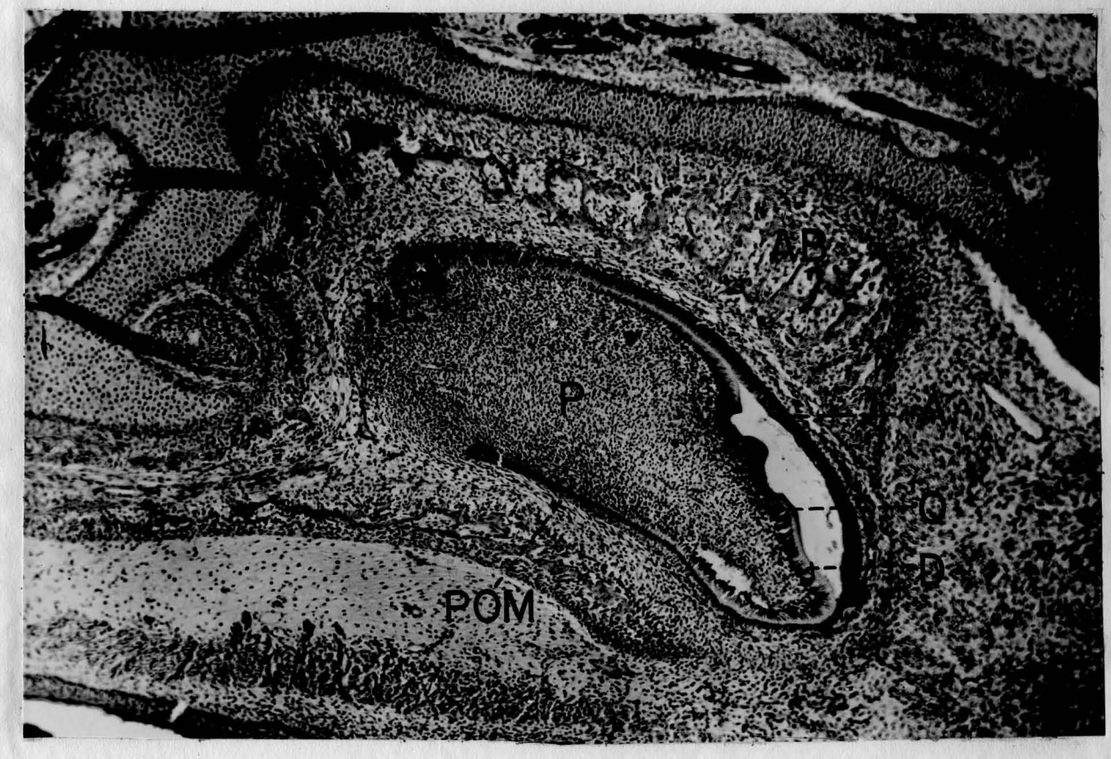

FIGURE 9

PLATE X

Figure 10. The dental papilla and enamel organ of a control fetus age 18 days. (x100).

Abbreviations: AB - Alveolar bone
DP - Dental papilla
EO - Enamel organ
OC - Oral cavity
OE - Oral epithelium
T - Tongue

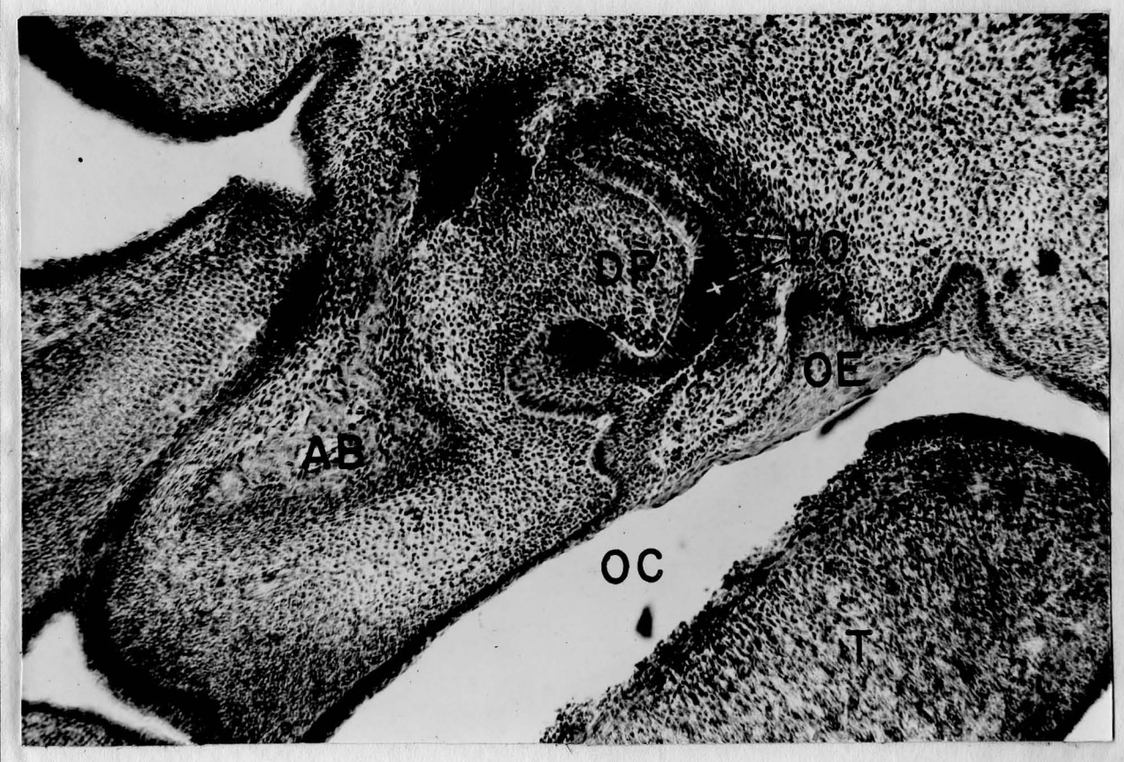

FIGURE 10

PLATE XI

Figure 11. The dental papilla of a fetus which had received 500 gamma of cortisone on the 17th day of gestation. Age 18 days. (x100).

Abbreviations: AB - Alveolar bone
DP - Dental papilla
EO - Enamel organ
OC - Oral cavity
OE - Oral epithelium
T - Tongue

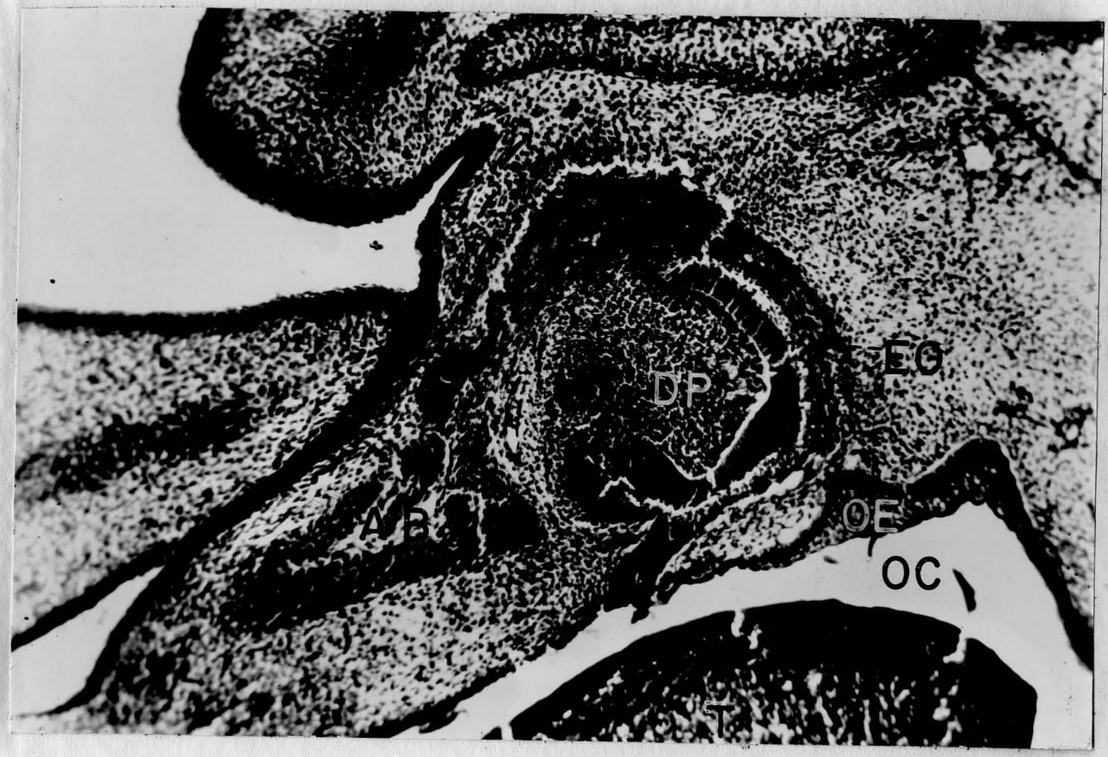

FIGURE 11

PLATE XII

Figure 12. The blood vessels in the pulp of an incisor of a control postnatal rat age 11 days. (x120).

Abbreviations: BV - Blood vessels
P - Pulp

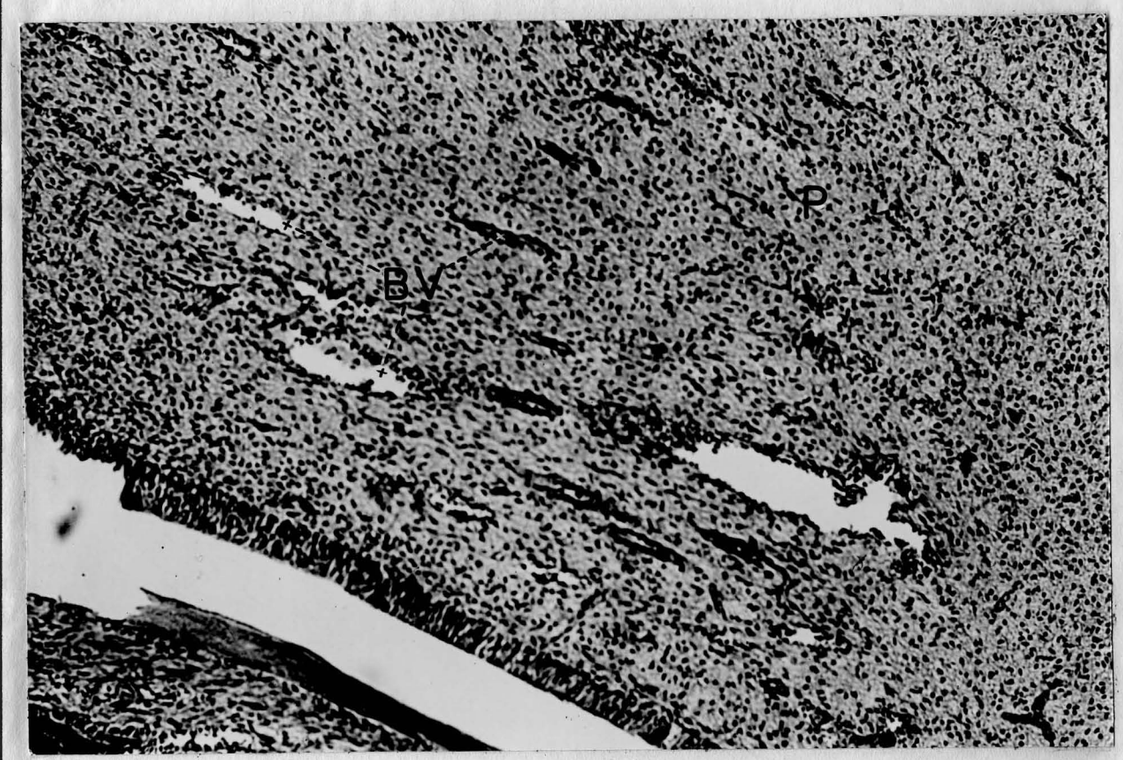

FIGURE 12

PLATE XIII

Figure 13. The blood vessels in the pulp of an incisor of a post-natal rat which had received daily injections of 1 mg of cortisone for 4 days beginning on the 7th day. Age 11 days. (x120).

Abbreviations: BV - Blood vessels
P - Pulp

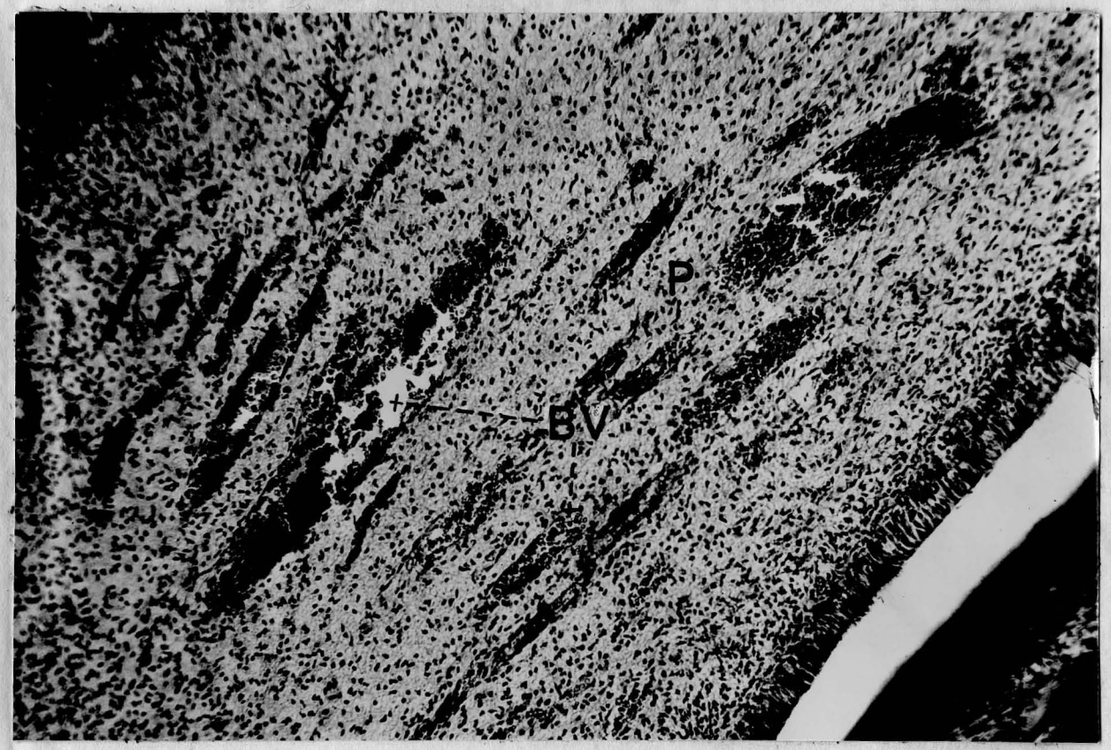

FIGURE 13

APPROVAL SHEET

The thesis submitted by Genia Mar-Yohana 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.

May 29, 1957
Date

L. V. Johnson
Signature of Adviser