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THE INFLUENCE OF DIETS LOW IN MAGNESIUM UPON THE HISTOLOGICAL APPEARANCE OF THE INCISOR TOOTH OF THE RAT

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Although it is known that the histological appearance of the tooth is altered in magnesium deficiency, little work has been done on this subject. Some histological studies of incisors and molars in rats under conditions of acute Mg deficiency have been described by Klein, Orent & McCollum [1935]. The chief changes found in the teeth were the appearance of striations in the dentine and proliferation of the parodontal tissues. The striations in the dentine were presumably due to an intermittent interference with the normal calcification process, and Klein *et al.* suggested that they were related to the convulsive attacks which are typical occurrences in acute Mg deficiency. According to Tufts & Greenberg [1938], however, the diet used was deficient not only in Mg but also in vitamin B_2 complex. Watchorn & McCance [1937], studying conditions arising in subacute Mg deficiency, confirmed the findings of Klein *et al.*, but showed that the argument presented by these workers to explain the observed dentinal changes was untenable, since striations were found in subacute deficiency when convulsions did not occur. They did not, however, present any alternative interpretation. Becks & Furuta [1939] found degeneration of the enamel organ in acute Mg deficiency, but, as will be shown subsequently, the changes they observed are probably not specific for this condition.

Advantage was taken of the concurrent lines of work at this Institute upon Mg metabolism and upon tooth formation, to observe more fully the dental changes which occur during dietary Mg deficiency, and the recovery process after return to a diet of normal Mg content. The plan of the experiment was very similar to that described in the preceding paper [Duckworth & Godden, 1940]. The diet and management of the animals was the same as that used by these authors.

Methods

Thirty-nine animals were placed on the deficient diet at weaning and four or more were killed and examined after 2, 4 and 6 days. A number of the remaining rats were then transferred on to the adequate diet and killed and examined after 2, 4, 6, 8 or 10 days. The remaining animals on the low Mg diet were allowed to go on till they died, the longest survival period being 23 days. Four control rats on the adequate diet from weaning were killed after 6 days and two further ones after 16 days on the diet. An adult female animal, about 1 year old, was placed on the low Mg diet and, after subsisting on it for several months, was killed and its teeth examined.

Longitudinal sections made of the upper incisor teeth were cut at 12μ and were stained with haematoxylin and eosin. The sections were examined with a micrometer eyepiece and a series of semi-quantitative measurements made. Only the labial side of the tooth was examined, but it was noted that the lingual side in general showed similar changes.

Results

Animals on the deficient diet

No change was seen until the animals had been on the Mg-deficient diet for at least 4 days.

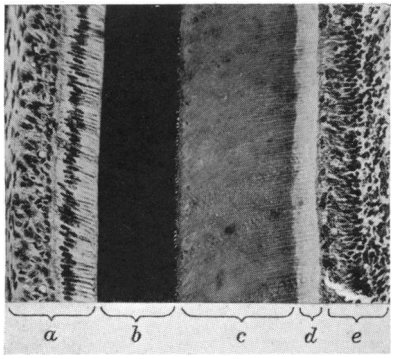
Dentine. After 4 days on the diet, the first characteristic change of Mg deficiency appeared. This was a sudden increase in the predentine width at a point about 3 mm. from the basal part of the tooth. This point will be referred to as the "predentine step" (Fig. 2). Beyond the predentine step the predentine width became roughly double. In one animal on the deficient diet for 4 days, the predentine was 15μ wide up to the step and 31μ wide beyond. After longer periods on the deficient diet, two steps appeared (Fig. 3), the width of the predentine being about three times the normal value beyond the second step. The distance from the base of the tooth to the first step was remarkably constant in all teeth, being about 3 mm. The predentine-dentine junction was very sharp throughout the whole tooth and showed none of the irregularity characteristic of Ca deficiency. The dentine formed during the deficiency did not, however, appear entirely normal. It had a translucent appearance, did not stain so deeply with haematoxylin, and the dentine tubules appeared unduly prominent. After 8 days on the deficient diet, the wide predentine showed the stratification of the predentine to which other authors have drawn attention. Careful examination of the teeth showed

this to be an abortive attempt at a homogeneous calcification of the, by now, very wide predentine, each stratification corresponding to a step in the more proximal part of the tooth. Thus if the predentine was followed from the proximal to the distal part, it was found to become suddenly wider beyond the step. At a variable distance distally, a line, staining deeply with haematoxylin, appeared in the predentine at a distance from the odontoblastic margin corresponding to the predentine width before the step. This line might continue to the tip of the tooth, or might disappear completely, or might reappear at intervals as the predentine was followed distally (Fig. 4). On the odontoblastic side the line was very sharp and straight, on the dentine side it merged gradually into the predentine. If two steps were present in the predentine, two lines were often found in the more distal part (Fig. 5). In no case, however, did fusion with the dentine occur.

In animals which had been on the deficient diet for some time, no definite predentine steps could be made out as they were masked by the stratification in the predentine. In the adult rat the dentine at the basal end of the tooth had a folded appearance very similar to that reported by Schour & van Dyke [1932] as occurring after hypophysectomy (Fig. 6; compare with Fig. 7). Prolonged vitamin A deficiency also causes similar changes [Wolbach & Howe, 1933].

The odontoblasts showed a progressive atrophy when the animals were on the deficient diet. The normal height of these cells is about 51 μ; after 4 days on the diet this was reduced to 40 μ and after 23 days to 33 μ. After 8 days on the deficient diet the odontoblasts in many places had not receded after laying down predentine and were embedded in this tissue, giving the line of odontoblasts a puckered appearance (Fig. 8). In two cases the odontoblasts had become calcified *in situ*, the pulp cavity appearing to be lined in places with osseous material (Fig. 9).

Enamel. After 6 days on the diet, the enamel organ showed large calcareous granules embedded in its substance at the basal end (Fig. 8). As the deficiency progressed these granules became more frequent, causing marked distortion of the enamel organ (Fig. 9). After 23 days on the diet the entire enamel organ showed atrophic changes. In the proximal part the four normal layers could no longer be distinguished and only ameloblasts and epithelial papillae remained. This part of the enamel organ indeed differed little in structure from the intermediate zone, and, in both, the epithelium and papillae had regressed in size. In the distal part the ameloblasts were replaced by a low cubical epithelium, and the shrunken epithelial papillae were seen on a background of



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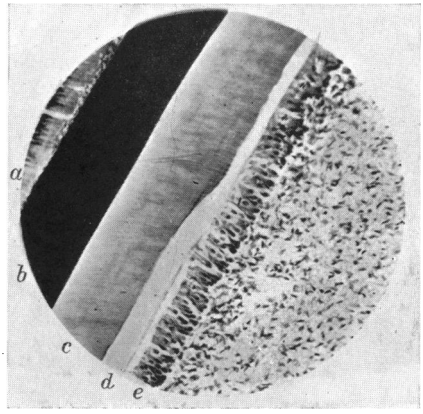

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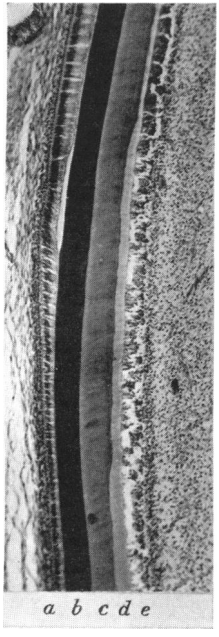

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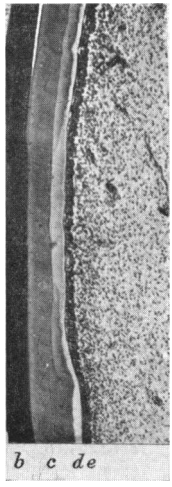

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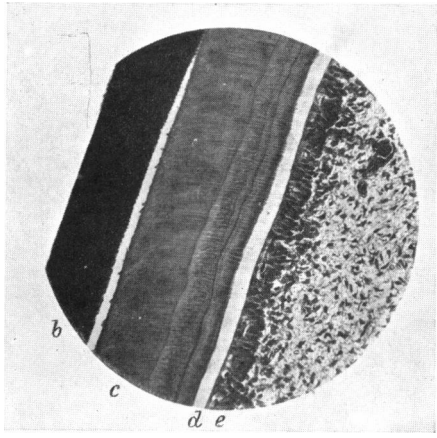

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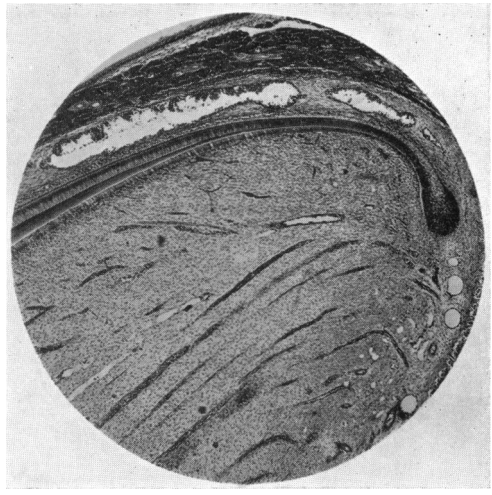

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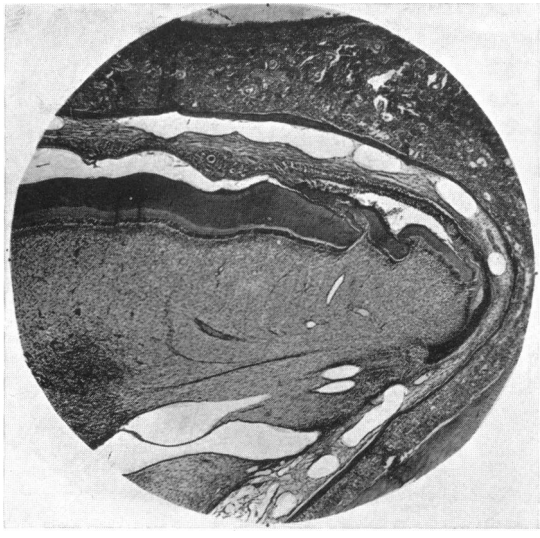

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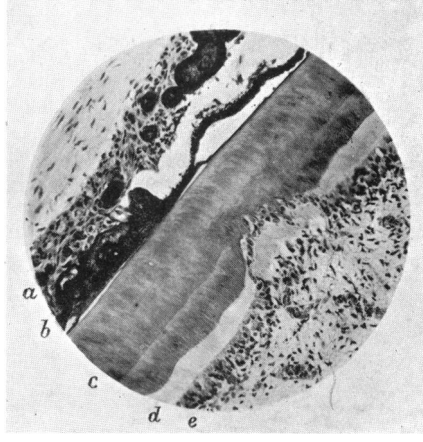

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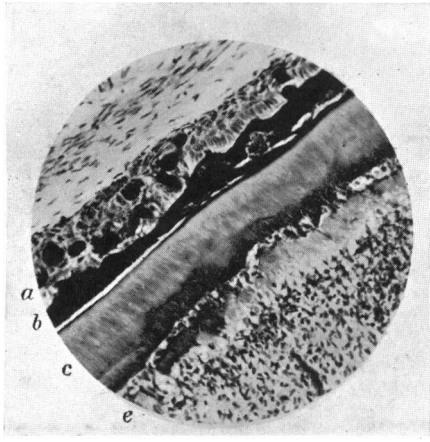

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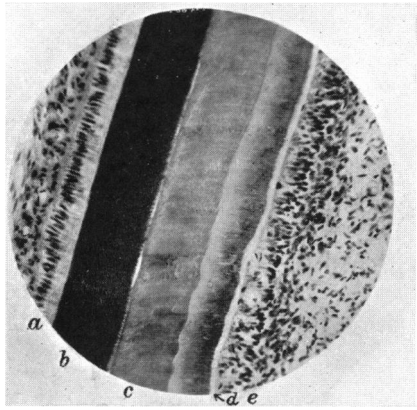

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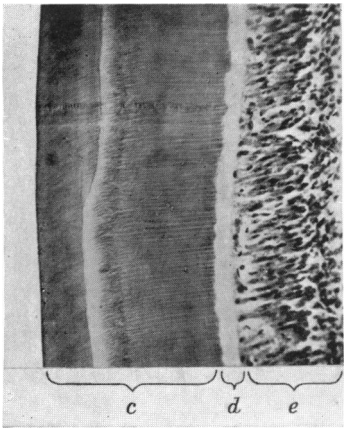

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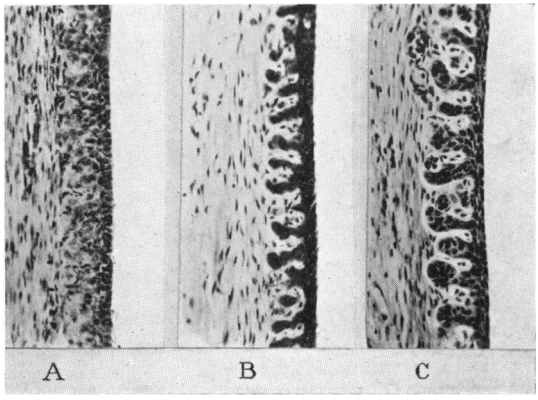

Fig. 12.

fibrous tissue. The amount of organic enamel present was greatly reduced. In the adult rat the enamel organ was recognizable as such only in its proximal part, and here it was very much shrunken and distorted by adventitious calcification. In the intermediate zone the ameloblasts were represented by a layer of cuboidal cells, and in the distal part had become completely flattened. The epithelial papillae had shrunk considerably in size and in many places disappeared altogether. There was virtually no organic enamel; a large enamel space showed, however, that enamel had been present before histological decalcification.

Molar teeth. The lower molar teeth of the rat 23 days on the deficient diet were also examined. The changes were less marked than those in the upper incisor, the dentine showing some stratification only. Part of the lower incisor was also present in this section and showed changes similar to those in the upper incisor.

Animals placed on the adequate diet after 6 days on the deficient diet. The teeth of these rats showed all the changes which had been present in those of animals 6 days on the deficient diet. As a result of the subsequent increase in Mg content of the diet, the abnormalities were covered or slowly displaced by new and more normal tissues.

Dentine. The response to the increased Mg content of the diet was seen after 2 days (Fig. 10). New dentine, recognizable by its staining deep blue with haematoxylin, was laid down along the whole length of the tooth and while the step and stratifications in the previous predentine were not removed, these structures gradually faded into the background as they were covered with new dentine, and moved distally with the growth of the tooth. Only the edge of the old predentine was calcified and no further attempt was made to calcify it or the step, the new dentine being laid down between these structures and the odontoblasts (Fig. 11). It is of interest to note that the new dentine extended to the extreme end of the tooth, an observation similar to that of Hevesy, Holst & Krogh [1937], who found that radioactive phosphorus, after administration to rats, was laid down in new dentine even at the extreme end of the incisor. The labial edge of the new dentine had no distinct margin but merged into the old predentine, indicating that the latter had been to some extent calcified when the animal was returned to the adequate diet. On the odontoblast side, the new dentine had the usual sharp margin with the predentine. Owing to the indistinct margin of the new dentine, it was not possible to measure with any accuracy the amount laid down. Rough figures showed that the incremental cycle was resumed; but the new predentine was at first abnormally narrow so that the amount of dentine

formed was less than normal until the animal had been on the adequate diet for about 6 days.

The predentine step moved slowly distally and was finally lost at the end of the tooth. After 4 days on the normal diet, the distance from the base of the tooth to the step was 4·6 mm. and after 8 days it had become 6·0 mm.

The odontoblasts rapidly recovered and regained their normal height, the new ones laid down at the basal end of the tooth being normal in all respects. Many of the more distal odontoblasts, however, had not recovered from the previous deficiency even after 10 days on the diet; these had been formed while the animal was still on the deficient diet and in places were deeply embedded in the new dentine.

Enamel organ. No new calcified granules were formed in the enamel organ, and those which were present and which had been formed during the deficiency period moved slowly distally the longer the animal was on the adequate diet.

Discussion

The histological findings show that Mg deficiency exerted a marked influence upon tooth calcification. In the early stages, this process was retarded in a characteristic way; later, while still on the deficient diet, attempts to return to the normal calcification cycle led to stratification in the predentine, and adventitious calcification of the enamel organ and of the odontoblasts.

In the early stages of the deficiency, the extreme proximal end of the tooth remained normal, the predentine step being formed 2–3 mm. from the epithelial sheath. This abrupt alteration in predentinal width was the most specific change found and has not been described as occurring in any other condition. On it hung all the other changes in the dentine. There is no doubt that this and subsequent changes in the predentine were intimately related to the progressive atrophy of the odontoblasts, already described by Watchorn & McCance [1937], which was first noted after 4 days on the deficient diet, the stage at which the predentine step was also first seen.

Why the predentine step should occur at a relatively constant distance from the base of the tooth cannot be explained. It is, however, well known that the vascularity of the pulp becomes poorer in the more distal parts, and that changes in the predentine are always more extreme distally. It may possibly be that, at the level of the step, the vascular supply began to be insufficient to provide enough Mg when the intake was already inadequate.

Beyond the step, the calcification cycle was upset in a quantitative way. In all the deficient teeth examined, the predentine width had the normal value of about 16μ up to the step. Beyond this, the predentine became virtually twice as wide. This indicated a change in rhythm from a 24 to a 48 hr. cycle. When two steps were present, the cycle took 72 hr. to complete. That this is the true explanation, and not that the teeth had ceased to calcify altogether, which seems at first more obvious, was shown by the fact that at 6 days, when the wide predentine was very marked, the teeth were still growing almost normally. This had further been considered in relation to another point. The chemical findings showed that while the Ca and P content of the teeth, reckoned in absolute amount, continued to increase, the Mg content remained constant. It was thus possible that what appeared histologically as predentine was actually abnormally calcified dentine, low in Mg. But Schour & Ham [1934] have conclusively shown that normal dentine stains with haematoxylin and uncalcified predentine with eosin; and it appears improbable that this wide predentine was in fact calcified, since later in the deficiency calcified matter staining normally with haematoxylin was found not only in the enamel organ and odontoblasts, but in the predentine itself, and this calcified matter presumably likewise contained little Mg. I therefore consider that the original interpretation is the correct one, that the 1-day calcification cycle required 2 and later 3 days for completion. The diminution in inorganic matter, expressed on the increased width of the predentine, was probably far too small to contribute to the slight loss of tooth weight observed, the more so, since comparison of the growth and weight figures of the tooth show them to be in almost the same proportions. The slight change in appearance noted in the dentine during the deficiency may well have been due to its low content of Mg.

The later changes in the tooth, while the animal was still on the deficient diet, appeared to be an attempt to return to normal calcification. The effort to resume the 16μ cycle in the predentine failed as the incremental stratifications did not fuse with each other or with the dentine. Calcification was so extreme that the odontoblasts became calcified *in situ* or became included in the predentine.

Along with the abnormal calcification of the odontoblasts, adventitious calcareous granules were found in the more proximal parts of the enamel organ at and after 6 days on the deficient diet. This represented the earliest stages in the atrophy of the enamel organ which was so conspicuous after 23 days' deficiency and in the adult animal. This change has also been described by Becks & Furuta [1939] in Mg deficiency. I do

not, however, consider this as a change specific to Mg deficiency. Schour & van Dyke [1932] found very similar changes after hypophysectomy, and Wolbach & Howe [1933] have reported changes in the enamel organ in vitamin A deficiency which the writer has found to be indistinguishable from those caused by Mg lack (Fig. 12). It is not altogether surprising that several apparently unrelated factors should affect a highly developed structure like the enamel organ, which is not only laying down a very specialized tissue but which also exhibits continual growth.

As the teeth sections were decalcified it is impossible to say if the enamel removed by this process was in any way changed. The organic enamel appeared normal in structure save where it was distorted by the calcification in the enamel organ. In late stages of the deficiency, however, the amount of organic enamel was definitely reduced, indicating a much earlier calcification of the enamel.

It has been found by a number of workers [see review, Duckworth, 1938–9] that one of the outstanding characteristics of Mg deficiency is its effect upon Ca metabolism, prolonged deficiency causing adventitious deposition of Ca salts in the body. In the present investigation, a prominent change found in the teeth was the presence of calcified material in the enamel organ and odontoblasts, and, as just stated, in later stages the enamel appeared to be calcified earlier than usual. These changes were seen after only 6 days on the deficient diet; it would thus appear that, as was found in studies of Ca metabolism, the tooth is a very sensitive index of abnormalities in Mg deficiency.

It is interesting to contrast the effect of Ca and P deficiency on the dentine with those of Mg lack. In Ca and P deficiency, the dentine-predentine junction is very irregular, owing to malfusion of the calcospherites, and this may lead to the formation of interglobular dentine; in Mg deficiency, the dentine-predentine junction is unusually straight and regular and no calcospherites are seen, the dentine being very homogeneous in appearance. The predentine may be wide in both conditions, but, whereas in Ca and P lack, the measurement may be of all values up to 90μ, in Mg deficiency the width is usually a multiple of that normally found. The wide predentine in Ca and P lack never shows the striations which are such a conspicuous feature of the late stages of Mg deficiency. Moreover, the stratifications caused by Ca and P lack, when they are present, occur in the dentine and not in the predentine, and are due to malfusion of calcospherites, being in fact a species of interglobular dentine. The predentine step is peculiar to Mg deficiency; vascular inclusions, conspicuous abnormalities in Ca and P deficiency, have never

been noted in Mg deficiency; and, lastly, the odontoblasts do not shrink in Ca and P deficiency.

The recovery process, when the animal was returned to the adequate diet, is interesting as showing that structures already formed during the deficiency, apparently could not be changed, but were covered by normal tissues and finally lost at the distal end of the tooth; only the edge of the old predentine was calcified and that but to a slight extent. All the abnormalities gradually moved to the far end of the tooth, the structures laid down behind them being entirely normal. The predentine step and the wide predentine were fully recognizable even after 10 days on the adequate diet, though they were by then deeply buried under a layer of new dentine. The new dentine became gradually wider with successive days on the adequate diet until after 10 days, it and the predentine together were about 135μ wide. The odontoblasts had recovered their full height after 6 days on the adequate diet, but it was evident that those laid down during the deficiency were permanently abnormal, since many of them were included in the dentine and predentine. Also, the new predentine first laid down beyond the step was abnormally narrow and the full width was not attained till the animal had been 6 days on the diet. This corresponded with the complete recovery of odontoblast height and accounted for the fact that the dentine plus predentine width at 10 days was lower than normal.

The rate of movement of the predentine step corresponded well with the rate of tooth growth obtained by marking the tooth [Duckworth & Godden, 1940]. Between the second and eighth days on the adequate diet, the step had moved 2·4 mm. This was at the rate of 2·8 mm. per week, the other method yielding a figure of 2·9 mm.

The calcified granules in the enamel organ behaved likewise and would ultimately have been lost. No new ones were formed after the animal had been returned to the adequate diet.

Erdheim's classical work in 1906 showed that parathyroidectomy exerted a profound influence upon the histology of tooth calcification, and this was later found to be due to interference with Ca metabolism. In the case of Mg deficiency, no such correlation is known to exist with any endocrine gland. It is true that certain changes bear a superficial resemblance to those induced by hypophysectomy. But it is far too early to do more than comment upon this likeness and suggest it as a possible approach for further work. It can only be considered as very striking that a lack of Mg, which enters into the composition of the tooth in very small amounts, should exert such a marked histological effect.

SUMMARY

A series of characteristic changes occur histologically in teeth during acute Mg deficiency. The calcification rhythm is upset and adventitious calcification occurs in abnormal situations. After restitution of Mg in the diet, the normal calcification cycle is immediately resumed.

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EXPLANATION OF PLATES I–III

PLATE I

a, enamel organ. *b*, enamel. *c*, dentine. *d*, predentine. *e*, odontoblasts.

Fig. 1. Longitudinal section of the basal end of the upper incisor of a 40-day stock rat (×120). This shows the normal structure of the predentine and dentine and also of the enamel organ.

Fig. 2. Longitudinal section of the basal end of the upper incisor of a rat 6 days on the Mg-deficient diet (×120). The predentine step is clearly seen.

Fig. 3. Longitudinal section of the basal end of the upper incisor of rat 6 days on the deficient diet (×47). Two steps can be seen in the predentine.

Fig. 4. Longitudinal section of the basal end of the upper incisor of a rat 8 days on the deficient diet (×47). At the lower end, the predentine is normal in width. A predentine step is then seen with some stratification. This gives way to predentine of double the normal width, while more distally, the stratification reappears in the predentine.

PLATE II

Fig. 5. Longitudinal section of the basal end of the upper incisor of a rat 9 days on the deficient diet (×120). A double layer of stratification in the predentine is clearly visible. The odontoblasts are atrophic.

Fig. 6. Longitudinal section of the basal end of the upper incisor of an adult rat maintained on the deficient diet for several months (×26). Note the folding of the dentine, the almost complete atrophy of the enamel organ and the absence of organic enamel.

Fig. 7. Longitudinal section of the basal end of the upper incisor tooth of a 40-day stock rat (× 27). This section shows the normal appearance of the structures at the basal end of the tooth.

Fig. 8. Longitudinal section of the basal end of the upper incisor of a rat 14 days on the deficient diet (× 120). This section shows the inclusion of odontoblasts in the predentine and the calcified material in the enamel organ. The enamel organ is completely disorganized at this point.

PLATE III

Fig. 9. Longitudinal section of the basal end of the upper incisor of a rat 23 days on the deficient diet (× 120). This section shows in more extreme form than does Fig. 8 the inclusion of odontoblasts in the predentine and the adventitious calcification of the enamel organ. A calcified matrix has also been laid down round the odontoblasts.

Fig. 10. Longitudinal section of the basal end of the upper incisor tooth of a rat 6 days on the deficient diet and 2 days on the Mg replacement diet (× 120). Note the new dentine laid down as a deeply staining band, between the odontoblasts and the predentine formed during the deficiency. The new predentine is abnormally narrow.

Fig. 11. Longitudinal section of the mid part of the upper incisor of a rat 6 days on the deficient diet and 8 days on the Mg replacement diet (× 120). Note the broad layer of new dentine and the normal predentine and odontoblasts. The step and predentine formed during the deficiency period are still distinctly visible.

Fig. 12. A. Longitudinal section of the distal part of the enamel organ in a normal rat (× 120). B. Longitudinal section of the distal part of the enamel organ of a rat 23 days on the Mg-deficient diet (× 120). C. Longitudinal section of the distal part of the enamel organ of a rat 54 days on a diet deficient in vitamin A (× 120). (From a section by Irving & Richards [1939].) The similarity of the changes in B and C is very marked.