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EXPERIMENTAL STUDIES IN CALCIFICATION

I. THE EFFECT OF A RACHITOGENIC DIET ON THE DENTAL TISSUES OF THE WHITE RAT *

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The purpose of this study was to investigate the effect of a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D upon the dental structures of the albino rat, and thus to establish a basis for subsequent experiments on the influence of parathyroid hormone, vitamin D, starvation, and injections of phosphate upon the dental tissues of the rachitic rat. This study was further indicated by conflicting findings reported in the literature regarding the occurrence of hypoplasia of the enamel in rickets.

REVIEW OF THE LITERATURE

While there are many reports on dental changes in experimental rickets (Erdheim,[1] Fleischmann,[2, 3] Gottlieb,[4] Bauer,[5] Mellanby,[6] and others), only investigations based on experimental diets comparable to the one used in this study, *e.g.*, diets high in calcium, low in phosphorus, and deficient in vitamin D, will be given primary consideration. Karshan and Rosebury [7] reported normal formation and calcification of the enamel and only slight and regional disturbances in the calcification of the dentin. They found an increased thickness of predentin only in the basal portion of the incisor. In the molars, wide predentin was confined chiefly to the roots and pulpal floor. Similarly, Lobeck [8] found in severe rickets only globular dentin and no changes in the enamel of the incisors. Becks and Ryder,[9] on the other hand, reported hypoplasia of the enamel in animals that had been on the rachitogenic diet for 4 weeks. Table I summarizes the data of the experimental studies on rickets.

* Received for publication, September 5, 1944.

Material and Methods

This study is based on material obtained from 33 white rats that were placed on a rachitogenic diet for a period of 1 to 56 days after weaning (at 21 days) (Table II). In addition, a study was made of 25 litter-mates, 21 to 77 days of age, that were fed a normal control diet. The Steenbock-Black [10] diet no. 2965, modified by the substitution of corn meal for whole yellow corn, was used. The experimental animals gained less weight than did the controls.

Table I

Data of Previous Experiments with Rachitogenic Diets Compared with Those of Present Investigation (High Calcium, Low Phosphorus, and Deficient in Vitamin D)

Author	Diet	Number of animals	Age at beginning	Duration of experiment	Histologic Findings		
					Alveolar bone	Incisor	
						Dentin	Enamel
			(days)	*(days)*			
Karshan and Rosebury[7]	Steenbock and Black No. 2965, modified by Epstein	?	33–41	38–57	Poorly calcified	Globulated predentin, apically	Not investigated
Lobeck[8]	McCollum No. 2638	12	0–28	18–126	Not investigated	Globulated	No changes
Becks and Ryder[9]	McCollum No. 3143	21	25	7–42	Poorly calcified	Poorly calcified	Hypoplasia
Weinmann and Schour	Steenbock and Black No. 2965 (modified)	33	21	1–56	Poorly calcified	Poorly calcified	No changes

The animals used in this and the subsequent studies were kindly made available by Drs. F. C. McLean and W. Bloom.

Table II

Summary of Histologic Findings in the Upper Jaw of 33 Rats on Rachitogenic Diet

Length of experiment	No. of rats	Incisor Average width*			Molar predentin	Cementoid		Cyst of enamel organ
		Predentin	Calcified dentin	Total dentin		Molar	Incisor	
(days)		*(microns)*	*(microns)*	*(microns)*				
1–4	7	15	111	126	Normal	Normal	Normal	None
5–6	6	18	112	130	Normal	Normal	Normal	+ (1)
7–8	3	23	97	120	Normal	Normal	Normal	None
21–56	17	64	70	134	2 to 3 times normal width	+ +	+ (13)	+ (8)

Numbers in parentheses indicate number of animals showing the disturbance.

* Measured at the level of completed enamel matrix (labial side).

*Histologic Preparation.** The heads of the experimental and control rats were removed and fixed in 10 per cent formalin immediately after sacrifice. The jaws were dissected and the upper incisors and molars were prepared for decalcified histologic sections. The incisors were cut in serial longitudinal sections and the molars in serial mesiodistal sections. The stains used were hematoxylin and eosin. A number of ground sections of the incisors also were prepared.

Microscopic Findings

Upper Molars

Enamel. The enamel formed during the experimental period showed no significant changes.

Dentin. The predentin showed an increase in width, so that at the end of the experiment (after 56 days on the rachitogenic diet) the predentin reached a width of 50 to 60 μ, which is about three times that of the normal animal (Table II). The greatest width of predentin was found at the floor of the pulpal chamber. The width of predentin increased from the first to the third molar.

At the beginning of the experiment, approximately two-thirds of root formation was completed in the first molar. In the third molar, root formation had just begun. The second molar showed an intermediate stage of development. Those portions of the roots which had been formed during the experimental diet consisted almost entirely of predentin (Fig. 11). The pulp and odontoblasts showed no significant changes.

Cementum. The newly formed layer of cementum (precementum) did not calcify and was considerably wider than in the normal animal. The sum of the thickness of cementoid (or precementum) and calcified cementum was the same as in normal control animals, indicating a normal rate of deposition. Resorption of the cementum was much more frequent in the experimental animals (58 per cent) than in normal animals (12 per cent). The resorption areas were localized to the cervical half of the distal root surfaces. They were shallow but might extend into the dentin.

Upper Incisor

Enamel. The enamel of the incisor showed no disturbance in its formation, maturation, or pigmentation. Hypoplasia of the enamel in the sense of a lack of enamel structure was not observed.

The *enamel organ* was normal in the basal half of the tooth. In the

* Dr. H. Pitluck assisted in the technical part of this study.

incisal portion, however, cysts were found in about 50 per cent of the animals which had been on the experimental diet for 4 weeks. These cysts developed between the inner and outer enamel epithelium and probably originated by proliferation from the stratum intermedium (Fig. 1). They remained attached to the enamel surface in a restricted area and extended basally as a result of the eruptive movement of the tooth (Figs. 2 to 4). In some cases connective tissue had penetrated the degenerating lining of the cyst and filled the entire cavity (Fig. 4). In severe cases compression and degeneration of the enamel organ occurred as a consequence of the narrowing of the periodontal space.

Dentin. In the first week of the experiment the only effect was the appearance of a darkly stained incremental band in the enamel-covered dentin. This calciotraumatic line was seen 24 hours after the beginning of the experiment (Fig. 5), and shifted gradually away from the pulp in accordance with the continued apposition of dentin (Figs. 6 to 8). The line was more distinct in the middle and in the incisal third than in the basal third. The experimental dentin showed slightly less homogeneous calcification (interglobular dentin) than the pre-experimental dentin. This change was more pronounced also in the enamel-covered dentin than in the cementum-covered dentin.

Predentin. Beginning with the second week of the experiment, both the formation (Table II) and the calcification of the dentin were markedly retarded. The retardation of calcification was more pronounced than that of formation and as a consequence the width of the predentin in the basal third sometimes increased to four times the normal width (measured at the level of the completion of the enamel matrix) (Table II). The layer of predentin was thickest in the basal and narrower in the central and incisal portions. The zone of intermediate dentin (Schour and Rogoff [11]), which in normal animals was found in the middle third of the labial dentin, was absent in two-thirds of the animals after the third week of the experiment. The basal end of the dentin which was formed by the most recently differentiated odontoblasts tended to remain entirely uncalcified (Figs. 9 and 10, basal predentin) and varied in length from 0.35 mm. to 1.8 mm. It was always longer on the concave than on the convex side.

In 6 advanced cases the lingual basal predentin did not show a gradually increased width in the anterior direction but was abruptly demarcated from the more anterior and partly calcified dentin by a step in the pulpal outline (Fig. 10) as if the odontoblasts underwent a sudden spurt of increased activity at the time and place of initial calcification of dentin.

Vascular inclusions in the dentin were absent in animals that were on the rachitogenic diet 3 weeks or less but were present in 6 of 15 rats that were on the rachitogenic diet for a longer period.

Differences in Reaction in Labial and Lingual Dentin. The differences in the behavior of the enamel-covered and the cementum-covered dentin were enhanced after the second week of the experiment. In the labial dentin the globules were large and remained separated from each other by interglobular dentin. In the lingual dentin the globules were small and irregular and the interglobular dentin was reduced to a minimum. It showed a characteristic striation.

Rate of Apposition. The formation of the dentin was appreciably retarded in experimental rickets so that the dentin was thinner than normal. The increase of the cross section of the tooth, however, which depends on epithelial and pulpal proliferation, continued at a normal rate. The result was a disharmony between the increase in the diameter of the tooth and increase in the thickness of its dentinal walls (Fig. 12, A and B).

Cementum. The calcified layer of the cementum of rachitic animals was very thin and of normal width. The cementoid layer, however, was thickened and contained some cells (Table II). The evenly spaced dental fibers of the periodental membrane were anchored in the cementoid as Sharpey's fibers and gave it a striated appearance.

Discussion

Differences in the Reactivity of the Calcified Structures. The disturbances of the calcified dental tissues in experimental rickets were confined to the dentin and cementum. The formation of predentin was retarded and the calcification of the cementum and the dentin was impaired. When a part of the predentin formed during the experiment calcified, the calcification was incomplete, as shown by the persistence of large areas of interglobular dentin. The layer of predentin increased in width. The calcification of the newly formed cementum either was lacking or considerably restricted.

The enamel seems to be entirely immune to the metabolic disturbances of the rickets produced by our experiment. Not only is the formation of enamel undisturbed, but the maturation (calcification) of the enamel proceeds normally. The difference in the behavior of the enamel from that of the other hard tissues (dentin, cementum, and bone) may be caused by the chemical difference of the matrix of these different tissues. No principal differences exist in this respect between bone, cementum, and dentin, all mesodermal tissues. The enamel matrix, however, as an epithelial product, shows tinctorial peculiarities, at least, which indicate a different chemical composition.

The rachitic changes in bones are more complex because, in addition to the disturbance in calcification, there are the secondary changes which are caused by the resistance of uncalcified cartilage and bone to resorption and which are entirely lacking in the dental tissues. Resorption constitutes an essential phase in the development and growth of bones but plays no part in the development and growth of the incisors and molars of the rat.

Retardation in the Calcification of the Dentin. In rickets the predentin is significantly wider in the apical third but tends to be approximately of normal width in the middle and incisal thirds.

Since, with the continuous eruption of the incisor, the basal third of the dentin eventually moves anteriorly into the middle portion of the tooth, and since in that portion of the tooth calcification tends to be normal, the disturbance in calcification must be one of retardation in the rate of calcification. The retardation in the rate of calcification of dentin is further indicated by the fact that the predentin is not only wider, but persists longer, than normally, especially at the lingual side (basal predentin).

Hypoplasia of Enamel. The absence of hypoplasia of the enamel in our material confirms the findings of Karshan and Rosebury [7] and of Lobeck.[8] The cystic degeneration of the enamel organ was confined to the incisal half of the incisor where the enamel had already been completed in its formation and calcification, and therefore should not be considered as an enamel defect (Gottlieb [4]).

Several authors [12–15] have found hypoplasia of the enamel in 20 to 25 per cent of cases of rickets, while Fleischmann,[2, 3] whose findings were based on histologic and post-mortem evidence, found no relationship between hypoplasia of the enamel and rickets.

It seems that rickets *per se,* which results from a diet high in calcium, low in phosphorus, and deficient in vitamin D, is not responsible for hypoplasia of the enamel.

Summary and Conclusions

This investigation was based on a study of the teeth of 33 white rats which were placed after weaning on a rachitogenic diet high in calcium but low in phosphorus and deficient in vitamin D for a period of from 1 to 56 days, and of 25 litter-mate controls.

The histologic findings were:

1. Enamel formation and calcification were normal. Hypoplasia of the enamel was absent. The enamel organ showed cystic degeneration in the incisal half of the incisor.

2. Dentin formation was retarded in rate. Calcification of dentin was

retarded and also disturbed. A calciotraumatic line was the immediate response which marked the beginning of the experiment. The newly formed dentin showed an interglobular texture. The enamel-covered dentin was more severely affected than the cementum-covered dentin.

3. Cementum formation was normal in rate but its calcification was defective. Shallow areas of resorption on the molar roots were more frequent than in normal animals.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Plate 135

Photomicrographs of decalcified, longitudinal, midsagittal sections of the labial anterior region of the upper incisor of rats weaned and placed on a rachitogenic diet at 21 days of age. The area is indicated in the insert in Figure 1. Al.B. = labial alveolar bone; Am. = ameloblasts. The space next to the ameloblasts was originally occupied by enamel which is lost during decalcification. All illustrations were prepared from sections stained with hematoxylin and eosin.

Fig. 1. Rat 404, 50 days old. Section shows solid proliferation (P) of the stratum intermedium and outer enamel epithelium and the normal smooth surface of the ameloblastic layer. L.A.P. = labial alveolar periosteum. × 119.

Fig. 2. Rat 1602, 52 days old. Multiple sites of proliferation (P) and cysts (Cy) of the stratum intermedium and outer enamel epithelium are indicated. × 88.

Fig. 3. Rat 1501, 49 days old. A single large cyst (Cy) has its wall formed by the stratum intermedium and the partly degenerated outer enamel epithelium. × 88.

Fig. 4. Rat 303, 54 days old. A large cyst is filled with connective tissue (C.T.), which has apparently penetrated through gaps (G) in the epithelium. The surface of the enamel is smooth. × 119.

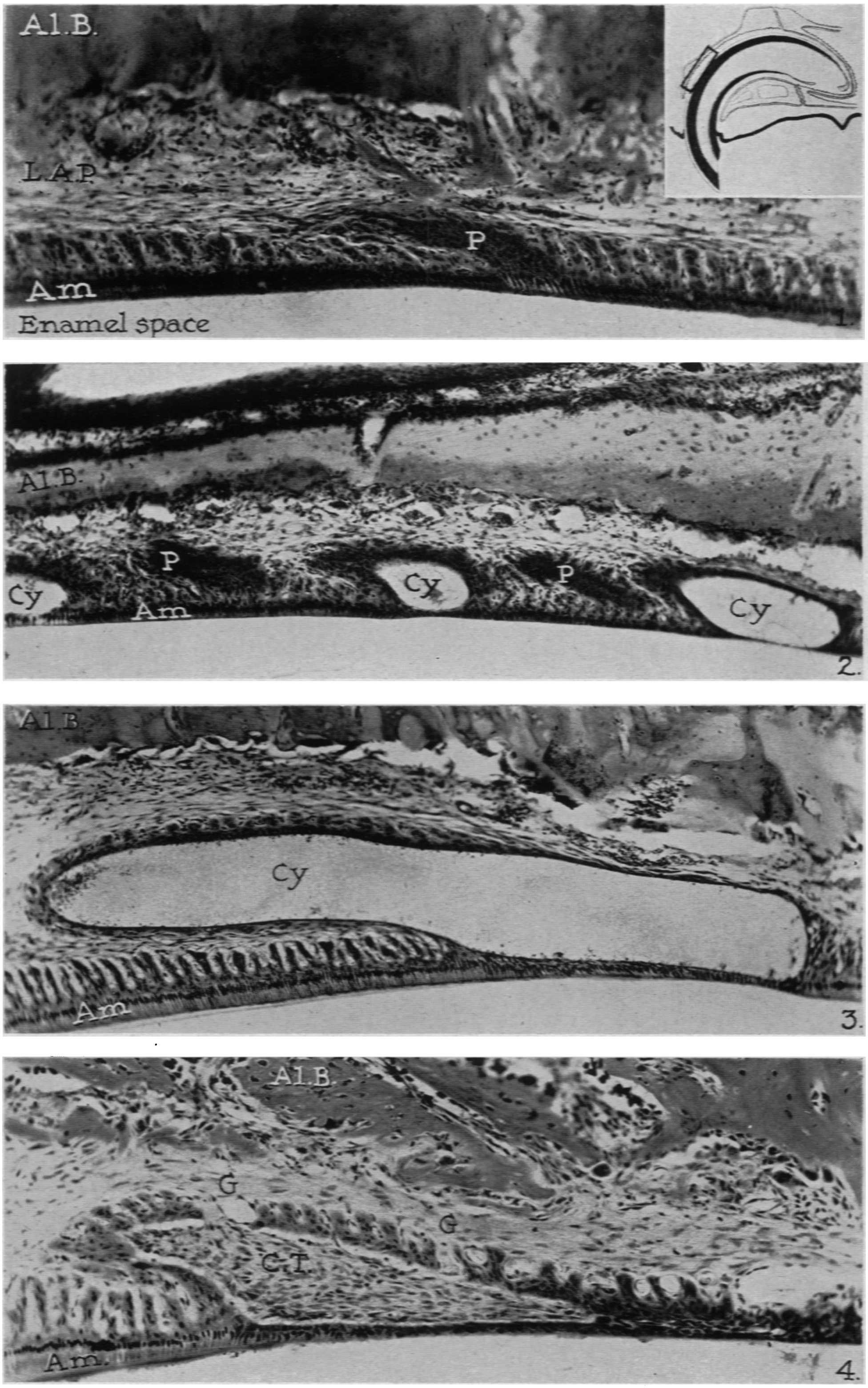

Al.B.
L.A.P.
P
Am
Enamel space
1.
Al.B.
P
Cy
Am
Cy
P
Cy
2.
Al.B.
Cy
Am
3.
Al.B.
G
G
C.T.
Am.
4.

PLATE 136

FIGS. 5–8. Photomicrographs of longitudinal sections of the labial dentinal wall from the middle third of the upper incisor. A dark-staining stripe in the dentin, the calciotraumatic line (X), represents the immediate response to the rachitogenic diet and marks the beginning of the experiment. × 102.

FIG. 5. Rat 3203, 22 days old, placed on rachitogenic diet for 24 hours. The calciotraumatic line (X) is next to the predentin.

FIG. 6. Rat 3205, 25 days old, placed on rachitogenic diet for 3 days. The calciotraumatic line (X) is 47 μ distant from the odontoblastic layer.

FIG. 7. Rat 3207, 27 days old, placed on rachitogenic diet for 5 days. The calciotraumatic line (X) is 84 μ distant from the odontoblastic layer.

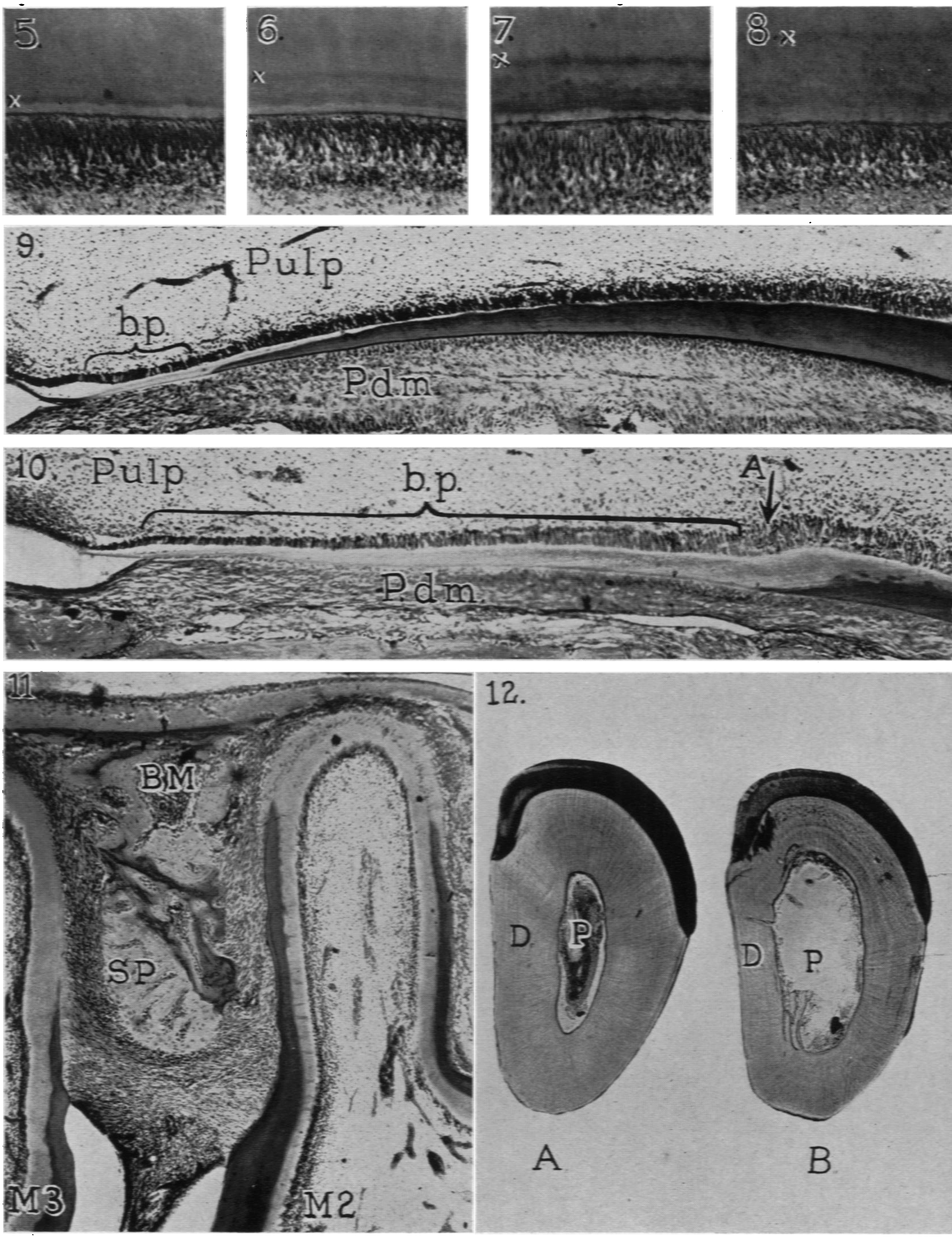
FIG. 8. Rat 3209, 29 days old, placed on rachitogenic diet for 7 days. The calciotraumatic line (X) is 112 μ from the odontoblastic layer.

FIG. 9. Photomicrograph of a longitudinal section of the lingual basal portion of the upper incisor of rat 1801, which was 67 days of age and was placed on the control basal diet after weaning at 21 days of age. Of note are the short uncalcified basal area, the basal predentin, b.p., and the smooth pulpal surface. P.d.m. = periodontal membrane. × 53.

FIG. 10. Photomicrograph of area corresponding to that of Figure 9 taken from rat 1802, which was 67 days of age and was fed the rachitogenic diet for 46 days. Here are seen the extensive uncalcified basal area (b.p.) and the sudden increase in dentin thickness at A. × 53.

FIG. 11. Photomicrograph of mesiodistal section of distal root of second (M2) and mesial root of third (M3) upper molar of rat 1902, 49 days of age, which was weaned at 21 days of age and then placed on a rachitogenic diet for 28 days. Section shows the difference in the calcification of dentin and cementum in the roots of the second and third molar, reduction of the bone marrow spaces (BM), and the eccentric location of the pre-experimental calcified core in the interdental alveolar septum (SP). × 58.

FIG. 12. Photomicrographs of transverse ground sections of the incisor of normal rat 1801 (A) and rachitic rat 1802 (B). The animals were litter-mates, 67 days old. The circumference of the teeth is essentially the same but the dentin (D) of the normal rat (A) is wider and the pulpal cavity (P) narrower than in the rachitic animal (B). × 58.



Weinmann and Schour

Effect of Rachitogenic Diet on Dental Tissues