THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME IX MAY, 1933 NUMBER 3

THE INCISOR TEETH OF ALBINO RATS AND GUINEA PIGS IN VITAMIN A DEFICIENCY AND REPAIR *

S. B. WOLBACH, M.D., AND PERCY R. HOWE, D.D.S.

(From the Department of Pathology, Harvard University Medical School, and the Forsyth Dental Infirmary, Boston, Mass.)

In 1925 [1] we described very briefly two outstanding effects of vitamin A deficiency upon the incisor teeth of white rats — atrophy and metaplasia of the enamel organ and atrophy of odontoblasts accompanied by atypical formations of dentine. Since then we have studied the teeth of vitamin A deficient guinea pigs and added considerably to our understanding of the deficiency changes by the study of the sequences of repair in white rats during recovery induced by the addition of butter fat to the diet. We emphasize the value of following recovery changes in all experimental studies of the consequences of vitamin deficiencies. In vitamin A deficiency, as in vitamin C deficiency (Wolbach and Howe [2]), cells of mesenchymal origin undergo changes in morphology, making identification inaccurate or impossible. In recovery we have found that such cells, osteoblasts and odontoblasts, resume function before morphological restoration is complete. The presence or absence of a deposit of matrix around cells by way of a recovery response and the character of the matrix if deposited, as well as the restored morphology of the cells, are decisive factors in identification of cells which concern us in studies of teeth in deficiencies.

Few histological studies have been made upon teeth in vitamin A deficiency. May Mellanby has written extensively and wisely upon the importance of vitamin A for the normal development of teeth. These papers fortunately are summarized in an excellent review [3]

* Received for publication February 2, 1933.

and present definite proof that defective or absent dentine and enamel formations are consequences of vitamin A deficiency. In her studies of human and experimental animal material, undecalcified ground sections were employed so that cell responses and histological sequences could not be followed. Marshall,[4] without giving histological details, attributed the formation of "pulp stones" (calcification in the pulp) in white rats to vitamin A deficiency. Coolidge [5] mentioned and illustrated irregularities of the dentine and the formation of osteoid masses free in the pulp or attached to the dentine in one white rat "of several hundred." The diet of this rat was not given and he gave no histological details, but as the illustrations are those of conditions constant in vitamin A deficiency, we believe that intentionally or inadvertently this rat's diet was deficient in vitamin A. More recently, Shibata [6] reported as consequences of vitamin A deficiency in white rats "abnormal formation of the enamel, dentin and cementum; degenerative changes such as atrophy and abnormality in enamel organ dental pulp and root membrane tissues; retardation of the eruption of the incisors; and the changes in the chemical compositions such as the decrease of phosphorous and calcium, increase of water magnesium, etc." Shibata refers to earlier papers of his but does not give the references so that we could not consult them for the histological descriptions which the above-mentioned paper lacks. It seems probable that many of the details we shall describe were seen by Shibata.

Our endeavor, as part of a program to achieve specific histopathological characterizations of the avitaminoses, has been to follow the sequences due to A deficiency in the teeth for the purpose of ascertaining the initial or direct (specific) effects, as contrasted with indirect or non-specific effects such as may be the consequences of secondary general disturbances in metabolism. Accordingly sections of decalcified skulls were used. Fixation was either in Zenker's liquid or 10 per cent formalin. Five per cent nitric acid in 70 per cent alcohol was used for decalcifying. Celloidin sections stained with hematoxylin and eosin were routinely used, but various special stains for connective tissue and reticulin were employed when indicated.

The diet used for the rats was that described by us in 1925. Whether or not the absence of vitamins C, D, and E was of importance we cannot say. We adhered to it because (1) scorbutic effects cannot be produced in rats; (2) vitamin D is not necessary to prevent

rickets in rats with properly balanced inorganic salts in their diets when kept under proper hygienic conditions; (3) we know of no effects of vitamin E deficiency outside of the testes in the male and the products of conception in the female; and (4) of greatest importance, the addition of vitamin A alone sufficed to bring about histological recovery.

The diet used for the guinea pigs was that described by us in 1928.[7] We varied the amount of orange juice in an attempt to find an amount which would prevent scorbutus and yet allow an A deficiency. Twenty cc. of orange juice daily is sufficient to prevent histological effects of vitamin A deficiency in any organ in guinea pigs of 300 to 500 gm. weight. Eight cc. daily does not prevent the epithelial metaplasia of vitamin A deficiency. Two cc. daily is sufficient to prevent scorbutic lesions in bones. The majority of our guinea pigs received 4 to 8 cc. of orange juice daily. This we emphasize because certain findings in the teeth of guinea pigs which were reported by Höjer [8] are apparently duplicated in guinea pigs on vitamin A deficient diet plus 4 to 8 cc. of orange juice daily.

Our studies were made from frontal plane sections through the skull at three or more levels each, including the upper incisor teeth. Owing to the curvature of the incisor tooth the frontal plane sections do not give true cross-sections, except in the midportion of the tooth. Allowance must be made for this in viewing the illustrations because the sections through anterior and posterior portions of the tooth cut the labial and lingual walls obliquely. The lower incisors were studied in sections at the level of the first molars in rats with different degrees of the deficiency. In guinea pigs and in rats during recovery the lower jaws were sectioned at three levels, one through the first molars, and two posterior to that.

The following tables serve to indicate the scope of our material.

The Incisor Teeth of Rats

The gross changes have not been accurately followed by us. Growth of the teeth seems to be commensurate with the growth of the skull, which shares in the marked retardation of the skeleton as a whole. The teeth lose the normal orange pigmentation and acquire a chalky-white appearance, due we believe to loss of the enamel which is the pigmented part, and to change in composition of the

TABLE I

Vitamin A Deficient Rats Used for Tooth Studies

No. of rat	Duration of deficient diet		Metaplasia all organs
	days		
227	61	Died	+
224	63	"	+++
207	77	"	++
209	82	"	+
205	89	"	++
206	89	"	++
220	91	"	+++
222	97	"	+++
203	98	"	++
201	100	Killed	++
202	101	"	+++
238	114	"	++
101	115	"	+
31	149	Died	++++
35	149	Killed	++
105	160	Died	+
107	167	"	+
106	171	"	+
103	180	"	++++
100	181	Killed	+

TABLE II

Vitamin A Deficient Rats in Recovery Used for Tooth Studies

No. of rat	Deficiency period	Repair period	Metaplasia of epitheliums	Weights in grams			
				Initial	Maximum	End of deficiency	Recovery
	days	*days*					
212	70	9	++	95	128	105	?
239	103	5	++	63	123	99	?
425	118	10	+	141	162	147	144
232	137	6	+	114	152	120	?
235	138	5	+	104	140	126	85
397	138	12	+	84	136	119	135
393	143	18	+	85	128	109	125
394	146	22	+	81	119	80	105
413	150	13	++	135	136	104	107+
399	151	15	+	86	140	123	142
400	151	20	++	81	129	107	129+
401	161	22	++	81	114	87	117
412	170	14	++	128	137	126	169
414	170	19	+++	143	146	112	160
50	185	43	++	?	?	?	?
417	187	7	+++	133	133	102	120

In the last column of weights + means that the recorded weight was that of several days previous to killing.

TABLE III

Vitamin A Deficient Guinea Pigs Used for Tooth Studies

No. of guinea pig	Duration of deficiency	Metaplasia all organs	Orange juice daily
	days		*cc.*
2	66	+	2
28	67	++	8–2*
29	67	++	8–2*
24	71	++	20–2*
38	101	?	16–4
23	105	+++	20–4–0**
42	105	+	16–8
39	110	+++	16–2
6	112	?	20–8
142	128	+++	4
45	144	?	20–8
15	153	++	20–4
46	158	?	20–8
143	160	++	4
72	161	++	16–2
8	163	++	20–8
30	287	0	20
41	299	++	20–5

* These three guinea pigs, after the orange juice was reduced to 4 cc. daily, received none for 2 weeks and then received 2 cc. daily for the final 2 weeks of life.

** This guinea pig received no orange juice for the final 2 weeks of life.

dentine. Also, the teeth are more brittle than normal. Striking histological changes were present in all the teeth of the rats listed in Table I so that a pronounced effect resulting in change of cross-sectional shape is apparent in from 60 to 70 days, possibly earlier. At all levels we find the dentine on the labial side of the tooth disproportionately thick, compared to the lingual, lateral and mesial sides. Figures 1 and 3 are typical of A deficiency. In advanced deficiency (Figs. 3 and 4) there may be gaps in the wall of dentine on either side, but always near the basal, open end of the tooth. An early effect of the deficiency is atrophy and cessation of function of the odontoblasts. The odontoblasts on the labial side (adjacent to the enamel organ) are affected last and this accounts for the final cross-sectional shape of the tooth. We have not determined the rate of growth of the incisors in vitamin A deficiency. In the normal albino rat (Addison and Appleton [9]) the rate of growth of the upper incisors is given as 2.2 mm. per week, of the lowers, 2.8 mm. per week.

Addison and Appleton also give the following lengths in millimeters of the incisors at different ages.

Age	Upper incisors	Lower incisors
23 days	12.8	18.1
41 "	15.0	21.7
10 weeks	18.3	25.5
15 "	20.3	26.4
5 months	23.3	29.4
8 "	23.7	29.9
10 "	26.2	31.3

If in vitamin A deficiency the *rate* of growth of the incisor teeth in *relation* to the growth of the skeleton as a whole is approximately that of the normal animal, it is probable, and we believe certain, that the entire tooth is replaced through growth during dietary periods covered by our observation. This must be kept in mind in studying sections taken at different lengths from the formative end of the tooth.

The enamel organ which extends for the entire length of the incisor tooth on its labial surface (elsewhere only about 1 mm. from the basal formative end) early undergoes atrophy and metaplasia (Fig. 2) and consists finally of atrophic remnants of the epithelial papillae and squamous cells replacing the ameloblasts and stratum intermedium (see Fig. 5 for contrast). The anterior part of the enamel organ is first affected but finally the whole length is involved, including the basal formative end (Rat 31, Table I). At the basal end there is continuous renewal of the enamel organ and in many rats with marked metaplasia in many organs practically normal structures were found here, though with distorted relationships, owing to pressure deformity of the tooth because of defective or absent dentine formation. According to Addison and Appleton the ameloblasts travel forward as the incisor grows and it is a fact of interest that for a brief period these cells, as well as the rest of the enamel organ, may retain normal structural appearances. The effect of the deficiency is most apparent after the ameloblasts have reached the region where they are normally most active in enamel formation. Toward the occlusal end, though still within the area of functional enamel organ, the metaplasia may become complete and as a result the enamel organ may be replaced by an epidermis-like structure with superficial keratinized cells. In middle and posterior parts we have not seen this degree of metaplasia. In the atrophy of the enamel organ (Fig. 5) the epithelial papillae are affected first. These structures shrink in size because of atrophy of cells and the surrounding connective tissues become less vascular. The ameloblasts diminish in size, become

coarsely granular and finally disappear. Cell outlines are lost and the ameloblast layer becomes represented by a layer of granular material and pyknotic nuclei, and then disappears. Cells presumably of the stratum intermedium persist as flat cells, usually two rows deep, until in very late stages in the anterior half of the tooth these develop numerous layers of flat cells, the most superficial of which occasionally become keratinized. Very rarely, in contrast to its occurrence in guinea pigs, after complete loss of ameloblasts globular formations of calcified material develop in the connective tissue adjacent to atrophied papillae (Fig. 6). This calcification following atrophy of the structure whose function it is to receive and segregate calcium salts from the blood stream, invites speculations regarding the possibilities of selective permeabilities of the capillaries supplying the epithelial papillae.

The sequences determined by study of rats in varying degrees of the deficiency are supported by the sequences of repair during recovery induced by feeding butter fat. The first evidence of recovery in the enamel organ is the plumping of cells of the epithelial papillae. This is accompanied by engorgement of capillaries and a less dense appearance of the connective tissue immediately adjacent to the papillae. The earliest response was in Rat 417 (Table II) after 7 days of treatment. Subsequently, and in a very few days, the surface cells, presumably of stratum intermedium origin, become cuboidal in shape and continue to assume more and more the appearance of ameloblasts (Rats 397 and 399, Table II, for example). In rats responding favorably to treatment, as shown by rapid gain of weight, the enamel organ is practically normal in appearance on the 19th day (Rat 414, Table II).

The details of changes in the pulp and odontoblasts are more difficult to follow than those in the enamel organ. Simultaneously with changes in the enamel organ occurs atrophy of the odontoblasts but, as we have said above, the odontoblasts on the labial side (adjacent to the enamel organ) retain their morphology and function for long periods. This accounts for the extraordinary thickness of the dentine on the labial side, as contrasted with the remainder of the tooth. It is naturally a pertinent suggestion that the enamel organ, even though undergoing atrophy and separated by a wall of dentine and enamel, still exerts an influence upon the maintenance of the odontoblasts comparable to that in the fetus and at the basal formative end.

Conversely, one may argue that all of the changes in the odontoblasts are secondary to atrophy of the enamel organ and withdrawal of its influence. At the basal formative end the odontoblasts undergo early atrophy, except on the convex or labial surface.

The atrophy of odontoblasts, with the exception of those opposite the enamel organ, proceeds rapidly and is complete usually when general histological changes in many organs are well established, and therefore in as short a period as 60 to 70 days. All cells resembling odontoblasts disappear and the internal surface of the dentine becomes bounded by cells that are indistinguishable from other connective tissue cells of the pulp until extreme thinning of the dentine has resulted (Fig. 7).

During the process of atrophy, odontoblasts, and rarely capillaries that supply them, become incorporated into the dentine because some cells remain functional for a longer time than others and by continuing to form it surround non-functioning cells with dentine. This detail is most strikingly seen when the odontoblasts on the labial side finally begin to atrophy (Fig. 8).

In rare instances, not accounted for by us, cells resembling osteoblasts in size and shape were present on the inner surface of the dentine and had deposited a thin interrupted layer of bone (secondary dentine?).

In the depths of the pulp small areas of osteoid tissue were frequently found in rats with fully developed deficiency. Going over the whole series of rats we worked out the sequences of pulp ossification as follows: first, minute spherical globules of hyaline matrix appear between the pulp cells and simultaneously narrow zones of similar material form about occasional capillaries; these deposits increase and incorporate pulp cells which assume the morphology of osteoblasts, and finally cells in contact with the periphery acquire the morphology of osteoblasts and presumably function as such.

In consequence of the atrophy of odontoblasts, as the tooth continues to grow, the dentine near the formative end becomes very thin, often sharply folded or pleated, or entirely absent (Figs. 3, 9, 10 and 13). Associated with these late consequences of the deficiency are aggregations of cells of two types, each responsible for the formation of masses of dentine (denticles) continuous with or separated from the tooth wall. In every instance where our sections passed through or near to the basal formative end, when these cell aggrega-

tions were present we found strikingly defective dentine formation associated with atrophic odontoblasts and much atrophied ameloblasts in Hertwig's sheath (the enamel organ at the basal formative end).

In some instances columnar cells were enclosed within folds of dentine and the relationships were such as to indicate positively that these cells were incorporated ameloblasts. The morphology indicated also that these cells were ameloblasts because they were cylindrical and radially arranged in contrast to the shrunken odontoblasts on the opposite side of the dentine. In regions of complete absence of the dentine at or close to the formative end there were frequently, in the gaps, gland-like clusters of cells that we are also forced to interpret as of enamel organ origin. Such gland-like formations also lie under the thin abnormal dentine (without canaliculi) at considerable distances from the basal end (Figs. 11, 12 and 15). One type of cell aggregate then we regard as of enamel organ origin and due to the fact that cells (presumably ameloblasts because, as we shall show below, they do not survive in repair) become incorporated by the pleating of a very thin and atypical dentine at the formative end.

The other type of cell aggregate is proliferative in type and consists of cells derived from the pulp that forms in regions of extremely thin or totally absent dentine. These cells are short, plump, basic staining, and resemble transitional forms between mesenchymal cells and osteoblasts. Their presence is accompanied by deposition of atypical dentine (osteodentine) upon the tooth wall (Figs. 3, 9, 10 and 11), although in very severe deficiency they form no matrix at all. These cell aggregates we regard as reparative in origin, comparable to callus formation in bone, and indicative that even in extreme vitamin A deficiency proliferative responses on the part of connective tissues may be energetic. We have further proof of this in the repair of wounds made for experimental purposes. All of our decisions regarding origins of cells were made after careful study of the histology of repair during recovery induced by substituting butter fat for lard in the diet.

An outline of the sequences observed in recovery is therefore indicated. As we have described above, the restoration of the enamel organ takes place first at the basal formative end. Very promptly the odontoblasts in juxtaposition to the enamel organ at this end recover and by the 7th day (Rat 417, Table II) in the region of Hert-

wig's sheath they have assumed normal morphology and function, as shown by the deposition of predentine with canaliculi. The predentine calcifies rapidly. Folds in pleated dentine fill in rapidly. Excess atypical dentine, so-called secondary dentine or osteodentine, the product of the proliferative pulp cell aggregates and denticles of ameloblast inclusion origin grow in size by the deposition of dentine upon them (Figs. 16, 17 and 18). The ultimate fate of these "denticles" we have not followed, but it seems reasonably certain that they continue to increase in size while advancing forward as the tooth grows.

The reappearance of columnar odontoblasts is represented in all stages by our series of repair experiment rats. It takes place first at the basal formative end and works forward, first upon the labial side of the tooth, presumably in response to the recovery of the enamel organ. Later it extends throughout the tooth. Predentine formation is active before the odontoblasts have acquired columnar shapes. Complete morphological recovery was almost achieved in 19 days in Rat 414, which showed a very satisfactory increase of weight in response to butter fat in the diet. There is little to record about the sequences of odontoblast recovery. The changes observed at different periods of recovery were quite like those to be seen in the formation of osteoblasts in granulation tissue in the repair of bone. Connective tissue cells in contact with the dentine become plumper and more basic in staining reaction. For a brief period they are polyhedral in shape and processed like young osteoblasts. Soon they become aligned more or less parallelly with their long diameters oblique or perpendicular to the dentine, but before there is any regularity in arrangement or uniformity in shape, predentine is deposited upon the old dentine. The sequences may be arranged in four stages: (1) increase of size, or at least of cytoplasm concentrated about the nucleus; (2) assumption of osteoblast-like morphology; (3) evidences of function and polarity, as evidenced by directional deposit of predentine; and finally, (4) further growth in size and acquisition of the normal shape.

Various stages of odontoblast recovery may be seen in one tooth because, as stated above, the recovery begins first opposite the enamel organ, *i. e.* at the basal end and labial surface. We have thus had ample opportunity in our recovery series to feel secure in com-

paring the recovery or reappearance of odontoblasts to the sequences seen in bone repair from fibroblast to osteoblast. Our observations lead us to regard, from the morphological viewpoint, the odontoblast as a polarized osteoblast.

The recovery sequences permit no doubt regarding the nature of the proliferative cell aggregates. They are of pulp connective tissue origin. The other types of cell aggregate in gland-like formation we have already concluded were of enamel organ origin. Again the morphological evidence is supplied by the sequences seen in recovery. The first change in recovery is an increase in size of the cells, some of which undergo mitosis (Fig. 12). The pulp cells in contact with these gland-like formations *respond, as do those in contact with the dentine,* and proceed to deposit predentine. We thus get the picture of epithelial cells in the pulp surrounded by dentine, in turn surrounded by odontoblasts. This is shown in low power at A and B in Figure 13 and at higher power in Figure 14. The enclosed epithelial cells undergo atrophy and disappear (Fig. 19). The last stage of degeneration is like that of the ameloblasts in the deficiency. The cells break up into coarse granules and the residue becomes calcified; pyknotic nuclei can be seen in denticles of this origin for some time after calcification of the cell débris (Fig. 18).

Excessive local formation of atypical dentine (denticles) is produced in vitamin A deficiency by focal proliferative responses of pulp cells. Such deposits are insignificant, but increase in the recovery period commensurate with rate and extent of recovery. Another type of excess dentine formation or denticle is initiated by the presence of cells of the enamel organ that become incorporated by folds of dentine or through total gaps in the dentine. In repair these cells stimulate the formation of odontoblasts from the adjacent pulp and hence denticle formation. We thus have brought new experimental evidence to support the prevailing opinion (Orban [10]) that cells of Hertwig's sheath may be carried forward into the pulp and stimulate the formation of odontoblasts from pulp cells, and consequently the formation of denticles. Fridrichovsky [11] described in human teeth and in rats' teeth (diet not stated) folds of dentine such as were of common occurrence in our series. These he regarded as due to developmental irregularities of Hertwig's sheath. He expressed the opinion that such folds could carry enamel organ cells

forward, and thus accounted for one source of denticle formation. Naturally he did not consider deficiency disease as a possible cause of the mechanism.

The Incisor Teeth of Guinea Pigs

In general the effect of vitamin A deficiency upon the incisor teeth of guinea pigs is similar to that in rats. There are some quantitative differences and some apparent contradictions appear to our interpretation of events from the teeth of rats. The outstanding difference is the rapidity and severity of the enamel organ changes. The apparent contradiction is exhibited in the prolonged persistence of the morphology and function of odontoblasts after marked degrees of enamel organ atrophy.

The durations of the deficient diet in the experiments with guinea pigs were on the whole shorter than those with rats, inasmuch as at the beginning the majority of the guinea pigs received sufficient orange juice to afford very considerable amounts of vitamin A. Twenty cc. of orange juice daily is completely protective against histological vitamin A effects in all tissues, except occasionally in the respiratory mucosa in the nares. Sixteen cc. daily is nearly protective. Therefore, the durations given in Table III are not those of completely deficient diets. For example, Guinea pig 38 received 16 cc. of orange juice daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Guinea pig 15 received 20 cc. for 6 days, 8 cc. for 90 days and 4 cc. for 57 days. Guinea pig 42 received 16 cc. for 55 days, 8 cc. for 50 days; Guinea pig 39 received 16 cc. for 63 days, 4 cc. for 7 days and 2 cc. for 40 days. Guinea pig 6 received 20 cc. for 70 days and 8 cc. for 39 days. Guinea pig 45 received 20 cc. for 69 days and 8 cc. for 75 days. Guinea pig 46 received 20 cc. for 28 days and 8 cc. for 130 days. Guinea pig 8 received 20 cc. for 52 days and 8 cc. for 11 days. Guinea pig 41 received 20 cc. for 209 days, 10 cc. for 15 days and 5 cc. for 75 days.

Gross evidence of scorbutus was absent in each of the guinea pigs in Table III. Histological evidence was found in the costochondral junctions of Guinea pig 23 which received no orange juice during the last two weeks of the experiment. These changes were so slight as to suggest that the retardation of growth secondary to the A deficiency was a factor, inasmuch as there was the typical athreptic type of

costochondral junction. In Guinea pigs 24, 28 and 29 there were microscopic evidences of repaired scorbutus in the teeth, such as we described in 1926.[2] The scorbutic factor, as possibly responsible for the changes we found in our vitamin A deficient guinea pigs, may be dismissed because of the fact that all of the changes we are describing were found in guinea pigs that had never received less than 8 cc. of orange juice daily and that exhibited no gross or microscopic evidences of scorbutus. This we emphasize because Höjer [8] has described and illustrated changes involving odontoblasts and dentine, with the production of osteoid or bony formations in the pulp and upon the dentine in latent or partial scorbutus. As our own work with scorbutus has been upon animals completely deprived of vitamin C we have as yet no basis for criticizing Höjer's conclusions. Such premises as we now have incline us to believe that in partial vitamin C deficiency Höjer's results would be duplicated. Both vitamin A and vitamin C deficiencies affect odontoblasts, though by different routes, but the effect upon dentine formation may be the same.

The Enamel Organ

The first demonstrable effect of the deficiency upon the incisor teeth is in the enamel organ. As in the case of the rat the external layer atrophies first. The ameloblasts become smaller and shorter. In rapidly progressing deficiency they finally undergo granular degeneration and disintegrate. Usually low cuboidal cells persist for long periods and we are not able to say positively whether they are atrophied ameloblasts or cells of the stratum intermedium, though we believe they are derived from the latter, inasmuch as often these cells have horizontal diameters nearly double the vertical, and it is improbable that atrophied cells should undergo such a change. Eventually the enamel organ completely disappears, unlike what occurs in rats. Before the atrophy is complete, globules of calcified matrix appear in the outer layer of the enamel organ between the inconspicuous epithelial papillae and within the epithelium itself. These globules increase in size and number, extending into the adjacent connective periodontal membrane. Similar calcified deposits form above the atrophic remains of the enamel epithelium and enclose epithelial cells (Figs. 20 and 21). When large, these calcified deposits take on the appearance of bone (Figs. 22 and 23), because

of the incorporated cells. However, the cells do not have the appearance of osteoblasts. They are without processes and in the largest plaques are surrounded by clear spaces reminiscent of cartilage rather than bone. Neither do these plaques become surrounded by osteoblasts. They resemble cementum rather than other examples of pathological bone formations, such as we are familiar with. They are often bounded on the deep side by cuboidal epithelial cells that laterally are continuous with atrophic cells of the enamel organ. The plaques increase by accretion. Globules of matrix continue to be deposited from the adjacent connective tissue.

The Odontoblasts

Atrophy of the odontoblasts occurs late. In no guinea pigs of our series was there complete cessation of dentine formation; always there were on the labial side a normal-appearing layer of odontoblasts and evidences of functional activity; on mesial and lateral sides only partial atrophy was found and hence the cross-sectional shape peculiar to the rat's incisors was not seen in the guinea pig. As in the case with rats the atrophy of odontoblasts appeared first on the lingual side of the tooth and never before advanced atrophy of the enamel organ had occurred. A very striking difference from occurrences in rat teeth is that coincident with the atrophy of odontoblasts, spicules of a modified dentine form (Fig. 24). This material, at first uncalcified and similar to predentine in all respects except that it contains no canaliculi, becomes finally calcified and continues to grow by peripheral deposit. In long-continued deficiencies, when these dentine-like deposits extend deeply into the pulp, they become surrounded by osteoblasts which they incorporate as they grow; further increase in size is like that of growth of osteoid tissue (Fig. 25).

The earliest formation of these amorphous dentine processes we found in regions of most marked atrophy of the odontoblasts and always upon the lingual side of the tooth. The initial stage we found to be a deposit of predentine-like matrix between atrophied odontoblasts that had lost their columnar form. It was evident that functional activity continued, but polarity of the odontoblasts was lost. In early formations but few cells are found enclosed in matrix. The outgrowths for a period are surrounded by other atrophic cells of the

odontoblast layer which retain their polarity. Finally, polarity is lost and the cells concerned in further growth are indistinguishable from osteoblasts.

The Pulp: The pulp remains practically unchanged. The only ossification we found in our series took origin in the manner described above as a continuation of growth of dentinoid processes.

The Cementum: As a rule in well established A deficiency there are local increases in thickness of the cementum, for the narrow, deeply calcified border surrounding the dentine is to be regarded as cementum. In fact the only cementum resembling bone we have seen in connection with the incisors of guinea pigs has been in A deficiency animals. The formation of cementum of appreciable thickness and enclosing cells is always found first upon the lingual side, but may extend on lateral and mesial sides forward to the enamel organ region as an irregular layer (Fig. 24). Frequently sharply circumscribed cementum formations were found, perhaps warranting application of the term cementicle (Fig. 26).

Discussion

Three facts are of assistance in interpreting vitamin A deficiency effects upon incisor teeth of rodents: (1) the enamel organ moves forward with the growth of the tooth so that the ameloblasts maintain throughout the same relative position to calcified structures; (2) the dentine increases in thickness progressively toward the anterior (occlusal) end, indicating activity of the odontoblasts extending nearly to the end of the tooth; and (3) the occlusal end of the pulp chamber is being constantly filled in by bony material (osteodentine), indicating a normal tendency on the part of pulp cells to produce osteoid matrix and cessation of a polar deposition of matrix on the part of the odontoblasts.

Fact 1 is of assistance in explaining why the earliest and maximum atrophy of the enamel organ is found at some distance from the formative end as a requirement of time for the deficiency to take effect. Fact 2, as dentine is not resorbed in consequence of the deficiency, explains the greater thickness of dentine for long periods in the deficiency toward the occlusal end. Fact 3 warrants the assumption that the deposit of osteodentine following atrophy of odontoblasts is an adaptation or premature stimulation of a normal sequence.

The enamel organ changes we have described in rats and guinea pigs are primary consequences of vitamin A deficiency common to many epithelial organs. The more rapidly produced and pronounced changes in guinea pigs, as compared with rats, are in conformity with the changes in other organs in these two animals (Wolbach and Howe [1]).

We prefer to interpret some effects upon odontoblasts as secondary to enamel organ atrophy, assuming that the latter, in teeth that are continuously growing, exerts an influence upon the former, as suggested by the early sequences in tooth formation and throughout life at the basal formative end of rodents' incisors. This interpretation is not supported by the facts that odontoblasts persist after disappearance of the enamel-forming organ in all stationary permanent teeth of animals, and that in incisor teeth of rodents the enamel-forming organ is continued forward only upon the labial side. Nevertheless, our observations show that enamel organ atrophy precedes atrophy and depolarization of odontoblasts. The fact that in rats odontoblasts remain morphologically and functionally active on the labial side, in apposition to the enamel organ, long after complete disappearance upon other surfaces, and that in this situation their eventual atrophy only follows enamel organ atrophy, demands the above interpretation. The finer sequences attending atrophy of the odontoblasts on the labial side, which we have carefully followed, here and there an odontoblast ceasing to deposit matrix upon its external pole, with continued deposition centrifugally, support strongly the argument that even though the odontoblast may be individually physiologically independent of the enamel organ, architecturally it is dependent. We have come to regard the odontoblast as a polarized osteoblast and the enamel organ as the polarizing agent. In guinea pigs the same changes occur, though in less evident degree. The persistence of normal odontoblasts upon the labial side is more pronounced than in rats. Odontoblast atrophy is less striking, yet illustrates more conspicuously in the sequences we have described the effects of the depolarization of odontoblasts, as shown by the extensions into the pulp of the osteodental processes (denticles) built up by these depolarized cells.

Late vitamin A deficiency effects in bones, as described by Pappenheimer,[12] and completely confirmed by us in unpublished studies, include complete cessation of growth. It may, therefore, be argued

that effects similar to those in bone should be expected in odontoblastic activities and that both are of the same nature. This we may admit without affecting the rôle of the enamel epithelium in the determination of the order of events. More direct attack based upon experimentally produced lesions of the enamel organ is needed to settle these problems.

The formation of bone in rats in the pulp proceeds, we have learned, as does the ossification in membranous bone formation, through direct change in character of the intercellular matrix, preceded by finely granular calcium deposition. Calcification in and adjacent to the atrophied enamel organ, followed by ossification, in guinea pigs may be regarded as a phenomenon having similar pathogenesis. In both instances the cells directly responsible for the elaboration of specific calcified structures, dentine and enamel, are partly or completely inactive, a fact that invites speculations along the lines of a continued extracapillary delivery of calcium and phosphorous compounds to the tissues, and therefore to questions of differentiated permeability of capillary endothelium. The high mineral content of enamel and its rapid growth rate present problems concerning concentration that make such speculations attractive.

We have presented two origins of osteodentine excrescences, or denticles, one occurring in both rats and guinea pigs by the depolarization of odontoblasts, and in the rat accompanied by a proliferative reparative response of pulp cells; the other, only in rats, induced by inclusions of ameloblasts in folds of recently formed dentine, late in the deficiency, close to the formative end. These solutions as to origin could not have been achieved without having for study many stages of repair.

We have observed no changes in the exceedingly thin calcified zone surrounding the dentine in rats which by some morphologists is regarded as cementum. In guinea pigs definite cementum overgrowths (cementicles) were frequently seen on the lingual side of the teeth in consequence of the deficiency, but we can offer no explanation except the suggestion that they formed in response to structural weakness of the bony socket as a part of the general athreptic skeletal effect of late vitamin A deficiency.

The application of our observations to human teeth obviously must be restricted to the formative period and particularly to teeth of the second dentition. Our inference, based upon studies of experi-

mental scorbutus,[2] and rickets (unpublished), is that vitamin A deficiency is the most important because of its effect upon the enamel organ and because recognition of this deficiency is always late and will continue to be so, unless other criteria than ocular effects are looked for by pediatricians. Defective enamel formation, ossification of the pulp, denticle and cementicle formation are all possible consequences of vitamin A deficiency in humans. Studies of unerupted teeth from infants in vitamin A deficiency, obtained post-mortem, are being made by Boyle,[13] and he has recorded observations that indicate we may safely apply some of our observations to the human.

Summary and Conclusions

1. The initial effect of vitamin A deficiency upon incisor teeth of rats and guinea pigs is upon the enamel organ. The ameloblasts respond earliest by atrophy, then the remainder of the organ atrophies; finally metaplasia and calcification, and, in the guinea pig, ossification occur.

2. Atrophy and depolarization of odontoblasts follow enamel organ changes. The odontoblasts survive longest on the side (labial) where in apposition to the enamel organ and in long-continued experiments gross deformities in the incisors of rats resulted from absent or deficient dentine formation.

3. Two types of denticle formation are described, one built up by depolarized odontoblasts, the other by inclusions of ameloblasts by the folding of imperfectly formed dentine at the formative end of the tooth.

4. Defective enamel formation and other poorly understood conditions in teeth, such as denticles, pulp bone and cementicles, may reasonably be regarded in the human as vitamin A deficiency possibilities.

5. Our observations indicate that in the incisor teeth of rodents the odontoblasts throughout life are influenced by the enamel organ.

6. As in other morphological problems concerning vitamin deficiencies, study of the sequences of repair was essential. We emphasize the importance of two types of control material, the normal and progressive stages in repair.

7. Our observations indicate that vitamin A deficiency is the most important of the known vitamin deficiencies in its effect upon tooth formation.

REFERENCES

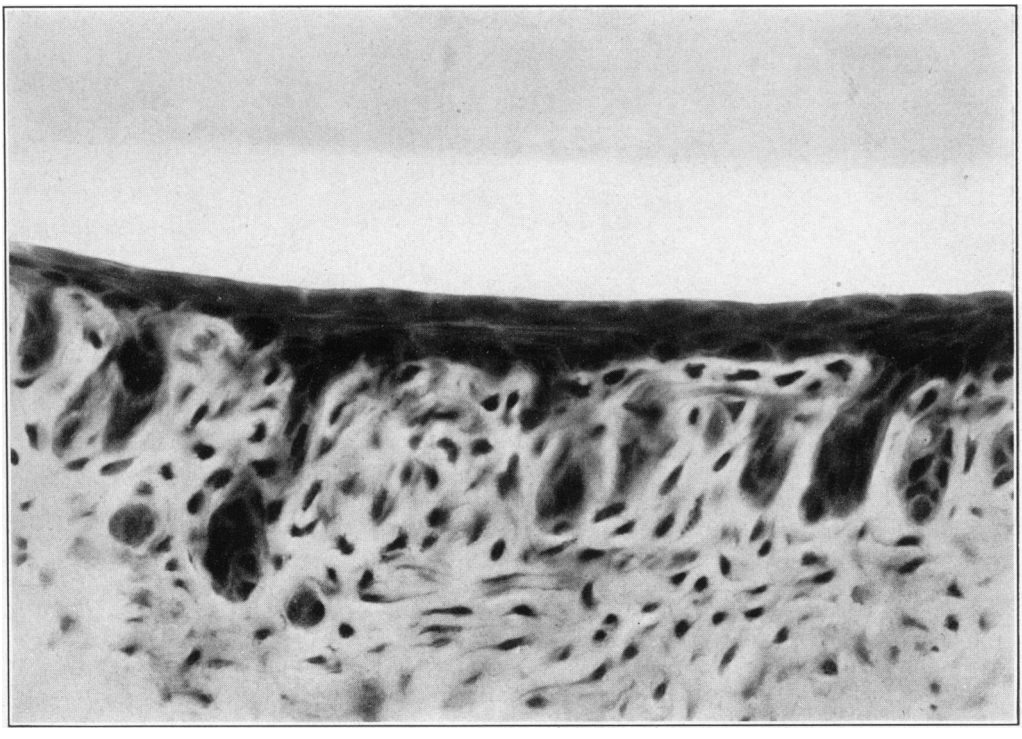
1. Wolbach, S. B., and Howe, P. R. Tissue changes following deprivation of fat soluble A vitamin. *J. Exper. Med.*, 1925, **42**, 753–777.
2. Wolbach, S. B., and Howe, P. R. Intercellular substances in experimental scorbutus. *Arch. Path. & Lab. Med.*, 1926, **1**, 1–24.
3. Mellanby, May. The influence of diet on the structure of the teeth. *Physiol. Rev.*, 1928, **8**, 545–577.
4. Marshall, J. A. Dental caries and pulp sequelae resulting from experimental diets. *J. Am. Dental Assoc.*, 1927, **14**, 3–37.
5. Coolidge, E. D. Heterotopic ossification in the pulp: a contribution to research on the biology of the dental pulp. *J. Am. Dental Assoc.*, 1929, **16**, 821–834.
6. Shibata, M. Effects of lack of vitamins on development of teeth. *Japanese J. Exper. Med.*, 1931, **9**, 21–32.
7. Wolbach, S. B., and Howe, Percy R. Vitamin A deficiency in the guinea-pig. *Arch. Path.*, 1928, **5**, 239–253.
8. Höjer, J. A. Studies in scurvy. *Acta. Paediat.*, 1924, Suppl. **3**.
9. Addison, W. H. F., and Appleton, J. L., Jr. The structure and growth of the incisor teeth of the albino rat. *J. Morphol.*, 1915, **26**, 43–96.
10. Orban, B. Dental Histology and Embryology. P. Blakiston's Son & Co., Inc., 1929, Ed. 2.
11. Fridrichovsky, J. Zur Histologie der Dentikel. *Ztschr. f. Stomatologie*, 1927, **25**, 124–157.
12. Hess, A. F., McCann, G. F., and Pappenheimer, A. M. Experimental rickets in rats. II. The failure of rats to develop rickets on a diet deficient in vitamine A. *J. Biol. Chem.*, 1921, **47**, 395–410.
13. Boyle, Paul E. Manifestations of vitamin A deficiency in a human tooth germ. Report of a case. *J. Dental Research*, 1933, **13**, No. 1.

DESCRIPTION OF PLATES

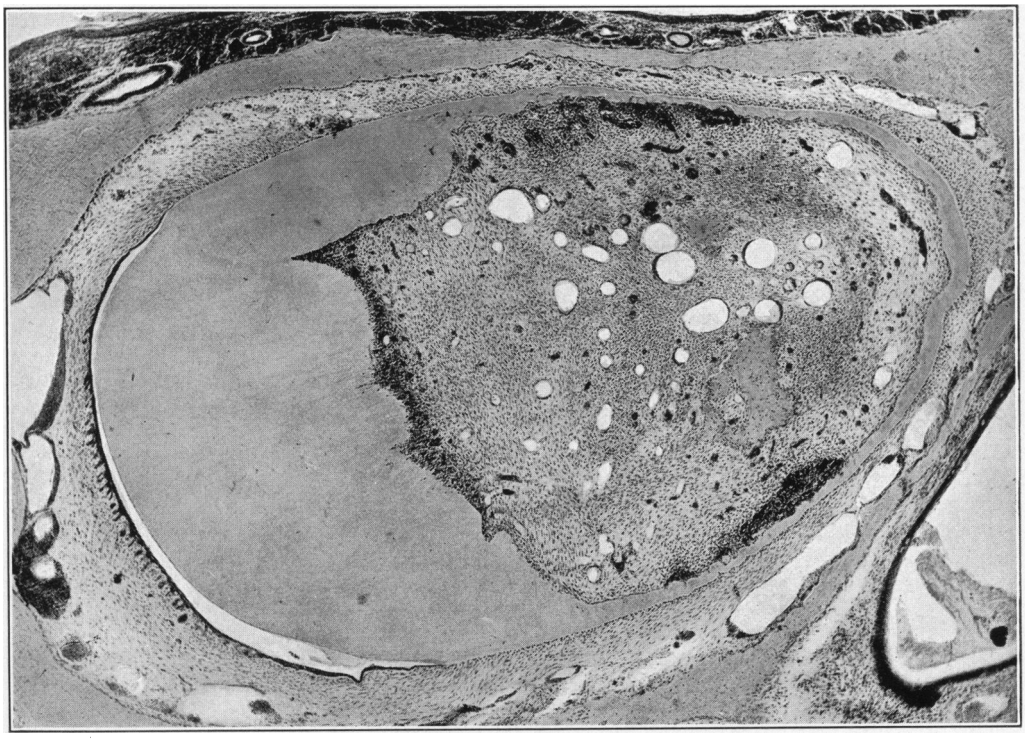
PLATE 38

FIG. 1. Rat 201. Upper incisor, typical of A deficiency in the rat. 101 days on vitamin A deficient diet. Atrophy and metaplasia of the enamel organ. Odontoblasts still present on labial side where the dentine is abnormally thick. Note thinness and folding of dentine on both sides of tooth where there are also many large areas of cellular proliferation derived from pulp cells and possibly from odontoblasts. Osteoid formation present in pulp. × 46.2.

FIG. 2. Rat 201. High power of enamel organ. Same preparation as Fig. 1. 101 days on vitamin A deficient diet. Note great atrophy of the epithelial papillae, absence of ameloblasts and the several layers of flattened cells upon the surface. × 588.



2



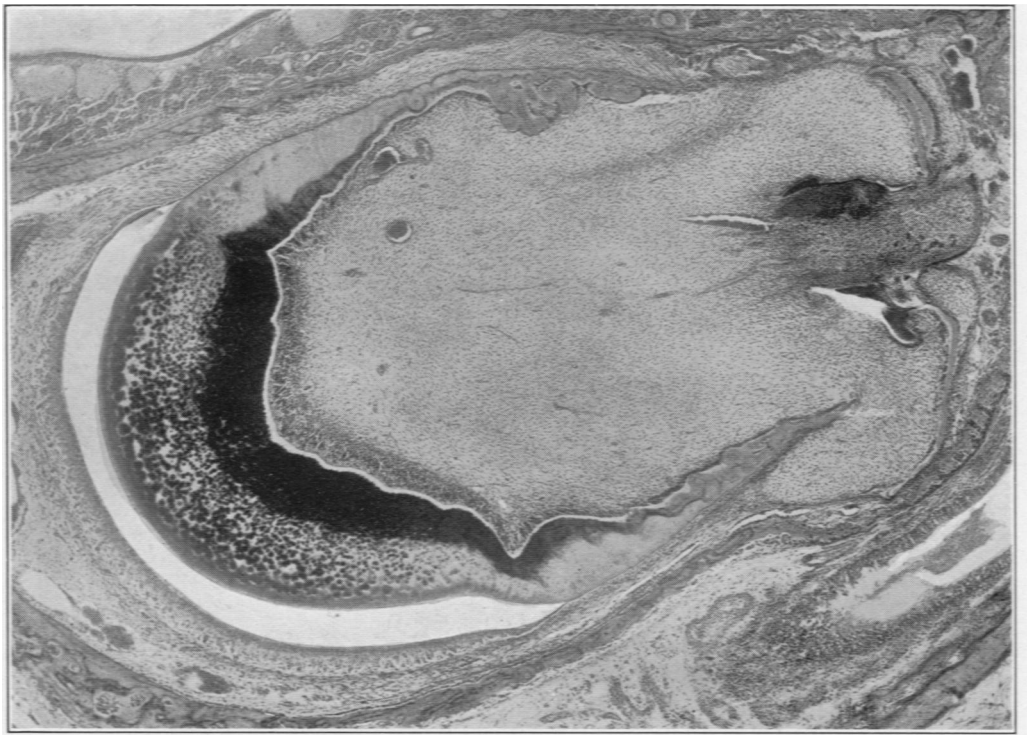
1

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

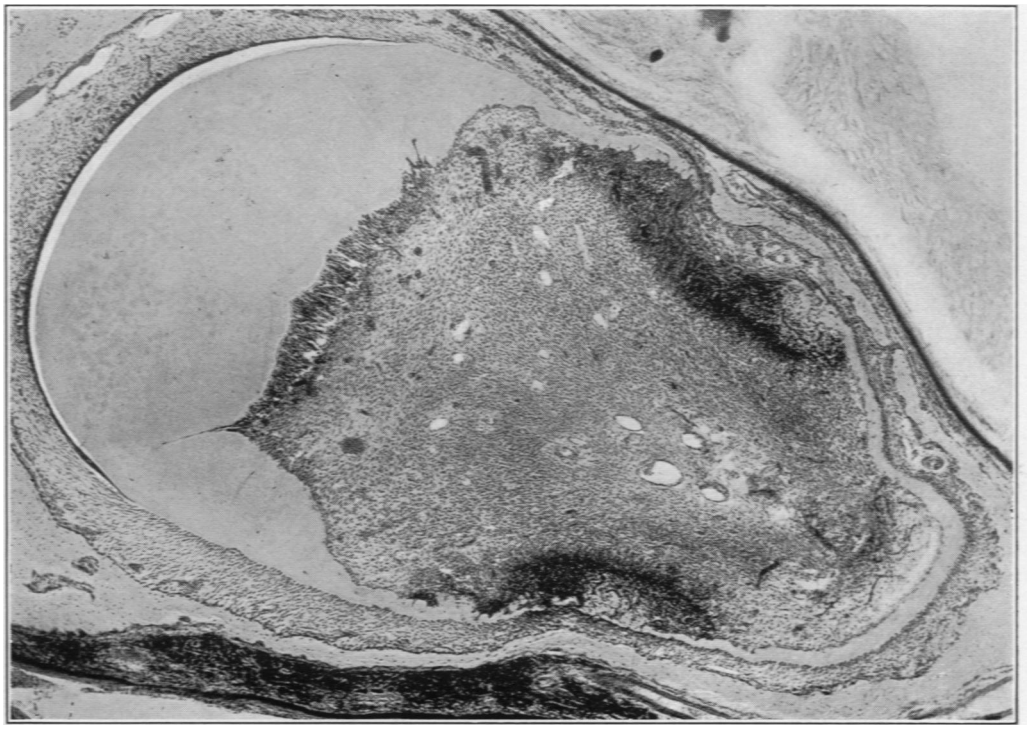
PLATE 39

FIG. 3. Rat 205. Upper incisor. 89 days on vitamin A deficient diet. Atrophy and metaplasia of enamel organ. Columnar odontoblasts still present on labial side. On mesial and lateral sides are gaps in the dentine which is elsewhere thin and much folded. Note the large areas of proliferative response composed of cells resembling osteoblasts. × 46.2.

FIG. 4. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. There is restoration of the enamel organ and heavy calcification of the dentine. The layer of recovering odontoblasts and newly deposited dentine (predentine) shows clearly on both sides, also folding and gaps of the dentine with gross distortion of the shape of the tooth. On the mesial side there are several groups of ameloblasts incorporated in folds of the dentine and further embedded by dentine formed during repair. × 46.2.



4



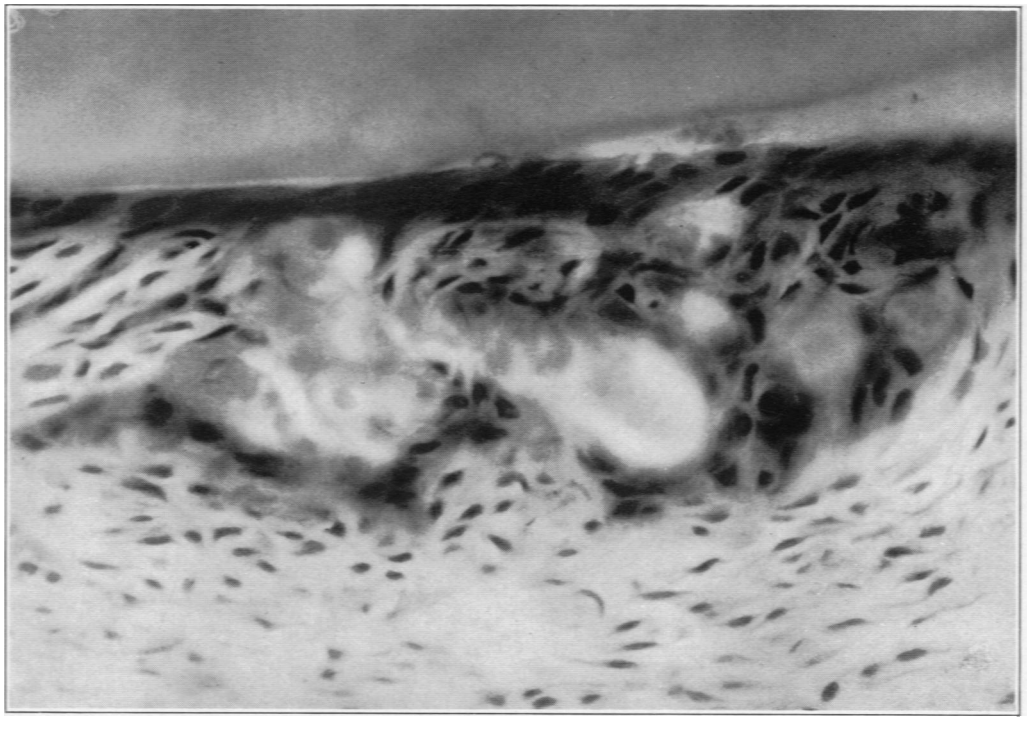
3

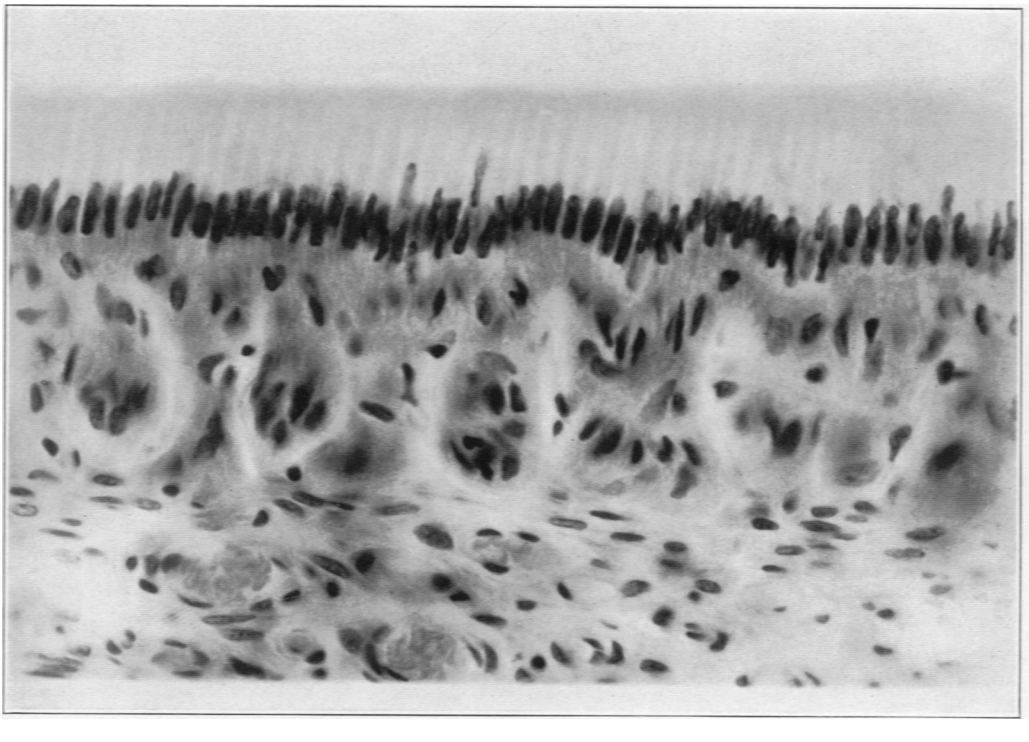
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

Plate 40

Fig. 5. The enamel organ of rat fed upon the control diet in which butter fat replaced the lard of the deficient diet. × 588.

Fig. 6. Rat 235. Upper incisor. 138 days on vitamin A deficient diet followed by 5 days with addition of butter fat. Shows extreme atrophy of enamel organ with globular deposits of calcified material in and adjacent to the atrophic remains of the epithelial papillae. × 588.



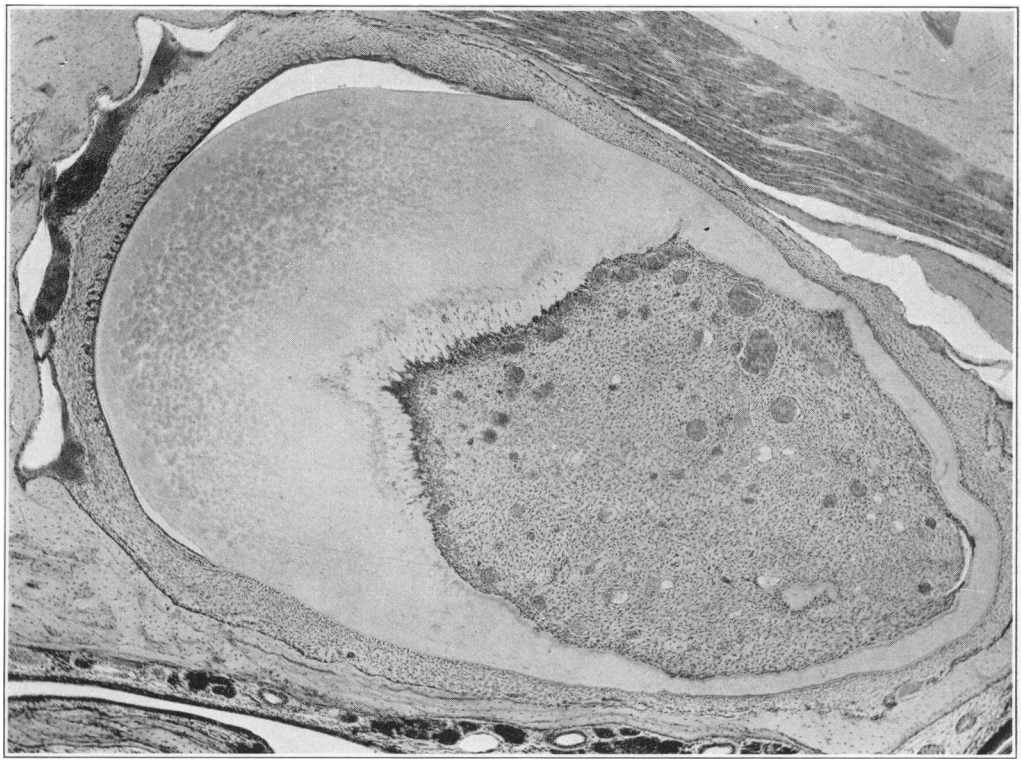


Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

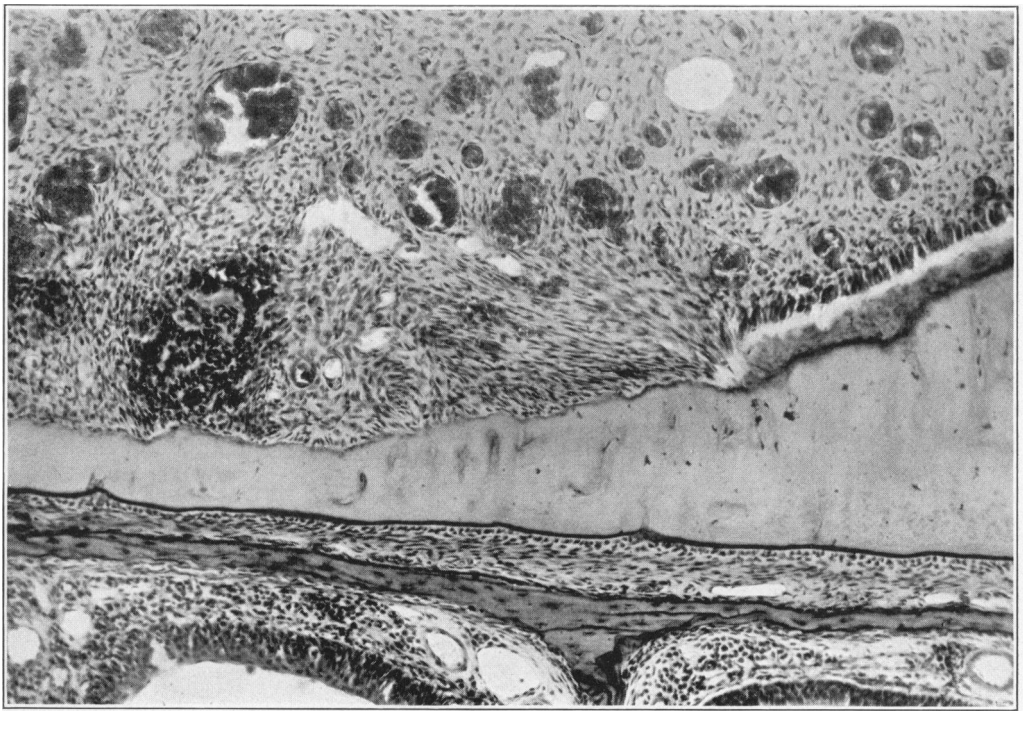
PLATE 41

FIG. 7. Rat 412. Upper incisor at junction of mesial and labial sides. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. The preparation illustrates the differences in behavior of the odontoblasts of the labial side from those on mesial and lateral sides. The odontoblasts of the first show deposition of dentine under influence of restored diet. On the mesial wall the dentine is bounded by connective tissue-like cells, though early repair is shown in places by traces of dentine deposit between cells, some of which are arranged perpendicularly to the dentine. Note also nuclei of cells incorporated in the dentine. × 147.

FIG. 8. Rat 235. Upper incisor. 138 days on A deficient diet followed by 5 days with addition of butter fat. Shows typical shape of vitamin A deficiency rat incisor. Complete atrophy with metaplasia of the enamel organ. Complete atrophy of odontoblasts except on the labial side where the atrophy is nearly complete. Continued function of some cells has resulted in the incorporation of others by the most recently deposited dentine. × 46.2.



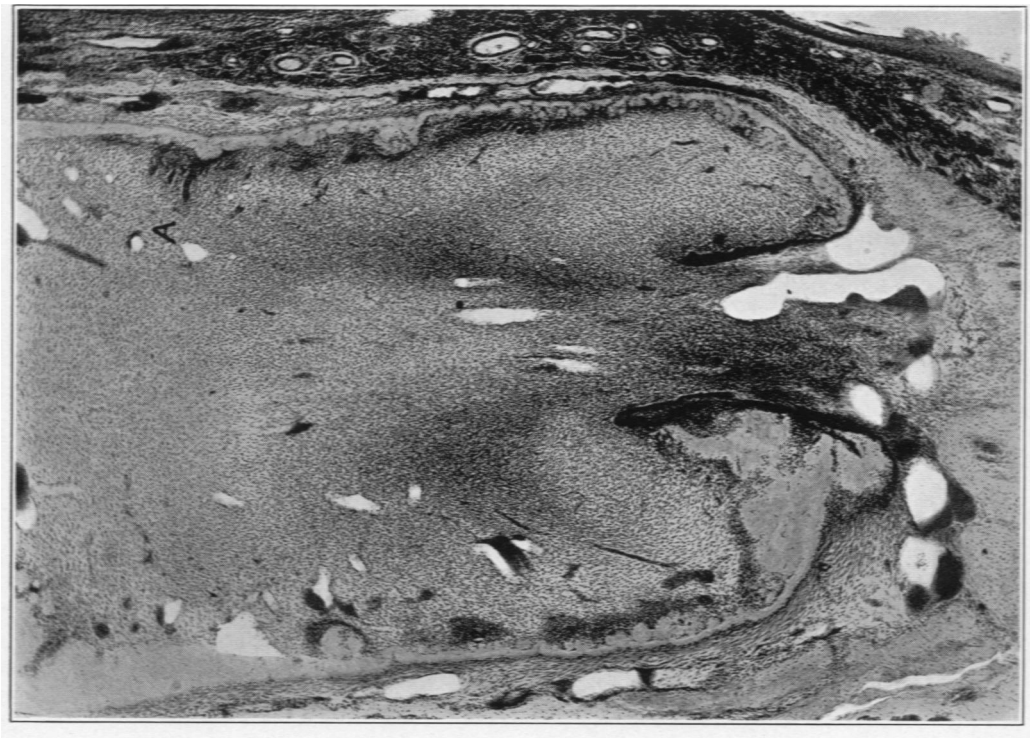
8

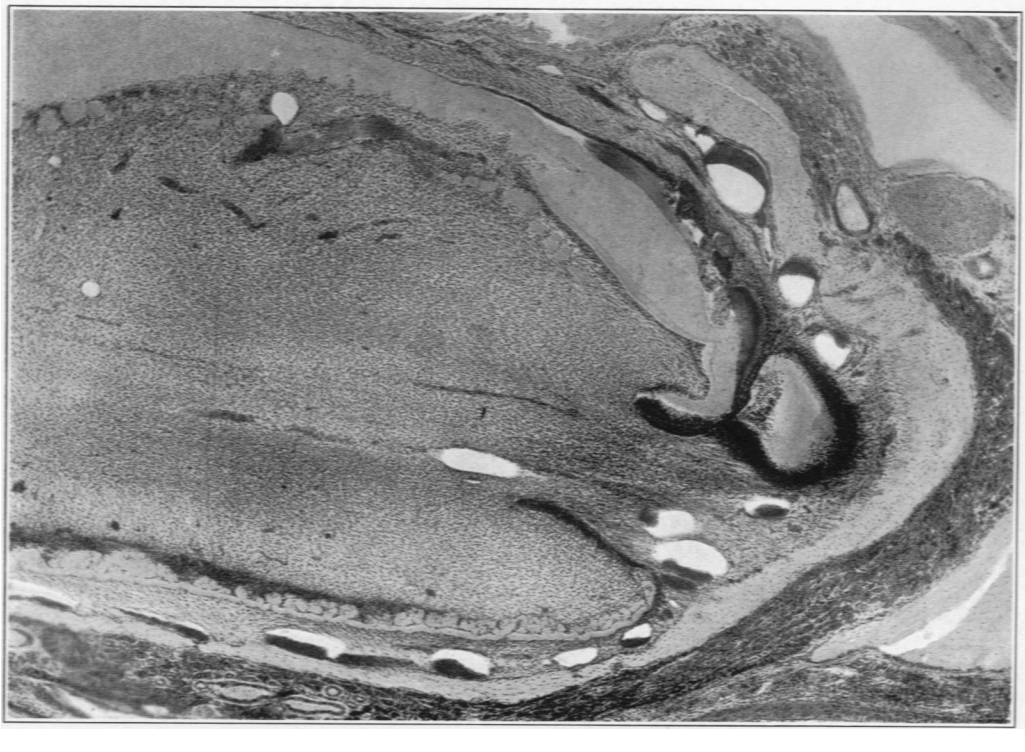


7

PLATE 42

FIGS. 9 and 10. Rat 103. Sections of both upper incisors through formative ends. 180 days on vitamin A deficient diet. Fig. 9 shows particularly well the folding of the dentine which makes possible the inclusion of ameloblasts. Both photographs show clusters of proliferated odontoblasts and in Fig. 10 the gland-like formations due to ameloblasts free in the pulp at A. For high power see Figs. 11 and 12. × 46.2.

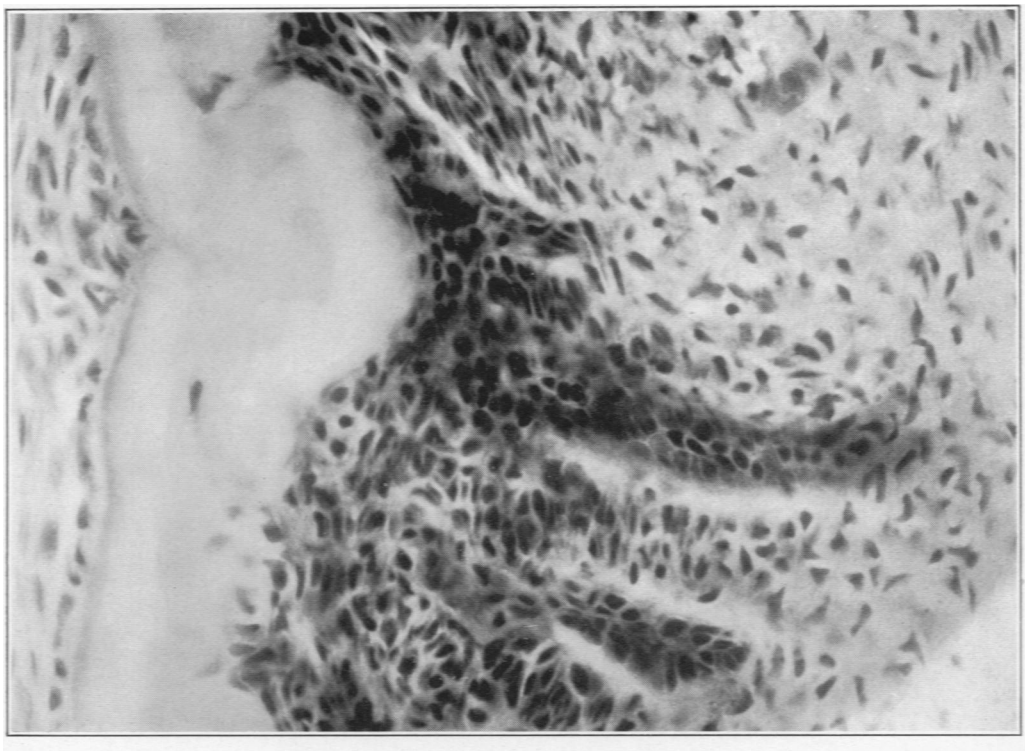




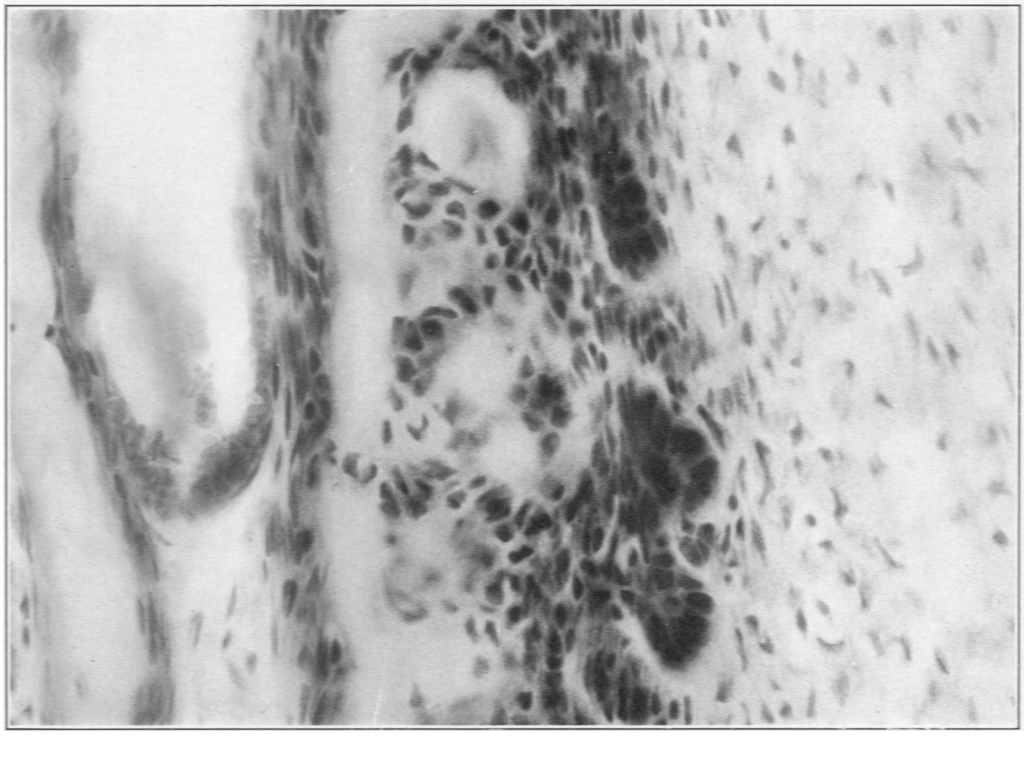
Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

PLATE 43

FIGS. 11 and 12. High power details of Fig. 10 showing inclusions of atrophied ameloblasts in the pulp. In Fig. 11 osteodentine is being formed by pulp cells external to the inclusions of ameloblasts. × 588.



12

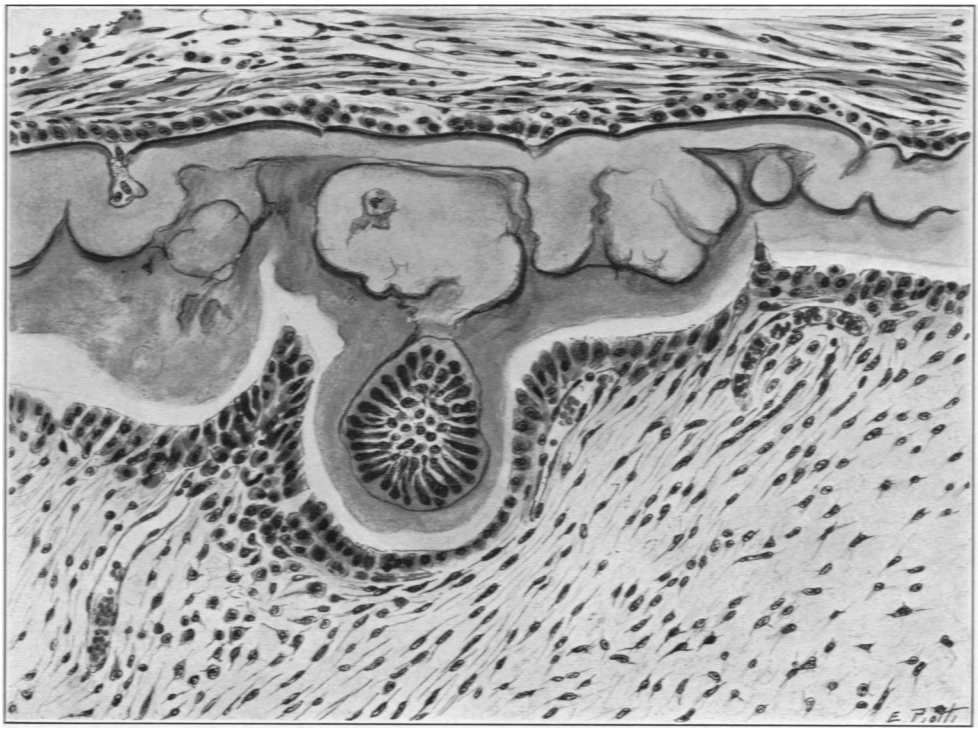


11

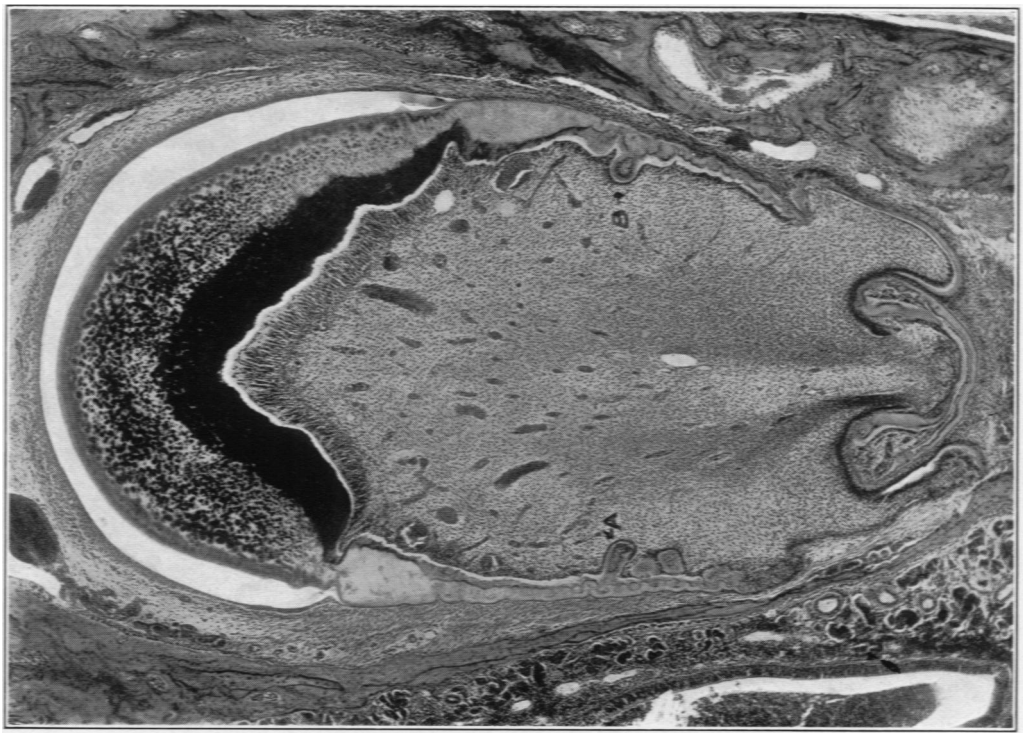
PLATE 44

FIG. 13. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. At A and B are ameloblast inclusions in the dentine. Newly formed dentine has filled spaces between the folds of dentine and has surrounded the inclusions of ameloblasts. For high power detail see Fig. 14. The restoration of odontoblasts is shown in both Figs. 13 and 14. Heavy calcification of the dentine on the labial side of the tooth is shown, a usual response to restoration of vitamin A. × 46.2.

FIG. 14. Drawing. A detail from Fig. 13 at B. Shows dentine formation about ameloblast inclusion as a result of restoration of vitamin A to diet. The odontoblasts have not yet recovered normal size and form, yet much dentine has been deposited and calcified. The irregular outline of the folded dentine of the deficiency period is less deeply calcified than the dentine deposited during the recovery period. The ameloblast inclusion was probably not surrounded by dentine during the deficiency period. × 300.



14



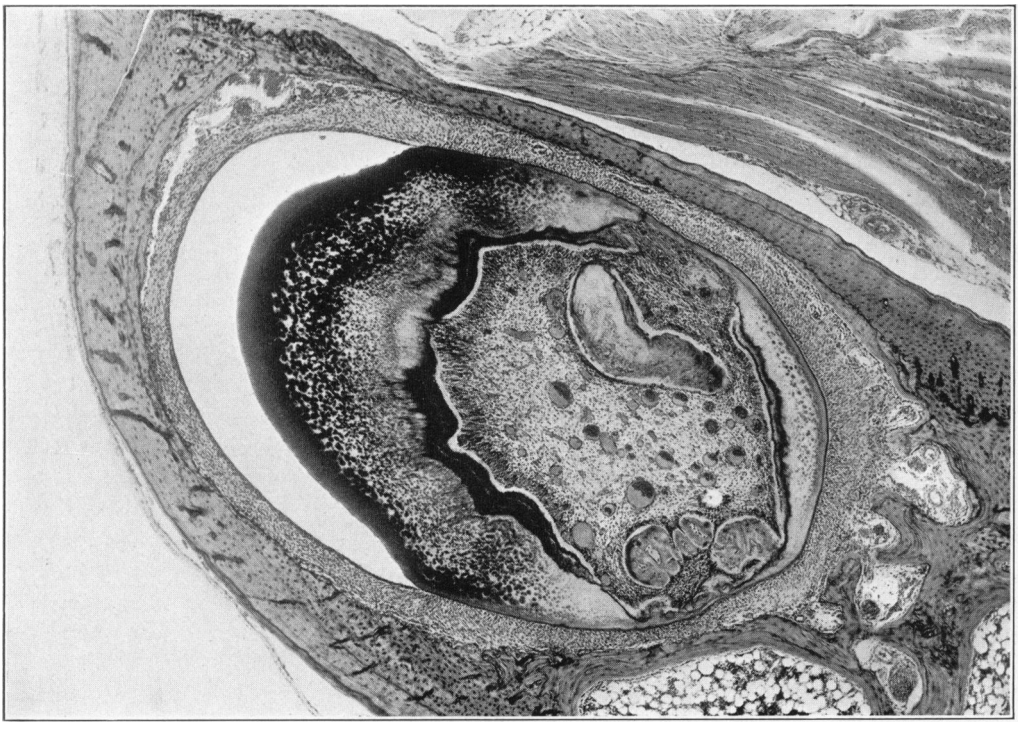
13

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

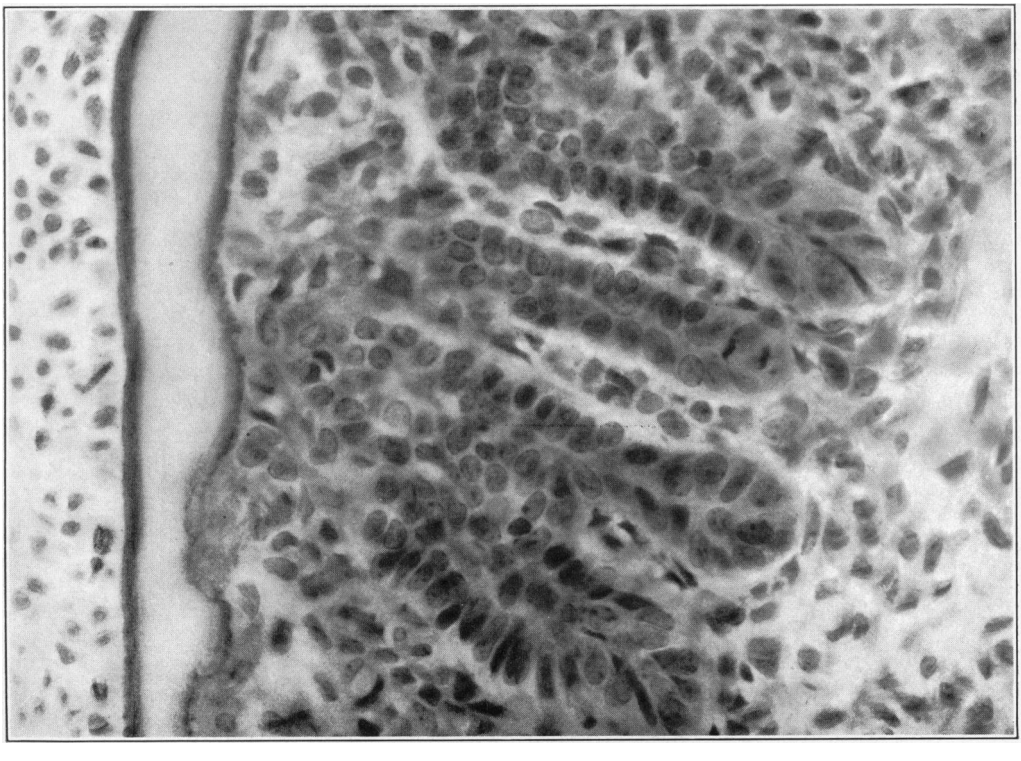
PLATE 45

FIG. 15. Rat 417. Upper incisor. 187 days on vitamin A deficient diet followed by 7 days with addition of butter fat. To show gland-like formation of ameloblast inclusions in pulp in recovery from atrophy. Note mitotic figure. × 588.

FIG. 16. Rat 412. Lower incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show growth of denticles in consequence of restored diet. Heavy calcium deposits in old dentine. × 46.2.



16

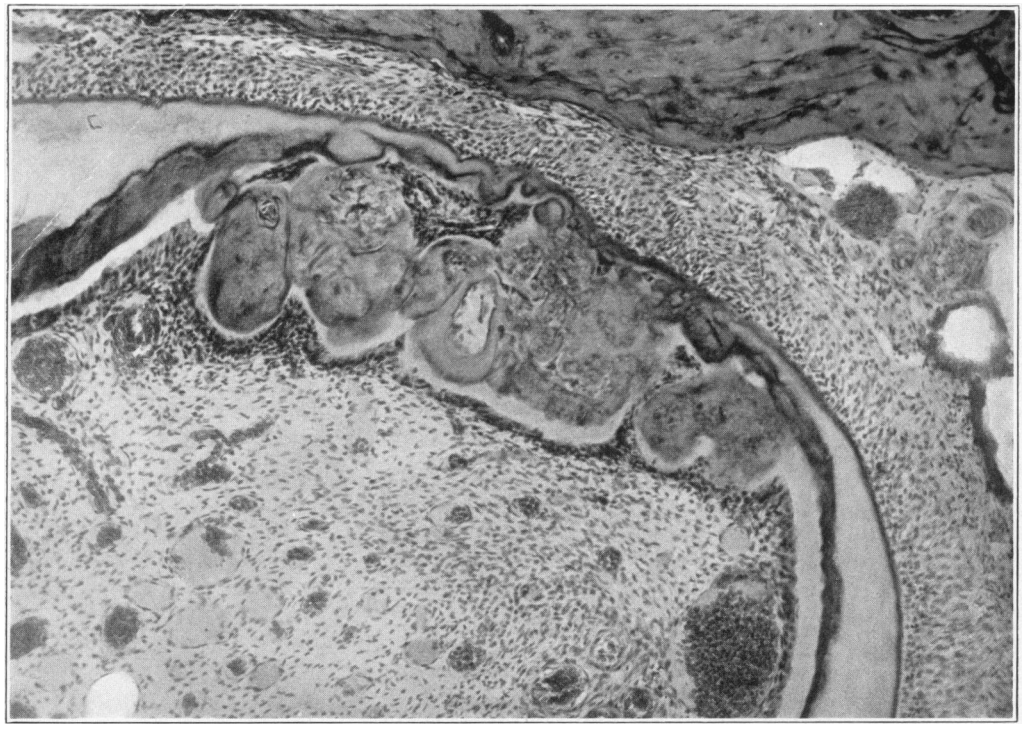


15

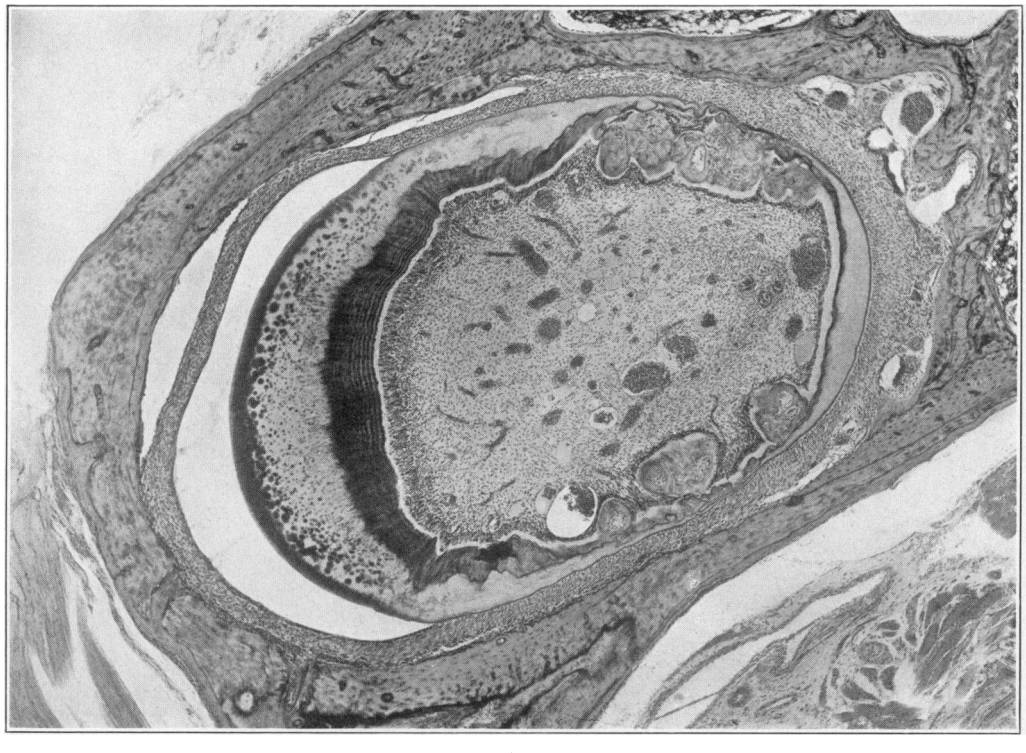
PLATE 46

FIG. 17. Rat 401. Upper incisor. 161 days on vitamin A deficient diet followed by 22 days with addition of butter fat. Denticles of both types showing large deposits of dentine formed since restoration of the diet. × 46.2.

FIG. 18. High power detail of Fig. 17 showing that the odontoblasts, though functionally active, are not yet completely restored in morphology. Inclusions of ameloblasts have disappeared. × 147.



18

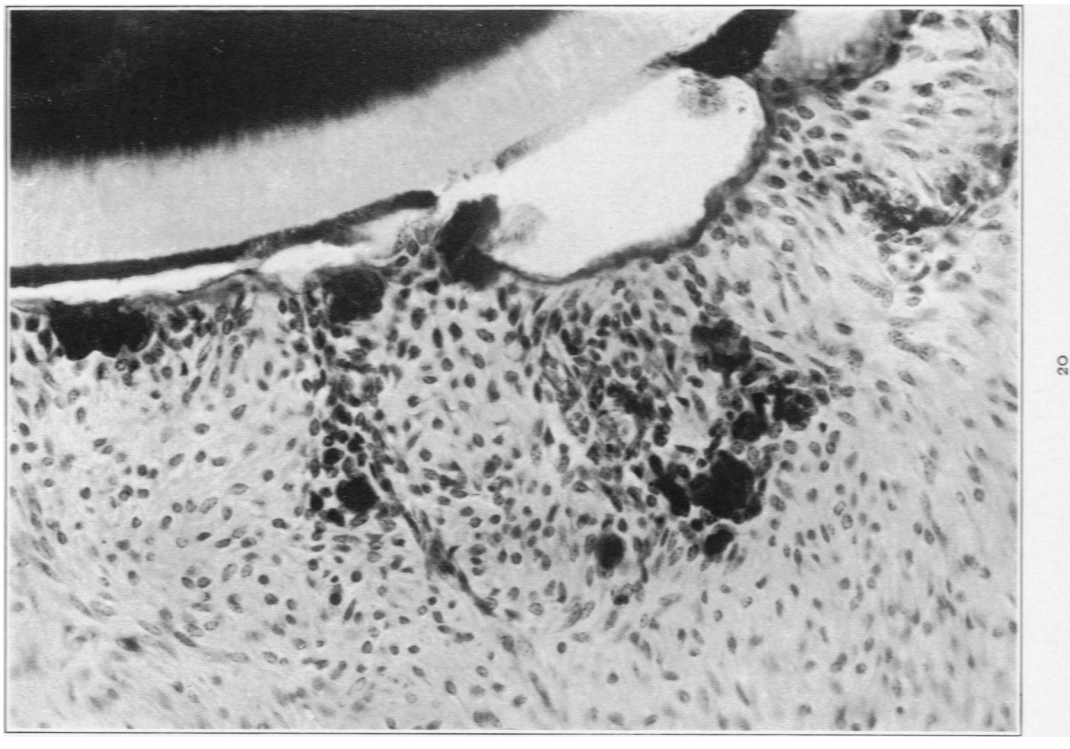


17

PLATE 47

FIG. 19. Rat 412. Upper incisor. 170 days on vitamin A deficient diet followed by 14 days with addition of butter fat. To show odontoblasts formed about ameloblast inclusions in the pulp. The former have deposited a considerable amount of dentine. The latter are atrophic. × 588.

FIG. 20. Guinea pig 142. 128 days on experimental diet without source of vitamin A other than 4 cc. of orange juice daily. Shows nearly complete atrophy and disappearance of enamel organ with calcified deposits in the atrophic remains of the enamel epithelium and external to it. × 294.



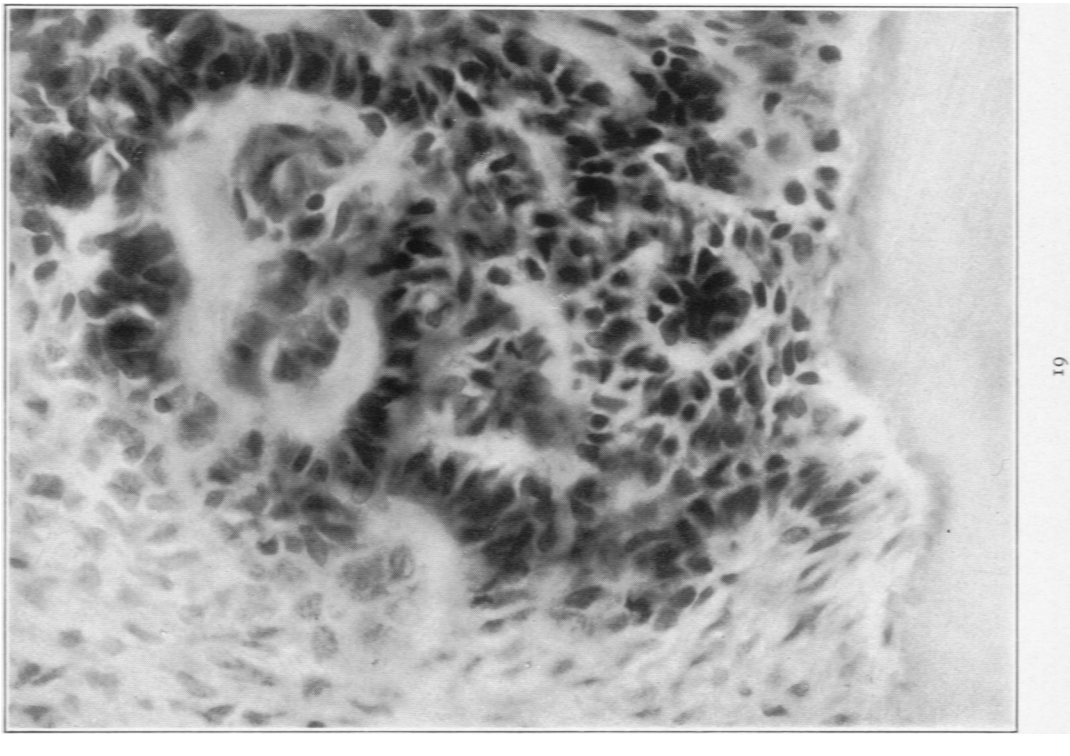
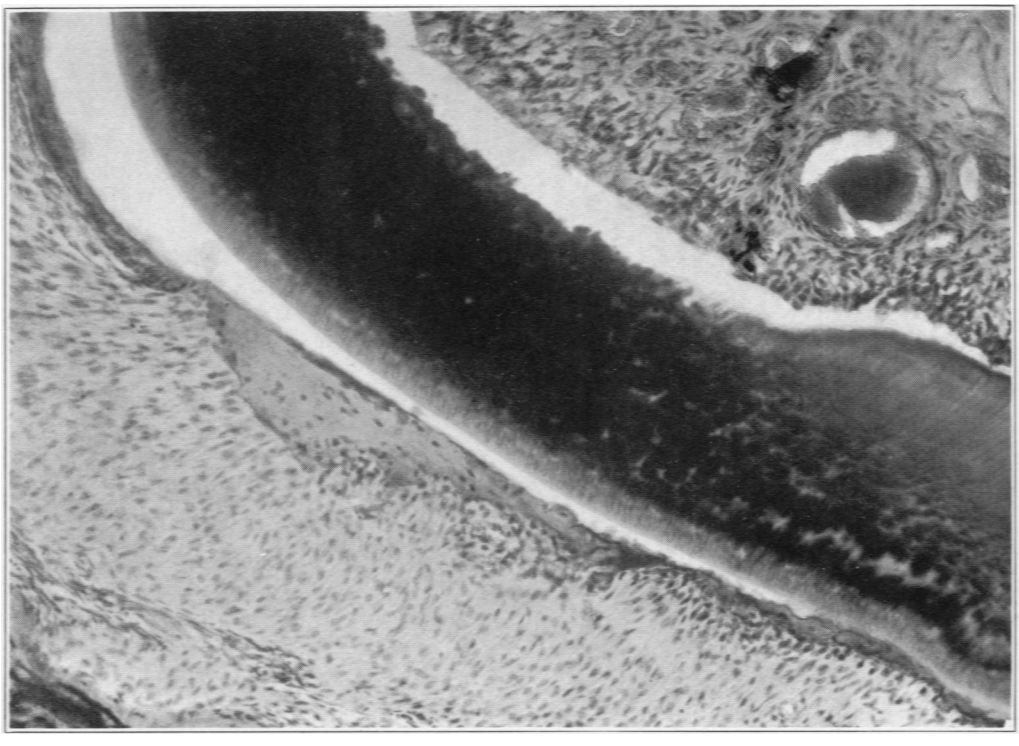


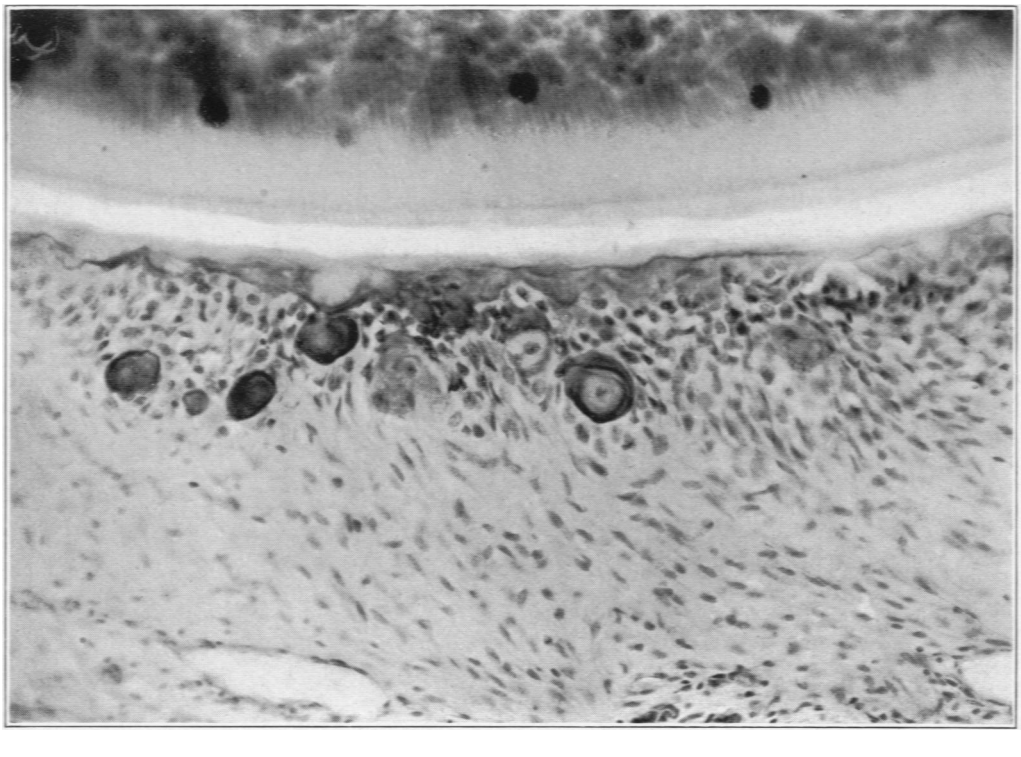
PLATE 48

FIG. 21. Guinea pig 142. Lower incisor. Extreme atrophy and calcification of enamel organ. × 294.

FIG. 22. Guinea pig 38. 91 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 56 days, 8 cc. daily for 21 days and 4 cc. daily for 24 days. Ossification of enamel organ. × 147.



22



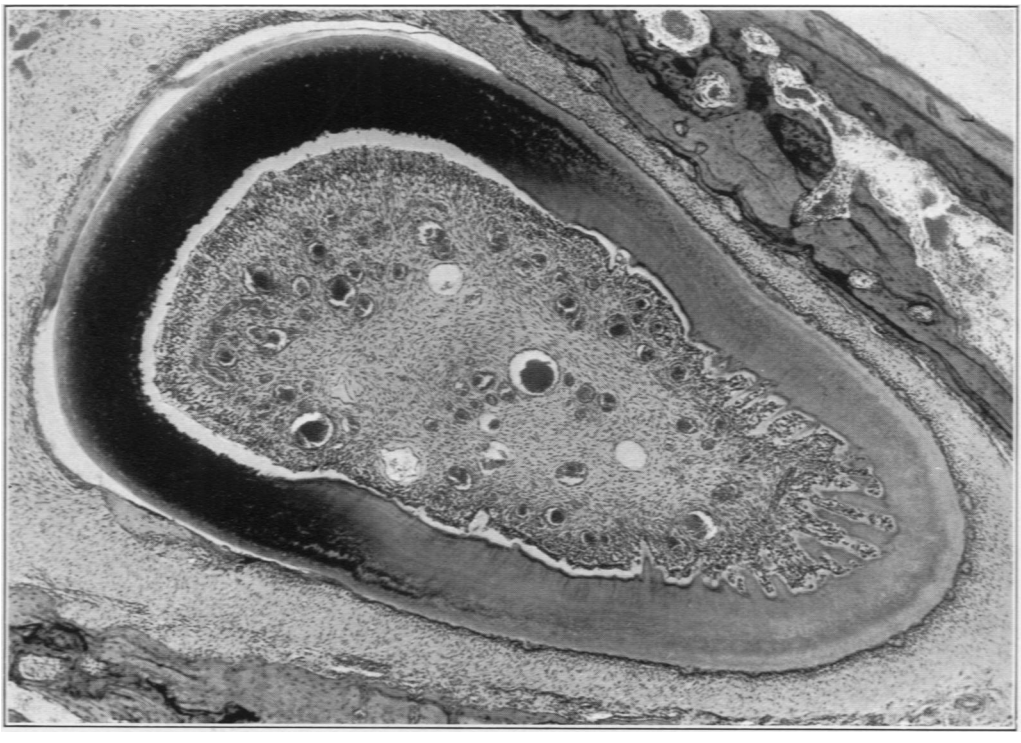
21

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair

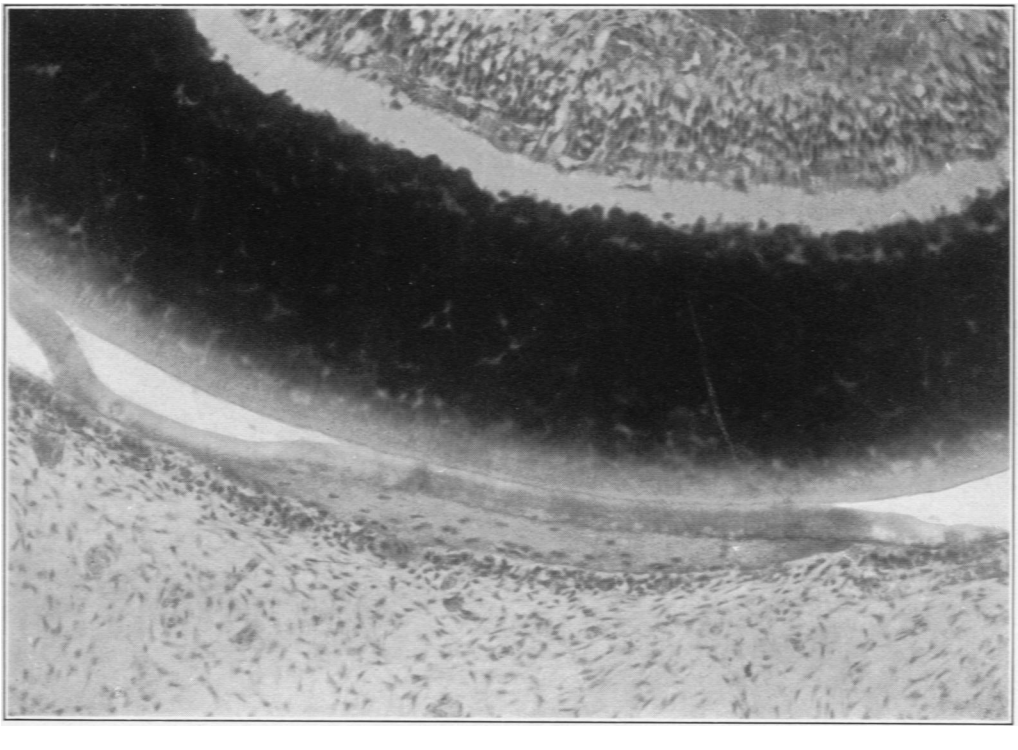
PLATE 49

FIG. 23. Guinea pig 38. Same preparation as Fig. 22. Another region. At each end of the bony plaque and external to it, remains of the enamel epithelium can be seen. × 147.

FIG. 24. Guinea pig 38. Lower power. Same preparation used for Figs. 22 and 23. Shows ossification of the enamel organ, cementicles, and on the lingual side, numerous ingrowths of osteodentine. × 46.2.



24



23

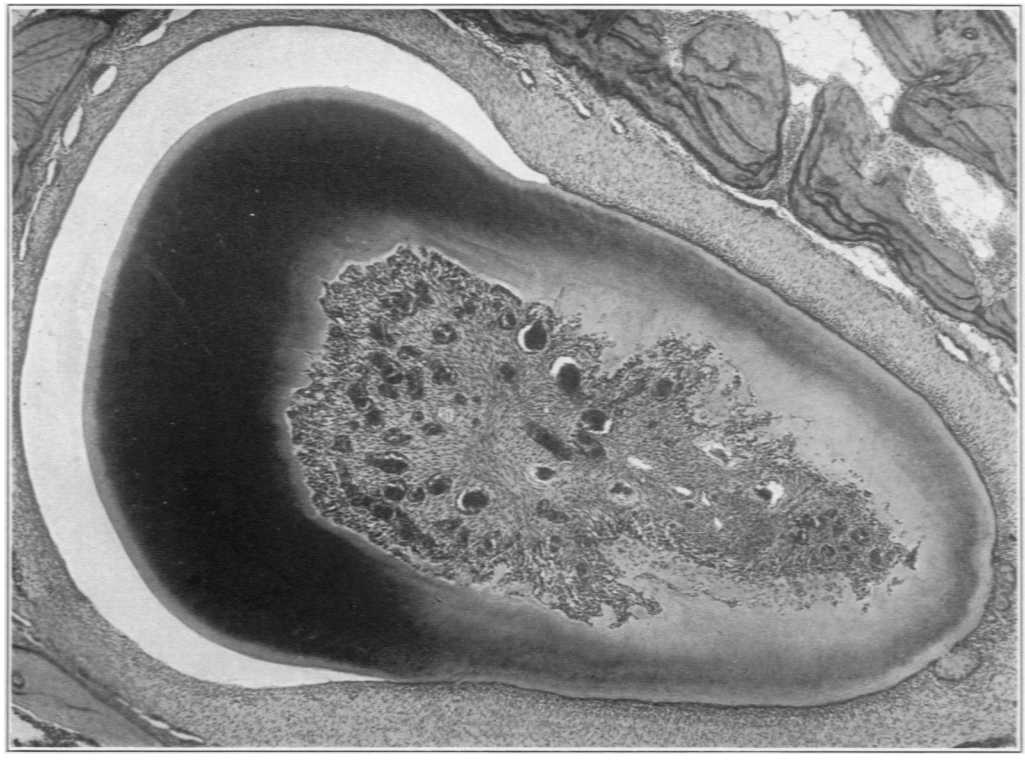
Wolbach and Howe

Incisor Teeth in Vitamin A Deficiency and Repair

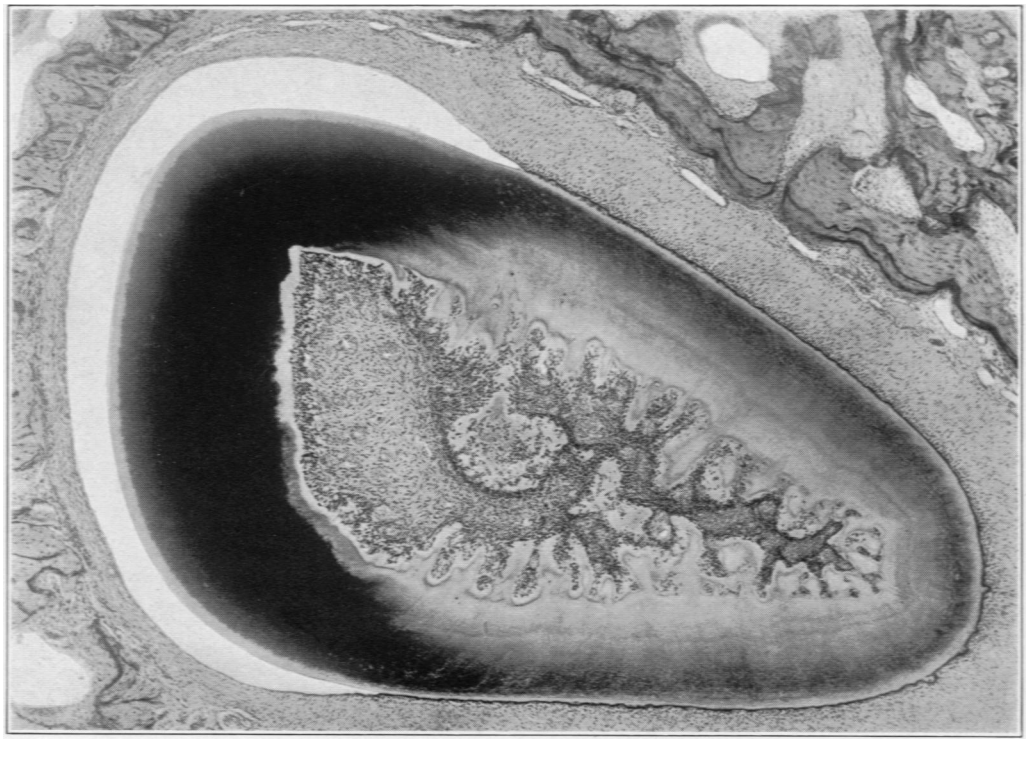
PLATE 50

FIG. 25. Guinea pig 41. Upper incisor. 299 days on experimental diet without source of vitamin A other than orange juice. For 207 days it received 20 cc. of orange juice daily, 10 cc. daily for 15 days and 5 cc. for the last 75 days. Shows nearly complete atrophy of enamel organ and of odontoblasts, except on labial side of tooth. In the guinea pig, in contrast to the rat, atrophying odontoblasts because of depolarization act like osteoblasts and deposit matrix centrifugally. The excrescences of osteodentine, or denticles, are of considerable size. × 46.2.

FIG. 26. Guinea pig 46. 158 days on experimental diet without source of vitamin A other than orange juice, of which it received 20 cc. daily for 28 days and 8 cc. daily for 130 days. Note cementum outgrowths, cementicles and atrophy of odontoblasts with osteodentine formations. × 46.2.



26



25

Wolbach and Howe Incisor Teeth in Vitamin A Deficiency and Repair