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EFFECT OF TETRACYCLINE ON THE ODONTOGENESIS OF RATS

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ABSTRACT

K. W. Shyu and Chung Hu (1971) *Effect of Tetracycline on the Odontogenesis of Rats* Bull. Inst. Zool. Academia Sinica **10**:(1) 7-16. Effect of tetracycline therapy on the development of dental tissues was studied on female Sprague-Dawley rats. Different dosages of tetracycline hydrochloride were injected intraperitoneally daily to three out of four groups of animals for various durations. The teeth of each rat were examined macroscopically after sacrifice. No apparent discoloration or hypoplastic defects, or any change of caries incidence after therapy were found. Various combinations of tissue changes were noticed in both ground and paraffin sections of the teeth of rats receiving tetracycline. The abnormalities of odontogenesis observed in this study consisted of prominent incremental lines, malcalcification of hard tissues, thick predentin, interglobular dentin, malformed enamel matrix, degeneration of odontogenic cells, pigmented enamel and hyperemic pulp. The interrelationship between the timing of medication and the maldevelopment of dentitions, and the mode of action of tetracycline were discussed.

Tetracyclines, a group of widely used antibiotics of broad spectrum, have been used clinically for about 20 years. The tetracycline family consists of tetracycline (achromycin), chlortetracycline (aureomycin), oxytetracycline (terramycin), rolitetracycline (reverin), demethylchlortetracycline (ledermycin) and several other derivatives. This group of antibiotics was found to cause certain side effects in recent years. Renal and hepatic impairment can be induced by excessive doses administrated intravenously (31). The breakdown products of the antibiotics may cause so called "Fanconi syndrome" (9, 10). From both clinical and animal studies, a number of investigators have found that these antibiotics can deposit in bones and teeth after extremely high doses (15, 16, 19, 25, 29, 37, 39, 41-49). This phenomenon arouses a great deal of interest in both medical and dental professions. The deposition of these antibiotics was only found in the formed hard tissues, such as teeth and bones, and was demonstrated mostly with fluorescent microscopy in these studies. From these previous investigations we know that the pigmentation and hypoplastic defects induced by the deposition of tetracyclines are usually found in the tissues which are forming during tetracycline therapy, the primary effect of the drug on teeth is most probably on odontogenic cells, but its mechanism is not well understood. Investigations designed to elucidate such interrelationship are far from abundant. The

present study is designed on this point of view and demonstrates some histological effects of tetracycline on both odontogenic cells and formed hard tissues of rat teeth.

MATERIALS AND MEOTHODS

Since the effect of tetracycline should be studied on developing teeth, the observation was made on rat teeth due to their continuous growth throughout the life, and their functional similarity of odontogenic cells with that of human teeth.

Fluorescent method used in most of the previous related studies would not be adopted in this study because, as Kutcher *et al* (19) had pointed out, the results of fluorescent studies were usually inconsistent and might give rise to a great deal of confusion and misunderstanding. Direct observation of the histological changes of the rat teeth after continuous administration of tetracycline was made instead of fluorescent microscopy after only few injections of the drug.

Fourty female *Sprague-Dawley* rats of outstanding physical condition were selected from a group of weaning rats after 20 days of feeding on laboratory diet (chicken feed, Taiwan Sugar Co., Taipei, Taiwan). Their average body weight was 170 gm. They were divided into four groups of ten each at random. Each rat was given a daily injection of different drugs intraperitoneally as follws:

Group A-the control group. Each rat was injected with 0.1 ml normal saline/200 gm body weight daily. Three to four rats each time were sacrificed by means of ether inhalation on the 26th, 40th and 60th day after first injection.

Group B-the group receiving average therapeutic dose. Five therapeutic doses (TD-one TD equivalent to 1 mg tetracycline/200 gm body weight) of freshly prepared solution of tetracycline hydrochloride (Taiwan Cyanamid Co., Taipei, Taiwan) were injected to each rat daily for five days, then followed by 1 TD daily up to the date of sacrifice. Three out of the ten animals were sacrificed on the 26th day (rat number B8, B9, B10). Rats B5, B6, B7 were sacrificed on the 40th day, and the other remaining rats B1, B2, B3 and rat B4 were sacrificed on the 60th day. The dose for rat B4 was increaced to 10 TD from 41th day to the date of sacrifice for 20 days in order to observe the tissue response to the abruptly changed dose.

Group C-the group receiving moderately heavy therapeutic dose. Ten TD of tetracycline to each rat for 5 days, then followed by 2 TD daily for 21 days. Rats C8, C9, C10 were sacrificed on the 26th day. The remaining rats were given 2TD per day from the 26th to 40th day. Rats C5, C6, C7 were sacrificed on the 40th day. The other remaining rats C1, C2, C3 were injected 2 TD but C4, 10 TD daily until they were sacrificed on the 60th day.

Group D-the group receiving excessively high dose. The rats were injected with 30 TD of tetracycline daily in the first 5 days. Rats D8, D9, D10 were sacrificed on the 6th day. Then, 10 TD daily was given to each remaining rat. They were sacrificed successively on the 26th (rats D6, D7), 40th day (rats D3, D4, D5) and 60th day (rats D1, D2).

Immediately after sacrifice, each rat was examined macroscopically for the number of dental caries, color and gross developmental defects of teeth.

Both the maxilla and the mandible, with their teeth *in situ*, of each rat were dissected out immediately and fixed in 10% formalin for 10 days. After fixation, the left halves of both jaws of each rat were decalcified, processed, embedded in paraffin wax and cut into bucco-lingual and mesio-distal sections of 6 microns and stained by hematoxylin and eosin; while the right halves were prepared into ground sections. All the sections were examined microscopically for

the changes of odontogenic cells as well as that of the hard tissues of the teeth. The results were recorded photomicrographically and analysed.

RESULTS

Macroscopical examination revealed no gross hypoplastic defect or tooth discoloration, nor apparent differences of caries incidence among groups of experimental animals.

In microscopic examination, various combinations of tissue changes were found in different groups of rats. All the rats administered tetracycline showed hyperemia of dental pulp.

There was no abnormal changes in the teeth of group A rats (Fig. 1).

In group B, no significant tissue changes were found in the teeth of all the rats except B4 which had been given 40 days of 1 TD followed by 20 days of 10 TD tetracycline and sacrificed on the 60th day. These tissue changes, evidently caused by the increased dosage, consisted of exaggerated incremental lines in its dentin (Fig. 2), degenerated odontoblasts forming a homogenous structureless belt (Fig. 3, 4), thick predentin between the degenerated odontoblasts and the primary dentin (Fig. 4), irregularly formed dentin (Fig. 3), malcalcified dentin (Fig. 4) and unevenly formed enamel matrix with accentuated incremental lines (Fig. 4).

In group C, rats sacrificed on the 26th day revealed interglobular dentin in both incisor (Fig. 5) and molar (Fig. 6) without any other significant findings. But in those animals sacrificed after 40th day, there were apparent abnormalities of odontogenesis. Faulty enamel matrix in various styles could be seen in many sections (Fig. 7, 8, 9, 10). Thick predentin and unevenly calcified dentin (Fig. 7), degeneration of ameloblasts (Fig. 8, 9, 10) and odontoblasts (Fig. 9) appeared in all of these rats.

In group D, the rats received excessively high dose (30 TD/day) for only short duration (5 days) also presented apparent changes in dental tissues, such as exaggerated incremental lines in dentin (Fig. 11), Wavy ingual dentin (Fig. 12) and markedly dilated blood vessels in both pulp and periodontal membrane (Fig. 12). Interglobular dentin, thickened predentin (Fig. 13, 14) and degenerated odontoblasts (Fig. 13) were noticed in the rats sacrificed on 26th day. Rats of the last sacrifice revealed, in addition to the forementioned findings, wavy and malformed buccal dentin (Fig. 15), interglobular dentin and pigmentation in enamel (Fig. 16).

DISCUSSION

Relationship between the severity of tissue changes and the dosage and duration of the administration of the drug is, in general, in proportion. The tissue changes caused by injections of different doses of tetracycline for different durations may be summarized as follows:

Doses and durations	Tissue changes
Control group	negative
1 TD for 60 days	negative
2 TD for 26 days	mild
2 TD for 40 or more days	moderate
1 TD for 40 days followed by 10 TD for 20 days	moderate
10 TD for more than 40 days	severe
30 TD for 5 days	severe
30 TD for 5 days followed by 10 TD for 20 or more days	most severe

The increasing severity of the tissue changes is apparently kept in pace with increased dosage or duration of the administration of the drug. Judging from the above evidence, large dose as high as 10 TD daily or long continuous administration of moderate dose, e.g. 2 TD, of tetracycline should be avoided for developing teeth.

DISCOLORATION OF TEETH is the most evident clinical sigh caused by tetracycline therapy. Shwachman and Schuster first reported this finding in 1956 (34). Afterwards, many investigators presented similar reports (4, 11, 35, 39, 41-48). Wallman and Hilton (38, 39) showed that the yellow discoloration was most apparent at the cervical portion of incisors and the site of developmental defects of cuspid and molars. They proved with the aid of physicochemical and fluoromicroscopic methods that the substance deposited in the dentin and the enamel was actually tetracycline. Other investigators (4, 11, 42-49) came to the same conclusion. Among the side effects of tetracyclines, pigmentation of the teeth was most frequently discussed in all the publications (4, 5, 7, 19, 27, 28, 32, 33, 37-39, 41, 42-49). Most of these reports were concerned with human teeth. However, in the present experiment, this phenomenon was not observed in rat incisors because they were always markedly pigmented physiologically. The only incisor which showed enamel pigmentation microscopically was that of rat D1, the animal received the largest dose and the longest term of therapy. All the molars of the experimental animals did not present discoloration either grossly or microscopically. This was probably due to relatively small dose used in this investigation. Discoloration itself carried no significance except from the viewpoint of esthetics. Its observation was not the main purpose of this study.

Fluorescence has been used as an indicator of tetracycline induced discoloration of teeth in quite a number of studies (2, 18, 24, 26, 30-33). This, as indicated by Kutcher and his associates (19), was usually inconsistent and has been the source of confusion and misunderstanding. Therefore, fluoromicroscopy has not been adopted as a means of interpreting the tetracycline effects on teeth.

HISTOLOGICAL CHANGES of dental tissues were the main objects in this investigation. Small therapeutic dose induced no tissue changes in teeth of rats when the antibiotics was given up to 40 days. However, long-term application of low therapeutic dose followed by 10 TD for 20 days produced prominent incremental lines indicating malformation of dentin, and degeneration of odontoblasts (Fig. 2, 3, 4). With moderately heavy dose, short-term drug administration caused only malcalcified interglobular dentin; but there were malcalcification (interglobular dentin, thick predentin, unevenly calcification), malformation (faulty enamel matrix) of dental tissues, and even degeneration of the formative cells of both dentin and enamel in the rats received long-term injections (Fig. 5-9). In the group treated with excessively high doses, all the rats, including those injected for only 5 days, presented apparent dental changes (Fig. 10-16). These results are in agreement with Lofgren (23) and indicate that a certain correlation exists between the total dose of tetracycline administered and the degree of dental changes. This correlation has also been presented in Kienitz's excellent review (15). In this study, administration of average therapeutic dose did not cause teeth discoloration and dental tissue changes in contrast to what was reported by Frankel *et al* (7, 8). It was likely that the location of the dental tissue changes depended on the timing of the medication, in another words, the stage of dental development determined the location of the abnormalities (40). The findings in this experiment support

the above mentioned hypothesis that long continuous administration of the drug induced total dystrophy (in most figures) and short duration injections produced only partial dystrophy in the developing portion (Fig. 11) of dental tissues. On the other hand, Kienitz suggested that the duration of treatment was only of minor importance (15). Since the dental hypoplasia was formed in the growing tissues during the administration of the antibiotics, many investigators therefore related the timing of medication to hypoplasia of deciduous or permanent dentitions (7, 15, 16, 28, 32, 39, 41, 46). Frankel and his coworker (7, 8) reported that tetracycline given to the infants before 11 months old would cause deciduous teeth changes, while in Kienitz's report in 1966 (16), he arrived at the conclusion that tetracyclines exerted influence on deciduous teeth in 4 months to 1 year old, and on permanent teeth development in 4-month to 4-year-old infants. Since deciduous teeth start their development in early fetal life, the use of tetracyclines should rationally be avoided in early pregnancy as Moynahan suggested (28) up to 4 years old of age.

THE MECHANISM of action of tetracyclines on calcified tissues has not yet been exactly known. The tetracyclines are all chelating agents. They can form a more or less stable complex with bivalent metal ions (2) and possess a special affinity to calcifying tissues (12-14, 31, 39). This might be the most possible mode of action on bones and teeth. Some investigators suggested that tetracyclines could directly combine with the ground substance or calcium of bones or teeth (4, 24). Others indicated that some malignant tumors were able to store more tetracyclines for longer period (18, 26, 30). Still others correlated certain diseases to the tetracycline discoloration and hypoplasia of teeth, such as prematurity of infants (6, 27, 39), cystic fibrosis of pancrease (35, 44-49), kernicterus (6, 15), and nephrotic syn drome (29). The dental abnormalities were likely not caused by these diseases, but rather resulted from the use of tetracyclines in treating them. The transplacental effect of tetracyclines on teeth was reported by Kline *et al* in 1964 (3, 17). However, none of the investigators has discussed any possible mechanism of the drug affecting on the tooth development before calcification or even before the formation of the matrix of the dental tissues. Such early influence was observed in this experiment in forms of degeneration of both ameloblasts and odontoblasts. Probably this influence is responsible for the hypoplastic defects of tooth caused by tetracycline therapy. Further investigation is needed to prove this hypothesis.

CARIES INCIDENCE-The interrelationship between tetracycline therapy and caries incidence has been discussed since the clinical application of the drug. Stephan (36), Zipkin and Larson (20-22, 50, 51) reported reduced dental caries incidence after tetracycline therapy and attributed the result to the bacteriocidal and bacteriostatic effects of the drug. On the contrary, many other investigators (1, 5, 15, 17) disclosed a high susceptibility of tetracycline-affected teeth to caries because of their hypoplastic defects. In other studies, Frankel (7, 8), Weyman and his coworker (42) reported no influence of tetracyclines on the incidence of dental caries. In this regard, opinions were widely divergent. No definite relationship has been demonstrated in the present experiment because there were only few dental caries detected from each group. Further study of long duration is needed to clarify this problem.

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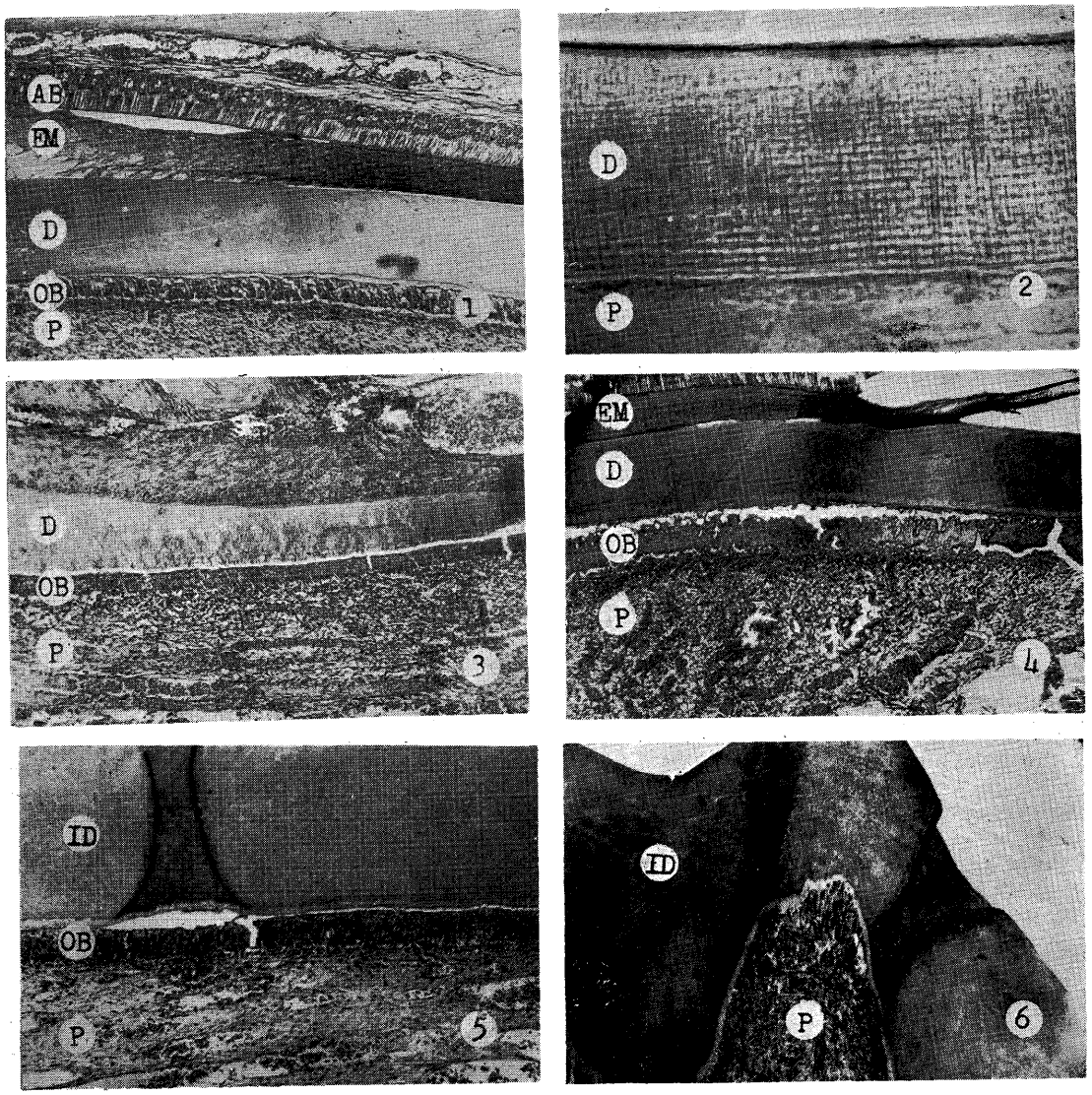

Fig. 1. Normal structure of an incisor of a control rat (× 100). Abbreviations: P-pulp; OB-odontoblast; PD-predentine; D-dentine; EM-enamel matrix; E-enamel; AB-ameloblast.

Fig. 2. Ground section of the incisor of rat B4 showing numerous exaggerated incremental lines in dentine (× 100).

Fig. 3. Lingual portion of the mandibular incisor of rat B4. Note the degenerated odontoblasts and malformed dentine (× 100).

Fig. 4. Labial portion of the mandibular incisor of B4. The odontblasts are degenerated to form a homogenous belt. Thick predentine, uneven dentine and malformed enamel matrix can be found (× 100).

Fig. 5. Mandibular incisor of rat C10. Note pulp hyperemia and interglobular dentine (× 100).

Fig. 6. A mandibular molar of rat C8 showing interglobular dentine (× 40).

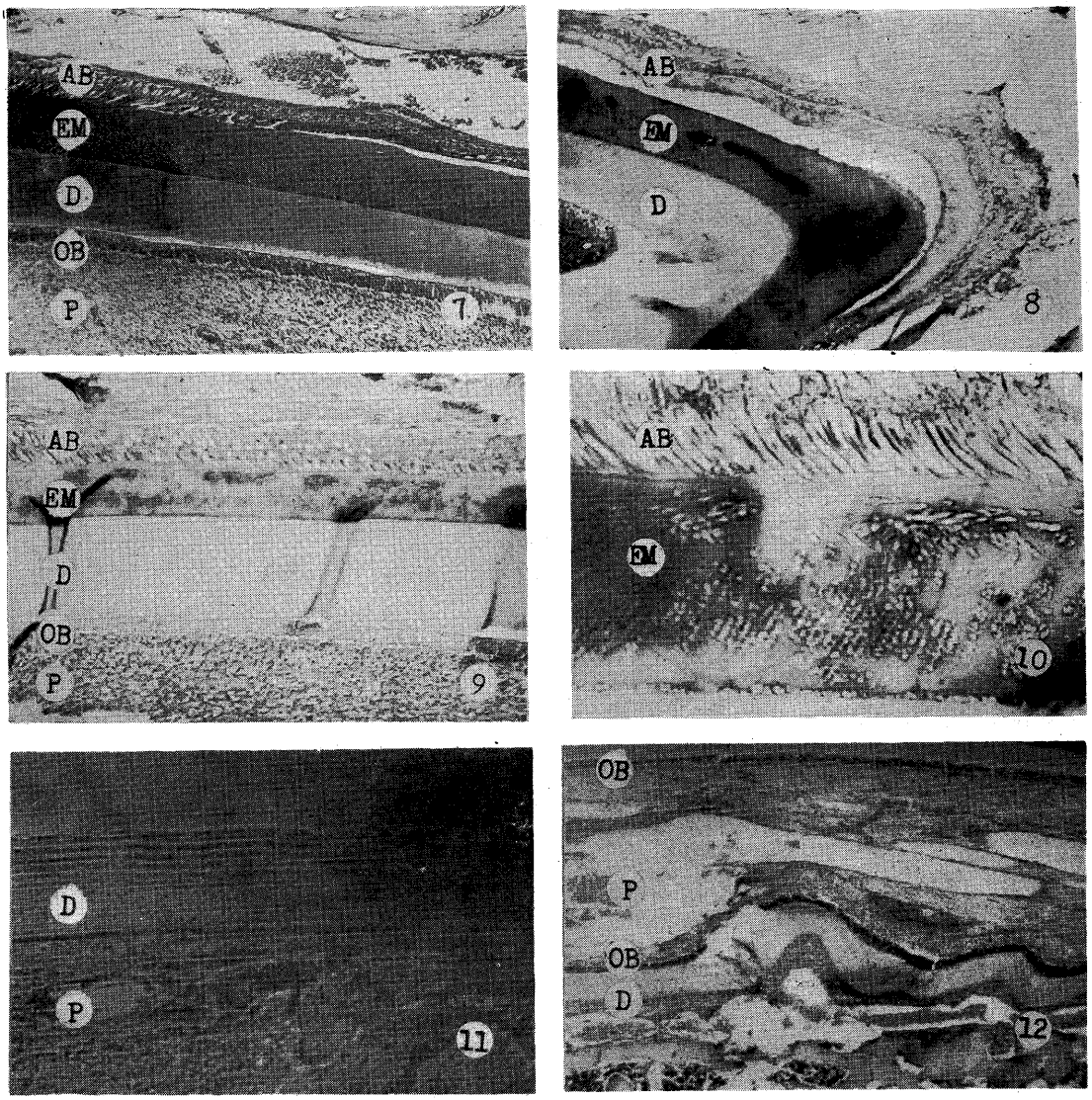

Fig. 7. Labial half of the mandibular incisor of rat C5 revealing thick predentine, uneven dentine and defective enamel matrix (× 100).

Fig. 8. The basal end of the mandibular incisor of rat C5. Note the malformed enamel matrix and the degeneration of ameloblasts (× 100).

Fig. 9. Labial portion of the mandibular incisor of rat C2 showing degenerated odontoblasts, defective enamel matrix and mildly degenerated ameloblasts (× 100).

Fig. 10. The high magnification of the enamel matrix in Fig. 9. The defects in enamel matrix and the degeneration of ameloblasts are clearly demonstrated (× 450).

Fig. 11. Ground section of the mandibular incisor of rat D8 showing accentuated incremental lines corresponding to the time of injections of tetracyclines (× 100).

Fig. 12. Lingual portion of the mandibular incisor of rat D9. Its dentine layer is wavy. The blood vessels in both pulp and periodontal membrane are dilated (× 100).

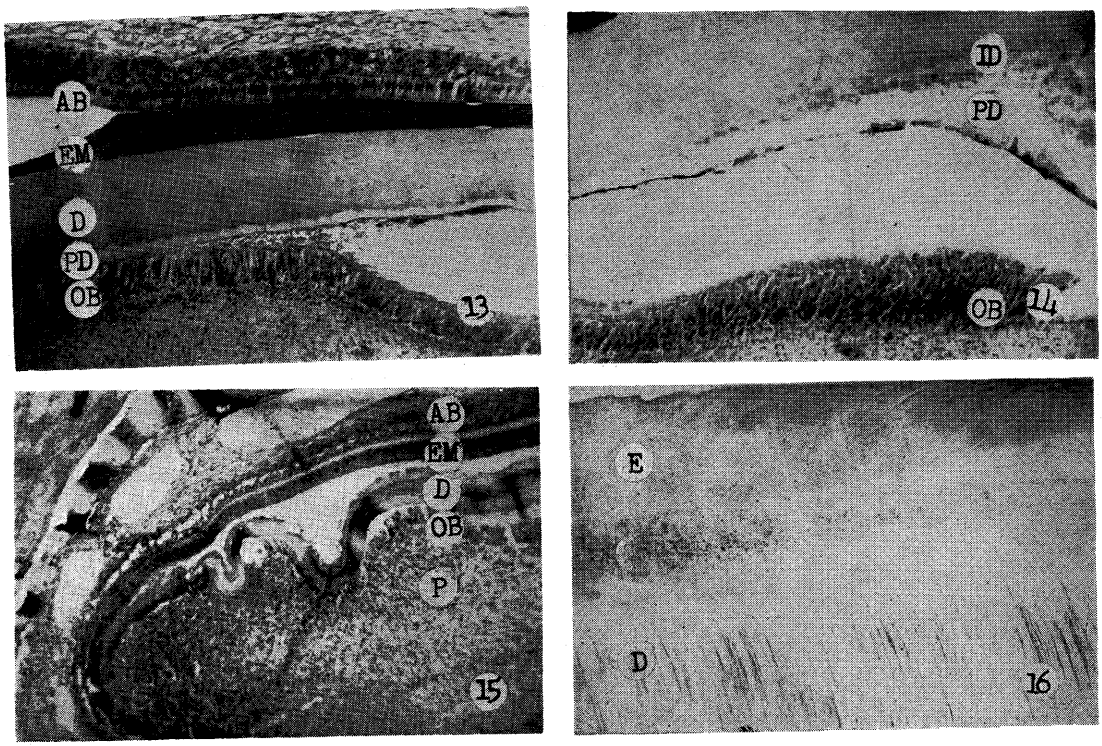

Fig. 13. Labial portion of the mandibular incisor of rat D6. Note the thick predentin, the interglobular dentine and the degenerating odontoblasts (× 100).

Fig. 14. The mandibular incisor of rat D7. The marked thick predentine and the interglobular dentine are apparent (× 100).

Fig. 15. Defective and wavy labial dentine at the basal end of the lower incisor of rat D2 (× 100).

Fig. 16. Cround section of the incisor of rat D1. Note the interglobular dentine and the pigmented enamel on the upper left (× 100).