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EXPERIMENTAL CHRONIC FLUOROSIS IN YOUNG RATS RECEIVING SUPPLEMENTARY DOSES OF VITAMIN D

by

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INTRODUCTION

Certain points of similarity between rickets and chronic, experimentally induced fluorosis in young animals have led some authors to suggest that fluorosis is of a rickets-like nature. This opinion has been voiced in publications by *Westin* (1935), *Bauer* (1945), and *Bélanger et al.* (1958).

In 1942 *East* called attention to the probable synergistic effect of fluoride and vitamin D, pointing out that *Hauch, Steenbock & Parsons* (1933), *Morgareidge & Finn* (1940), and *Kempf & Nelson* (1941) had demonstrated a more satisfactory therapeutical effect on rickets when fluoride and vitamin D were administered together than when they were given separately. Furthermore, *Morgareidge & Finn* (1940) found that the manner in which the fluoride was supplied to the rats was of decisive importance. If it was mixed into the food (300 p.p.m. F^-) a synergistic effect with vitamin D occurred, while the action of vitamin D was destroyed if a fluoride solution was administered dropwise once a day.

Table 1

Survey of results of experiments in which extra doses of vitamin D were given to fluorosed animals

a) Chaneles	1929	Rats	Better growth and conditions in teeth
b) Armstrong	1933	Rats	No effect
c) Schour & Smith	1934	Rats	No effect
d) Smith	1936	Rats	No effect
e) Maschcrpa & Lusignani	1936	Guinea-pigs	Tendency to maintain Ca-content in bone
f) Muñoz	1936	Rats	No effect
g) Tempestini & Cannava	1938	Rats & guinea-pigs	No effect
h) Simada	1939	Rabbits	No effect
i) Bauer	1945	Puppies	No effect
k) Tempestini	1949	Rats & guinea-pigs	Better conditions in teeth of guinea-pigs
m) Hennon & alii	1961	Rats	No effect

Other investigators have examined whether supplementary doses of vitamin D might have a curative or mitigating effect on chronic fluorosis. These investigations are summed up in Table 1. Various vitamin D preparations were used, e.g. cod-liver oil, Viosterol (U.S.P. used as synonym for calciferol), and Vigantol®. The doses applied have varied. *Armstrong* (1933) gave 4 drops of 10 D cod-liver oil per day to rats fed a diet containing 0.1 per cent sodium fluoride (450 p.p.m. F^-). *Smith* (1936) gave 2—4 per cent cod-liver oil in the food or 1 drop of Viosterol per day. *Tempestini & Cannava* (1938) gave 1—2 drops of Vigantol daily, while *Bauer* (1945) and *Schour & Smith* (1934) merely stated that a supplement of vitamin D was given. One author, *Chaneles* (1929), irradiated the animals daily, for 10—20 minutes, using a Hanau lamp.

In experiments in which animals with fluorosis received supplementary doses of vitamin D (Table 1, a—m) the interest has been focussed particularly on: the growth of the animals (a, b), the macro- and microscopical conditions of the incisor teeth (a, b, c, d, f, h, k), the condition of the bones, and the histological structure of bone (h, i). A few of the authors have determined

the contents in the bone ash of Ca, P, CO_2, and F (e, f, m).

It is also apparent from Table 1 that two authors found that the course of the fluorosis was mitigated when supplementary doses of vitamin D were administered whereas other authors failed to obtain any such effect.

In an attempt to determine whether or not vitamin D has a mitigating effect on fluorosis an experiment was set up. The main purpose of this experiment was to compare, clinically and histologically, the incisors and the periodontium of the incisors and molars of the animals fed a fluoride-rich diet with or without supplemental vitamin D. In a few cases the changes were correlated with findings in normal rats.

MATERIAL AND METHOD

Determination of the fluoride dose

On the basis of experience gained from earlier experiments with 0.05 per cent sodium fluoride (225 p.p.m. F^-) added to the diet of rats, *Pindborg* (1950) and *Lindemann et al.* (1959), it was decided to add 0.05 per cent sodium fluoride to the basic diet. The animals were maintained on a basic diet, Meco-Dumex's diet no. 6, comprising 32.5 per cent skimmed milk, 41.4 per cent potato starch, 8 per cent dry yeast, 13 per cent peanut oil, 5 per cent sharkliver oil, and 0.1 per cent ferrotartrate.

Determination of supplementary doses of vitamin D

In order to cure experimentally induced rat rickets the Meco-Dumex laboratories have found a daily dose of 0.8 I.U. of vitamin D sufficient. In the present investigation it was decided to use a somewhat larger dose, viz. 10 I.U. of vitamin D per day. All animals received the vitamin by pipette. Animals in one group received the basic diet + 225 p.p.m. F^-, animals in the other group had the same fluoride-rich diet + 10 I.U. of vitamin D.

Twenty 38—39-day-old white rats were used in the experiment. Fifty per cent of the animals in each of the two groups were sacrified 28 days, and fifty per cent 64 days after the beginning of the experiment. The animals were caged separately

and received a weighed, sufficient, daily amount of food. Food left over was weighed the following day thus making it possible to know approximately the amount of food and fluoride consumed by the individual animals. The animals were weighed at the beginning of the experiment and subsequently once a week. The experimental data are recorded in Tables 2 and 3.

Table 2

Data of 39 days old rats given basic diet + 225 p.p.m. F^- for 28 days and for 64 days

Sex	Exp. period in days	Weight at start of exp.	Weight at end of exp.
♀		46	77
♀		51	95
♀	28	47	77
♂		54	100
♀		50	75
♂		56	180
♂		62	185
♂	64	56	164
♂		58	118
♀		55	105

Table 3

Data of 38—39 days old rats given basic diet + 225 p.p.m. F^- + 10 I.U. vitamin D for 28 days and for 64 days

Sex	Exp. period in days	Weight at start of exp.	Weight at end of exp.
♀		48	87
♂		61	137
♂	28	53	105
♂		50	106
♀		48	83
♂		53	138
♂		53	161
♂	64	61	180
♂		60	207
♂		57	195

The incisor teeth were examined clinically on two occasions, the first 13 days after the experiment had started, the second after sacrifice by ether of the animals. The heads were fixed in 4 per cent formaldehyde. The upper and lower jaws were dissected out, decalcified in 5 per cent nitric acid, and embedded in celloidin. Generally they were cut longitudinally, but in two cases the upper jaws were cut transversely. The sections were stained by haemalum-eosin and Hansen's connective tissue stain.

RESULTS

Ingestion of food and increase of weight

The food uptake and weight increase of male rats in the two diet-groups were calculated on the basis of a 28-day experimental period in order to obtain as many data as possible, Table 4. The figures did not differ significantly between the two groups. Student's t-test gave $t = 0.34$ for ingestion of food and $t = 0.84$ for increase in weight.

Table 4

Food consumption and gain in weight of male rats during 28 days of experiment

Basic diet + 225 p.p.m. F^-		Basic diet + 225 p.p.m. F^- + 10 I.U. vitamin D	
Food consumption gram	Gain in weight gram	Food consumption gram	Gain in weight gram
263	46	269	76
276	66	233	52
295	63	288	56
239	56	230	40
219	44	247	51
		273	59
		288	80
		279	74

Incisor teeth

Clinically, the incisors of rats in the two diet groups were not found to differ whether examined after 13, 28, or 64 days. In both groups the lower incisors showed evidence of beginning loss

of pigment after 13 days on the diet. After 64 days transverse hypoplastic striae typical of advanced fluorosis were visible in the enamel of all teeth, independent of type of diet.

Histological examination of rats receiving 225 p.p.m. F^- in the diet for 28 and 64 days

Enamel and enamel organ

It was characteristic that the enamel did not become completely acid-soluble at any site. The enamel matrix persisted more or less right to the gingiva, and accentuated incremental lines and numerous hypoplastic defects were noted. Proliferations from the stratum papillare into the labial periodontium were observed; occasionally cyst formation was seen in these proliferations, Figs. 1 and 2. Because of the persistent enamel matrix the sector, termed the fifth sector by *Pindborg & Weinmann* (1959), was not well-defined. Consequently it was difficult to decide if the ameloblasts, which normally become reduced in height in the

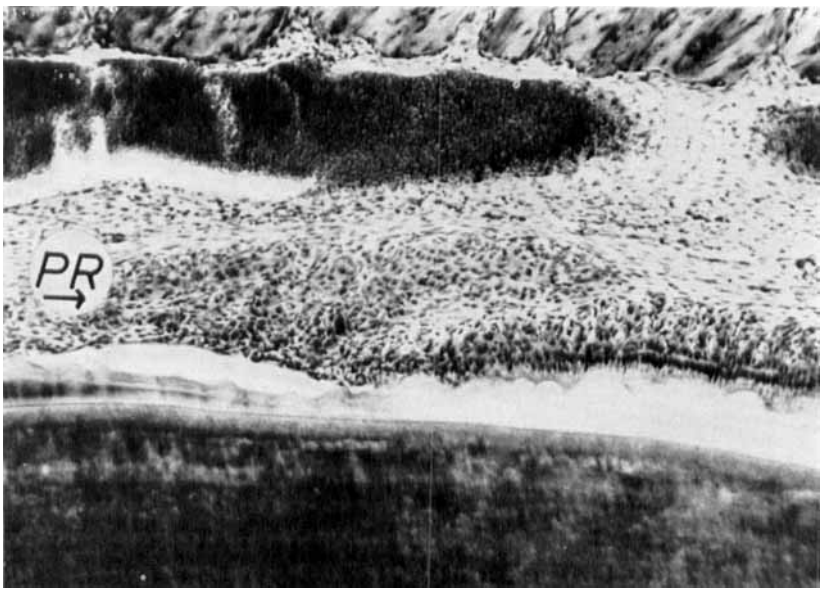


Fig. 1. Persistence of enamel matrix, accentuation of incremental lines and hypoplastic defects. Note proliferation from stratum papillare (PR), varying width and loose structure of labial premaxillary periodontal membrane with hyperaemia. Part of section of upper incisor from 67 days old rat on diet containing 225 p.p.m. F^- for 28 days. Orig. magnif. × 80.

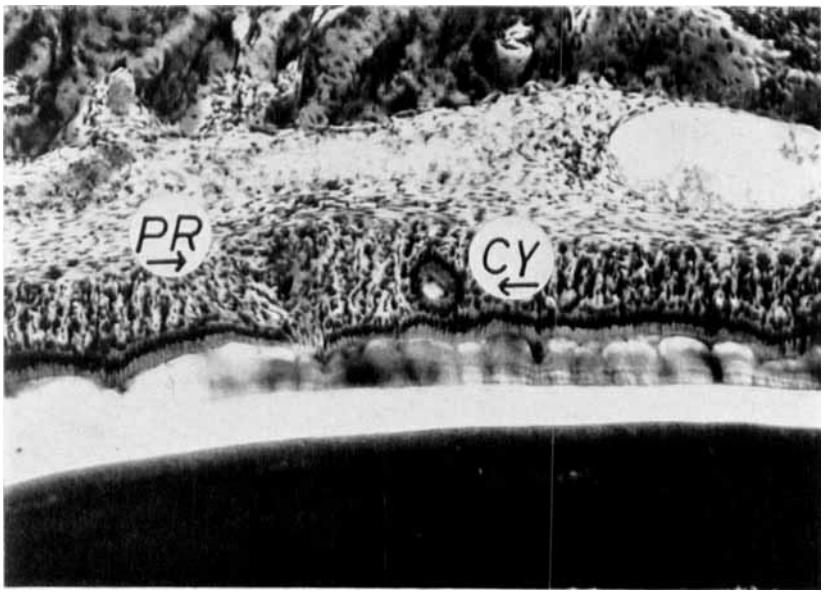


Fig. 2. Persistence of enamel matrix with hypoplastic defects and a small proliferation from stratum papillare (PR). At (CY) a small cyst is seen. Note desorganization of labial periodontal membrane. Part of section of upper incisor from 67 days old rat on diet containing 225 p.p.m. F^- for 28 days. Orig. magnif. × 80.

incisal end of this sector, atrophied prematurely, although this was the impression gained. Prior to the atrophy, the ameloblasts were irregularly arranged and of different heights; immediately proceeding the atrophy eosinophilic grains were demonstrable in the ameloblasts. In rats which had been on the experimental diet for 64 days the changes were more severe. The enamel was more hypoplastic and the proliferations from, and cysts in, the stratum papillare more numerous. In four out of five animals the enamel matrix was replaced by a thin basophilic zone.

Dentine and predentine

Poor mineralization of the dentine, particularly on the labial aspect, was encountered in all of the rats. The mineralization occurred in much smaller globuli than usual and thus the transition between predentine and dentine appeared granular. In addition, accentuated incremental lines were seen. The labial predentine which normally measures 10—20 μ across was much wider,

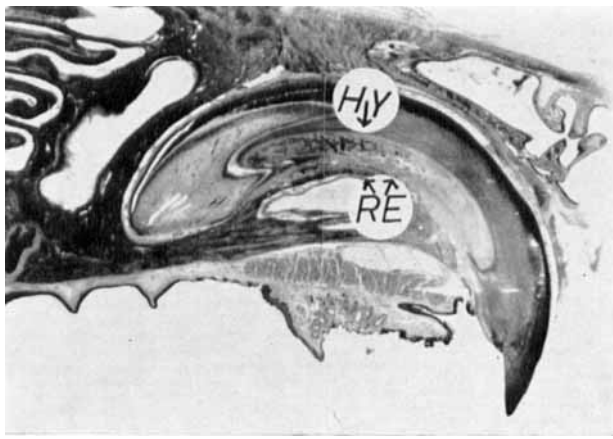


Fig. 3. Section with big lingual recess (RE). Note hyperaemia (HY). Section of upper incisor from 103 days old rat on diet containing 225 p.p.m. F^- for 64 days. Orig. magnif. × 7.

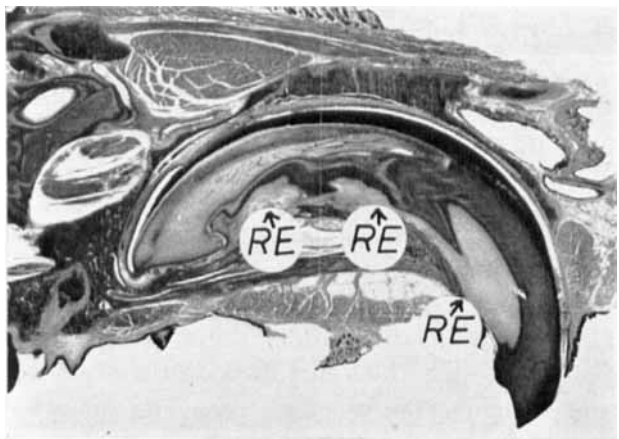


Fig. 3 a. Section with 3 lingual recesses (RE). Section of upper incisor from 102 days old rat on diet containing 225 p.p.m. F^- + 10 I.U. vitamin D daily for 64 days. Orig. magnif. × 7.

ranging from 36 μ up to 48 μ in rats in the 28-day experiment, and up to 70 μ in rats in the 64-day experiment. The lingual dentine was difficult to control and judge because of the frequent occurrence of lingual recesses, Figs. 3 and 3 a.

Periodontium of the incisor teeth

Marked hyperaemia was observed in the labial periodontal membrane, the width of which varied greatly, Figs. 1 and 2. In

areas with proliferations from the stratum papillare and with highly hyperaemic vessels the periodontal connective tissue was sparse. Where the proliferations and hyperaemias were less pronounced the connective tissue occupied a larger area. In this latter instance disintegration of the normal solid, fibrillar and well-oriented connective tissue was noted. The structure was loose, irregular, of a reticular character, and edema was seen in some areas.

In areas of the lingual periodontal membrane in which the incisors were free of recesses the structure remained normal. At the sites of the recesses the hyperaemia was intense, and here and there spheroid formations of osteoid tissue were found, Fig. 6.

In the alveolar bone at the upper incisors the processes of apposition and resorption occurred at the sites at which the normal, physiological apposition and resorption take place, *Sicher & Weinmann* (1944). All newly formed bone, however, was characterized by an irregular structure and an almost complete ab-

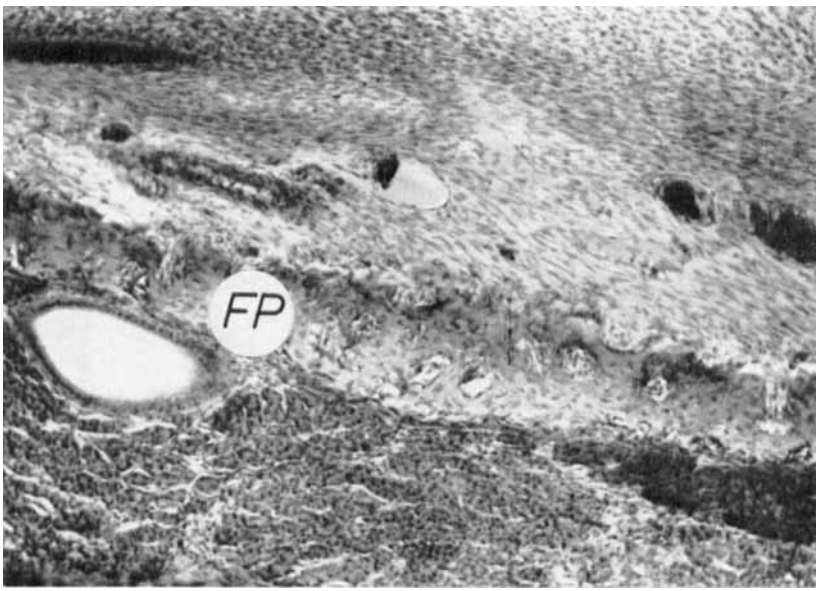


Fig. 4. Fundic plate from upper incisor (FP). Note irregular bone structure with coarse osteocytes and abnormal width of fundic plate. Part of section from 67 days old rat on diet containing 225 p.p.m. F^- for 28 days. Orig. magnif. × 80.

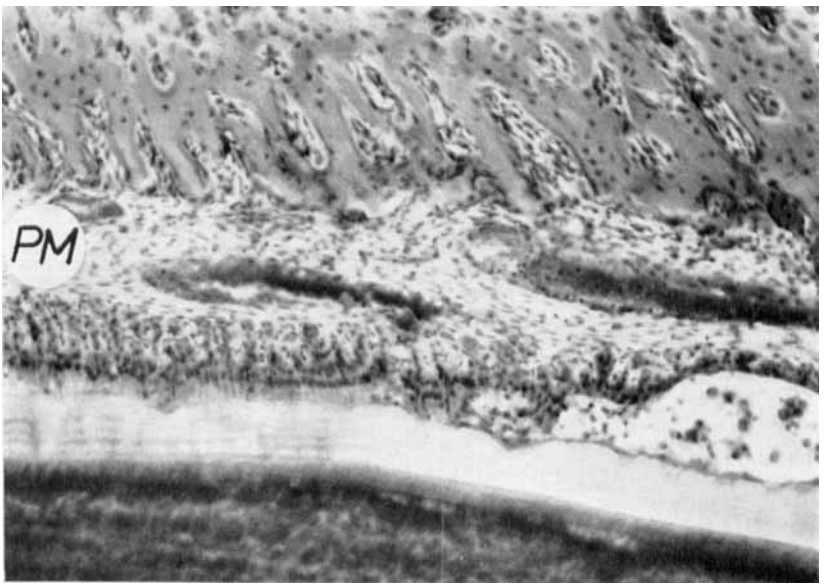


Fig. 5. Irregular structure of labial premaxillary bone with inclusions of connective tissue. Note also the irregular width of periodontal membrane (PM) and the irregular bone surface. Part of section of upper incisor from 67 days old rat on diet containing 225 p.p.m. F^- for 28 days. Orig. magnif. × 80.

sence of lamellae. In Fig. 4 which illustrates the lingual maxillary bone from the basal portion of an upper incisor, immature bone containing crude osteocytes replaces the normal lamellar structure. According to *Weinmann & Schour* (1945) the width of this fundic plate is normally 50 μ. In the area here depicted the width is about 150 μ, although the sites of resorption and apposition were normal.

Occasionally, the labial premaxillary alveolar bone contained numerous connective tissue inclusions. Many of these inclusions were connected with the periodontal membrane due to normal resorption in this area. As a result the surface of the alveolar bone facing the tooth was very irregular, Fig. 5. On the free surface of the labial premaxilla rather wide, osteoid margins were present, indicating a retardation of the mineralization process. The vessels located in the bone of the labial premaxilla were encircled by osteoid tissue.

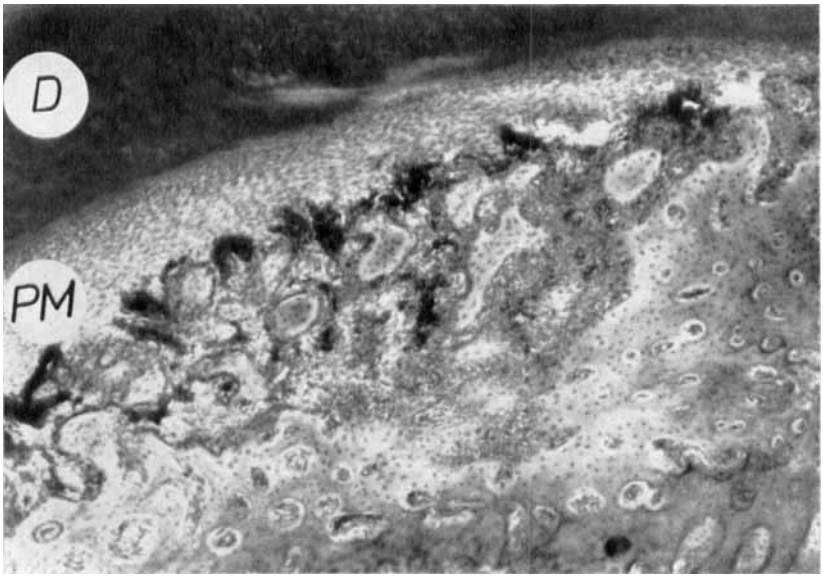


Fig. 6. Lingual premaxillary alveolar bone with formation of new bone in the periodontal tissue (PM) in a lingual recess. Note hyperaemic areas. Lingual dentine (D). Part of section from 67 days old rat on diet containing 225 p.p.m. F^- for 28 days. Orig. magnif. × 80.

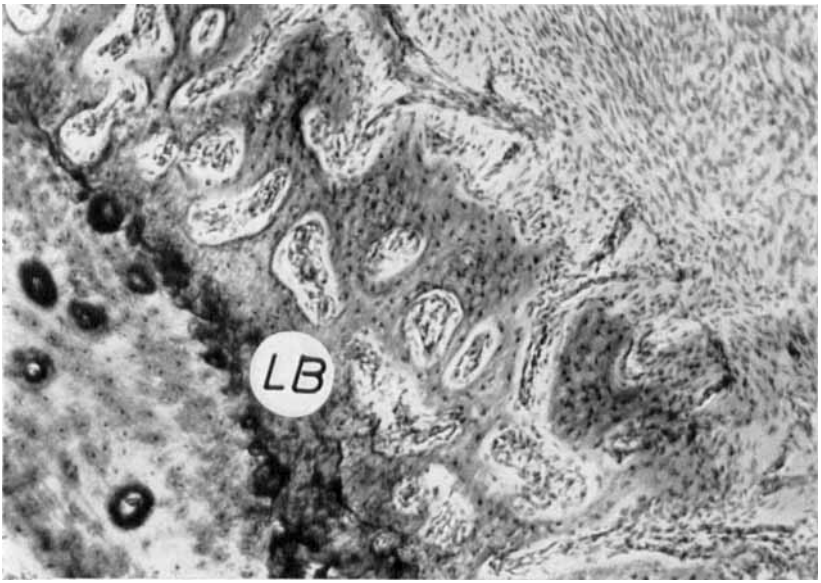


Fig. 6 a. Lingual premaxillary alveolar bone (LB) in the gingival region. Note osteoid borders, irregular structure with coarse osteocytes and inclusions of connective tissue. Part of section from 103 days old rat on diet containing 225 p.p.m. F^- + 10 I.U. vitamin D for 64 days. Orig. magnif. × 80.

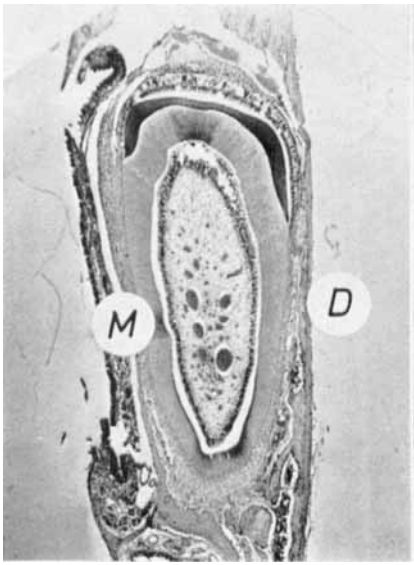


Fig. 7.

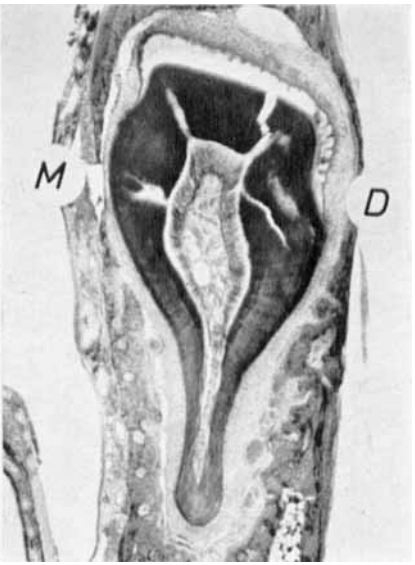


Fig. 8.

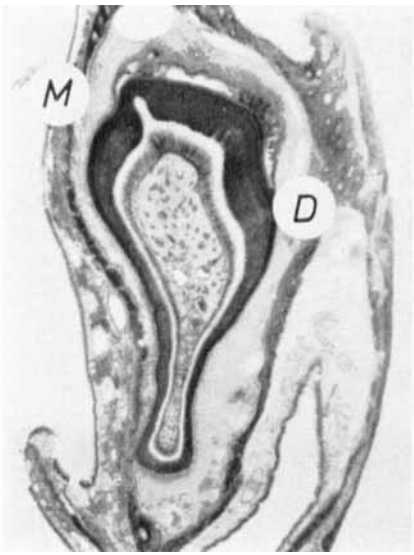


Fig. 9.

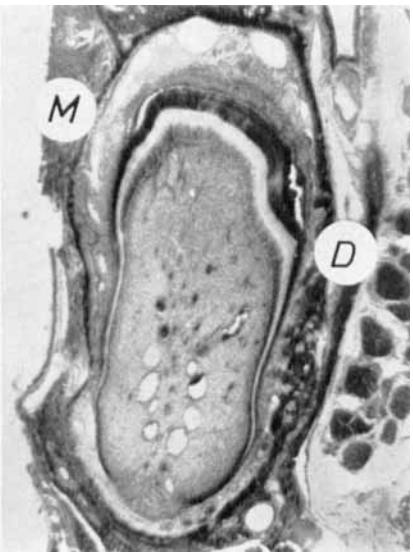


Fig. 10.

Fig. 7. Cross section of upper incisor from 3 months old normal rat. M = mesial, D = distal. Paraffin section. Orig. magnif. × 20.

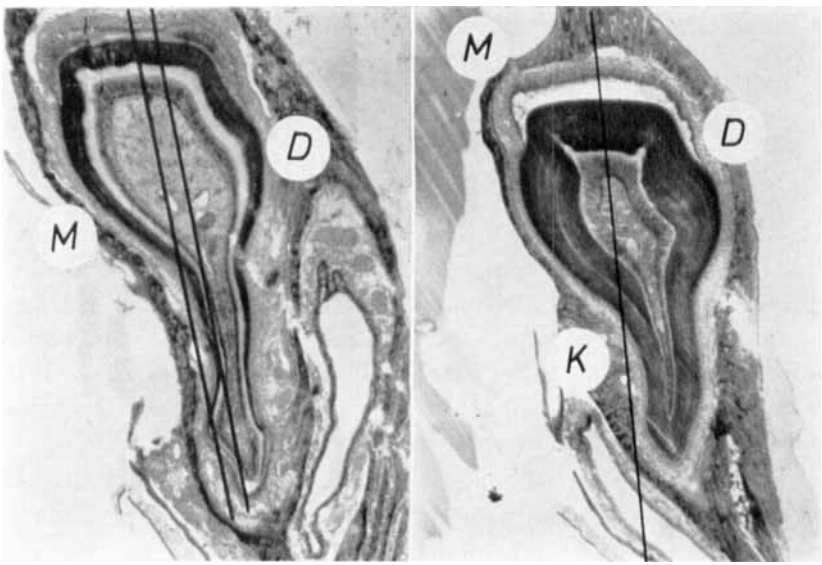
Figs. 8, 9 and 10. Different cross sections of the same upper incisor. Note varying form of tooth and varying width of periodontal membrane. 103 days old rat on diet containing 225 p.p.m. F^- for 64 days. Orig. magnif. × 20.

In the lingual premaxillary bone apposition occurred at normal sites, but rather wide osteoid margins were present, indicating a retarded mineralization. Facing the lingual periodontal membrane bone apposition was marked by a presence of recesses, Figs. 3 and 3 a. At almost all sites at which recesses were noted the formation of immature bone tissue was intense and with margins of osteoid tissue, Figs. 6 and 6 a.

Cross sections of an upper incisor from a rat on experimental diet for 64 days disclosed rather unusual conditions. The cementum-covered proximal surfaces which normally are slightly convex and caudally converging, Fig. 7, were markedly concave, Figs. 8 and 9. In Fig. 10, depicting a more basal area than the above, a more normal, although irregular, outline of the tooth is seen.

Molar teeth and molar periodontium

In areas in which development of secondary cementum occurred, large amounts of precementum were seen. Bone apposi-



Figs. 11 and 12. Different cross sections of the same upper incisor. Note varying form of tooth and varying width of periodontal membrane. In Fig. 12 the bone tissue (K) is seen to follow the new form of the tooth. The drawn lines indicate different sagittal sections and show how lingual recesses probably originate. 102 days old rat on diet containing 225 p.p.m. F^- + 10 I.U. vitamin D for 64 days. Orig. magnif. × 20.

tion and resorption occurred at normal sites. Here too the structure of the newly formed bone tissue was often irregular and less lamellar than in normal rats. Margins of osteoid tissue in areas of apposition were seen chiefly in animals in the 64-day experiment.

Histological examination of rats receiving 225 p.p.m. F^- in the diet + 10 I.U. of vitamin D

No signs of healing or mitigation of the pathological processes were seen in the rats whether the experiment covered 28 or 64 days. This applied to all tissues examined. Examination of cross sections of an upper incisor from a rat on experimental diet for 64 days revealed the same morphological changes as those observed in a rat on experimental diet for 64 days without supplies of vitamin D, Figs. 11 and 12.

DISCUSSION

Any indication that vitamin D in the applied concentrations and types of dosage have a curative or preventive effect on pathological conditions to develop in animals fed a diet containing 0.05 per cent sodium fluoride (225 p.p.m. F^-) has not been found in the present study. This is in conformity with the results obtained by e.g. *Armstrong* (1933), *Schour & Smith* (1934), *Smith* (1936), and *Bauer* (1945). The present author is of the opinion that it is confusing to call chronic fluorosis a rickets-like condition. The pathological processes here discussed have nothing in common with the processes which according to *Weinmann & Schour* (1945) occur in the alveolar bone or in the dentine of rats with rickets. As pointed out by *Weinmann & Sicher* (1955) it may, at most, be a matter of delayed mineralization.

In the present experimental series the development of lingual recesses, also described by *Hoffmann, Schuck & Furuta* (1942) and *Pindborg* (1950), represented a frequent occurrence. A study of the cross sectioned incisors may provide an explanation of the very peculiar pictures seen on sagittal sections of the lingual dentine and periodontium, see e.g. Figs. 3, 3 a and 6, 6 a. It might be more pertinent to use the term: proximal "recesses". The rea-

son why osteoid tissue and bone tissue is visible in the lingual periodontal membrane corresponding to the recesses is that the shape of the incisors makes it very difficult to obtain completely sagittal sections of the enamel-covered as well as the lingual, cementum-covered parts of the tooth, cf. Figs. 11 and 12 indicating directions of sectioning. For reasons of compensatory bone formation at the site of the markedly concave proximal surfaces such sagittal sections will not infrequently include some of this newly formed bone tissue delayed in mineralization. Furthermore it was noted that the very same tooth did not maintain the same shape throughout its course in the jaw, Figs. 8, 9, and 10, and Figs. 11 and 12. No doubt, this change of shape indicates that the continuously erupting incisor is encountering great difficulties during eruption; these difficulties may be responsible for the retarded eruption described by *Smith* (1934). The occasionally intense hyperaemia observed in the lingual periodontium may also be ascribable to adverse circumstances of eruption. Besides it may be that the hypoplastic defects in the enamel (in the more advanced cases of fluorosis the enamel could be absent) and the proliferations from the stratum papillare, could be responsible also for a delayed eruption and for the hyperaemia and occasional disintegration of the labial periodontal membrane in the premaxilla.

SUMMARY

The author investigated whether supplementary, daily doses of 10 I.U. of vitamin D to young, fluorine-intoxicated rats would have a mitigating influence on the course of chronic fluorosis. Ingestion of food, increase in weight, the clinical appearance of the incisors, and the histological picture of the incisor teeth and the alveolar bone were found to remain uninfluenced by the administrations of vitamin D. The author draws the conclusion that supplementary doses of vitamin D have no therapeutical or mitigating effect on fluorosis induced under the conditions of the experiments.

The author is of the opinion that chronic fluorosis is responsible for morphological changes of rat incisors since cross sec-

tions of the incisors disclose unusual conditions in the cementum-covered portion of the teeth. The phenomena, previously referred to as oral recesses in the lingual dentine, are explainable on the basis of these morphological changes which remain equally uninfluenced by supplementary doses of vitamin D.

RÉSUMÉ

FLUOROSE CHRONIQUE EXPÉRIMENTALE CHEZ DE JEUNES RATS RECEVANT DES DOSES SUPPLÉMENTAIRES DE VITAMINE D

L'auteur a cherché à déterminer si l'administration supplémentaire quotidienne de 10 U.I. de vitamine D à de jeunes rats soumis à une intoxication au fluor pouvait atténuer le développement de la fluorose chronique. Il est apparu que l'ingestion des aliments, l'augmentation de poids, l'apparence clinique des incisives et l'aspect histologique des incisives et de l'os alvéolaire n'étaient pas influencés par l'administration de vitamine D. L'auteur conclut que les doses supplémentaires de vitamine D n'ont ni action thérapeutique ni action atténuante sur la fluorose provoquée dans des conditions correspondantes à celles des expériences présentes.

L'auteur pense que la fluorose chronique est responsable d'altérations morphologiques des incisives du rat, les coupes transversales des incisives mettant en évidence des anomalies au niveau de la portion couverte de cément des dents. Les phénomènes antérieurement désignés sous le nom d'invaginations linguales dans la dentine linguale peuvent être expliqués en s'appuyant sur ces altérations morphologiques qui ne sont pas non plus influencées par les doses supplémentaires de vitamine D.

ZUSAMMENFASSUNG

VITAMIN-D-ZUSATZ BEI EXPERIMENTELLER CHRONISCHER FLUOROSE JUNGER RATTEN

In der Arbeit wird untersucht, ob sich die experimentelle Fluorose junger Ratten durch eine extra Dosis Vitamin D von 10 i. E. pro Tag mildern lässt. Es zeigte sich, dass der extra Zuschuss von Vitamin D keinen Einfluss hatte, weder auf die Nahrungs-

aufnahme und Gewichtszunahme der Tiere noch auf die klinische Erscheinung und das histologische Bild der Incisiven und des Alveolarknochens. Die Verfasserin konkludiert, dass eine Vitamin D Gabe über den normalen Bedarf hinaus keine heilende oder mildernde Wirkung auf die chronische Fluorose hat, so wie sie im Experiment erzeugt wurde.

Die Verfasserin ist der Ansicht, dass die chronische Fluorose für die morphologische Veränderungen der Ratteninzisiven verantwortlich zu machen ist, die sich in Querschnitten dieser Zähne als einen eigentümlichen Befund in deren zementbekleideten Partien zeigen. Was man früher als orale Rezesse im lingualen Dentin bezeichnete, lässt sich auf der Basis dieser morphologischen Veränderungen erklären, und diese Veränderungen sind durch eine zusätzliche Dosis von Vitamin D nicht zu beeinflussen.

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