XI. *The Effects of Vitamin C Deficiency on Tooth Structure in Guinea-Pigs.*

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[Plates 56–61.]

Introduction.

Much attention has been given in the last few years to the effects of deficiency of vitamins D and A on dental and parodontal structure; extended clinical tests have been carried out; and the deduction has been drawn that a deficiency of these factors is a not infrequent cause of such common dental ailments as caries and pyorrhœa alveolaris. Vitamin C has received relatively little consideration in this connection; in fact doubt is expressed as to whether it has any practical significance for clinical dental disease, and difference of opinion exists even on the fundamental issue as to whether a deficiency of the vitamin is in any way injurious to the teeth. Thus, on the one side, Mrs. Mellanby (1929) found that lack of vitamin C had no influence on tooth structure in puppies, and concluded it was "improbable that the actual structure of human teeth is greatly affected by a deficient intake of vitamin C." At the other extreme Howe (1920, 1921, 1923) claimed that by feeding guinea-pigs on a scorbutic diet he had been able to produce with regularity all of the better-known dental lesions seen clinically in humans, including alveolar resorption, spongy gums, pockets and pus formation, together with caries and irregularities in the teeth themselves. He drew the deduction that vitamin C deficiency is an important factor in the ætiology of human dental disease. It will be generally conceded that further work is necessary to clear up the present unsatisfactory position.

With reference first to Mrs. Mellanby's experiments on dogs just alluded to, it has to be pointed out that one of us (Harris, 1931) has recently shown that dogs as a species (unlike humans, monkeys and guinea-pigs) do not need vitamin C. They are able to synthesize it in their body when none is present in the food, and hence cannot suffer vitamin C deficiency.* It follows that observations on dogs are not relevant to

* Histological examination by our colleague, Dr. J. R. M. Innes, of the bones and teeth of dogs kept for prolonged periods on vitamin C free diets showed a perfectly normal structure.

the question under discussion, and experiments to determine the effects of vitamin C deprivation must be carried out on guinea-pigs or, preferably, on monkeys.

HOWE's results, if they can be confirmed, are obviously of great importance. But his work demands repetition under more carefully controlled conditions, since the complex deficiencies in the diets, which he employed, were not accurately standardized, nor were the diseased teeth subjected to full histological study. HOWE's conclusions have been criticized by a number of writers, including HÖJER and WESTIN (1925), who speak of his " fantastic descriptions in their unscientific form." However, it seems to us that credit is due to HOWE for having succeeded, even if only by empirical means, in producing the most severe dental abnormalities—judging by the photographs which he has published—and it would be a step in the right direction if these abnormalities could be reproduced, however arbitrarily, with the aim of subsequently tracing the dietary errors responsible for them. It should be emphasized that two important claims are made by HOWE : that he is able, consistently, first to produce experimental dental caries, and, secondly, to cure experimental pyorrhœa.

HANKE (1929, 1930, 1933), working in collaboration with seventeen dental practitioners of Chicago in a large-scale clinical test, claims to have treated pyorrhœa and dental caries in humans with great success by means of massive doses of orange juice. According to his claims, X-ray examination showed a reduction in the thickness of the periodontal membrane, and the gingival tissues rapidly became healthy again. He leaves it open whether the action of the orange juice is due to its vitamin C or to some other unspecified constituent.

Now it is generally accepted by students of nutrition that many sections of the community, living on diets containing little or no fresh fruit and green vegetables, must in fact be near the border line of vitamin C requirements, or actually receiving sub-optimal amounts. The idea that a partial want of vitamin C of this kind may cause dental disease gains some support from the observation made by ZILVA and WELLS (1919) that the teeth were the first of the body tissues to show any abnormality in the development of scurvy in guinea-pigs deprived of all vitamin C. Since then histological studies on the effects of vitamin C deficiency upon the teeth have been made by other workers, including HÖJER and WESTIN (1925), TOVERUD (1923), KEY and ELPHICK (1931), also working with guinea-pigs. We felt it was desirable, however, to examine in greater detail and with improved technique the nature of the abnormalities, including the lesions (if any) occurring in the enamel and the cementum (hitherto these had usually been overlooked), more especially to find the effects of long-continued slight deficiencies of vitamin C or of frequently repeated deprivations—in order to correspond the better with the conditions actually prevailing in human experience. The guinea-pig, of course, differs in some important respects from man in its dental structure and physiology (*e.g.*, persistent growth), and the present work can only be regarded as a preliminary to further investigations to be carried out on monkeys. In the meantime, we think it significant that we have found that vitamin C deficiency in the guinea-pig does in fact

give rise to a failure in ameloblast function. This is the very kind of abnormality which, when it occurs in a small group of cells in developing human teeth, gives rise to the enamel lamellæ in which caries often originates. It may reasonably be anticipated that the same result will ensue in monkeys deprived of vitamin C during infancy.

A second and more immediate object of the investigation was in relation to enquiries in which one of us (L. J. H.) is interested as to the mode of action of vitamin C. The highly involved and somewhat contradictory set of abnormalities as described by past workers, including the formation of " pulp bone," the alleged transformation of odontoblasts to osteoblasts, and the new appearance of the latter in the pulp simultaneously with their degeneration in other sites, seemed difficult to reconcile with any simple theory of vitamin C action—*e.g.*, that it is needed primarily for the functional activity of the odontoblasts and similar cells (HARRIS, 1933). As a result of the present investigation we have been able to confirm this latter point of view. This leads to a fundamentally different interpretation of the abnormalities, and is based on a conception which, as we believe, greatly simplifies the issue and furnishes a straightforward and intelligible explanation of the effects of vitamin C deficiency on dental structure. Among other things it accounts very satisfactorily for the surprising and very striking difference in the appearance of the teeth in acute scurvy as compared with chronic subscurvy.

METHOD.

Groups of guinea-pigs, generally young growing animals, were fed at the Nutritional Laboratory, Cambridge, on a standard scurvy-producing basal diet :—

Bran	80	parts by weight
Oats	720	,,
Dried egg yolk	40	,,
Salts	8·4	,,
Cod liver oil	1%	

supplemented with varying amounts of cabbage for different lengths of time. These groups included :—(1) Animals given repeated intermittent attacks of acute scurvy ; (2) animals suffering from long-continued (" chronic ") subscurvy of varying degrees of severity (*i.e.*, restricted amounts of cabbage fed) ; (3) animals in the process of recovering from scurvy after different intervals of time ; (4) controls on diets qualitatively complete but restricted in quantity to the amount eaten by the vitamin C deficient groups ; etc. There is no need to give further details here, as it will be more convenient to mention the supplement given in each group when describing the result of the experiment in the text. A number of experiments was also carried out in which the basal diet itself was less completely balanced, in order to conform better with HOWE'S conditions, and consisted of rolled oats and spray-dried skim milk. Prior to the experimental period all animals were fed for about 10 days on the above-mentioned basal diet, supplemented with sufficient cabbage (about 15 gm. per day), and any which

failed to thrive on the diet were discarded. When it was desired to examine the teeth, the animals were guillotined, and the jaws placed in formol saline and despatched to the Hale Research Laboratory of the Royal Dental Hospital of London for sections to be cut.

Results.

The effects of the various diets upon the teeth and their supporting structures can only be described in close connection with the figures themselves. The description of plates has accordingly been expanded so that it serves the triple purpose of explaining the figures, defining the diet administered to each animal, and recording the effect upon the teeth. The figures can, therefore, most conveniently be examined at this point.

In every case where the deprivation of vitamin C was severe enough to produce results which could be observed by the histological methods employed it was found that the odontoblasts, ameloblasts, cementoblasts, osteoblasts, osteoclasts, and bone corpuscles had undergone degeneration and were sometimes entirely destroyed. The severity of this degeneration depended upon the extent to which vitamin C was excluded from the diet.

The effects observed upon the hard tissues were the usual physiological sequelæ of such degenerations and corresponding reactions are regularly observed when degenerations occur in these cells from other causes fig. 14, Plate 59.

Discussion.

In many respects the findings confirm those of earlier workers, though our interpretation does not coincide with theirs, and the method of presenting the results as photomicrographs of longitudinal sections of the cheek teeth seems to us to be important.

The Normal Guinea-pig's Tooth.

Before discussing the phenomena observed it is necessary to refer briefly to the physiology and growth of the normal guinea-pig's tooth.

The teeth of the guinea-pig, both cheek teeth and incisors, exhibit persistent growth. In any tooth at a given moment the dental tissues may, therefore, be observed, either in their embryonic developmental stage, or in a state of maturity, or again in a condition of senility and degeneration.

As growth progresses three phases may be observed :—first entirely new dentine and enamel is developed at the root end of the tooth by odontoblasts which are just differentiating from the indifferent cells of the dental papilla and by ameloblasts which are proliferating from the depth of the tooth band. Secondly, higher up the tooth, further deposits of enamel and dentine are being laid down on the outer surface and inner surface respectively of the enamel and dentine already deposited. Thirdly, at the older end of the pulp the odontoblasts and primary dentine are dying and calcific

scar tissue or secondary dentine is being deposited so that it occludes the whole pulp canal towards the biting end of the tooth.

The result is, therefore, that a threefold growth is in progress: the tooth is elongating and being pushed up to compensate for the wear on its biting surface, the pulp chamber at any given point is becoming narrower by the deposition of fresh dentine on its walls, and the total diameter of the tooth is increasing at the growing end, where active enamel formation is in progress, by the deposition of enamel on the outer surface of that already formed. The cheek teeth of a normal guinea-pig take about 40 days to complete one cycle of their growth from growing end to biting edge.

When a tooth is examined to determine the result of a special diet upon its structure it is important to know which part of that tooth was already formed when the special diet began to take effect, and which part was formed after this date, since it will be shown that the structural effects of hypovitaminosis C on the *hard* tissues are restricted to the part of the tooth which is formed after the diet has taken effect upon the metabolism of the animal. This occurs some ten days after the diet is first administered.

For this purpose it is essential to examine a longitudinal section of the tooth, fig. 1, since a transverse section may happen to be taken through a part of the tooth which was formed before the experimental diet was started, and, in any case, it is not easy in a transverse section to say how much of the tooth exhibited in the section was formed before, and how much after, the experimental period.

In carrying out the present investigation not only were all teeth cut in longitudinal section (except certain controls), but serial sections were prepared of each tooth so that the record should be complete.

In fig. 1 a longitudinal section of one fold of a cheek tooth is shown, *cf.* fig. 2, Plate 58. The enamel, dentine, secondary dentine and pulp are indicated, and the diagram is comparable with figs. 3, 4, Plate 56, etc. The thick black line is drawn through that part of the enamel dentine and secondary dentine which was in process of formation at one particular point of time. The hard tissues below this line have, therefore, been formed since, and those above

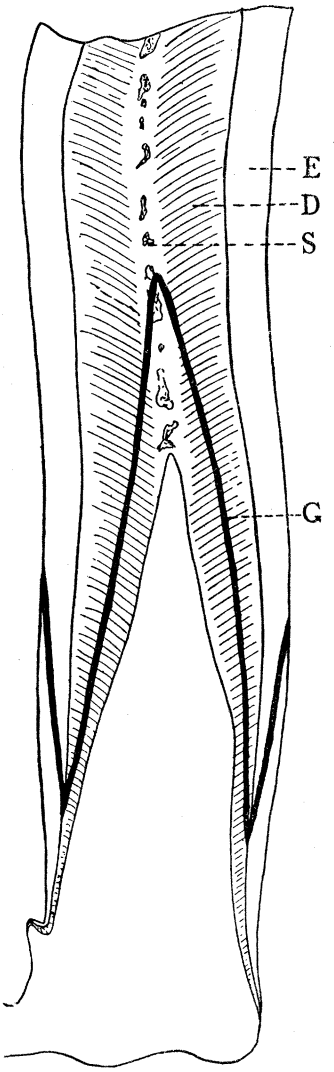

FIG. 1.—Diagram of one fold of a guinea-pig's cheek tooth, relatively shortened for convenience, and comparable with figs. 3, 4, 5, etc. E. = enamel; D. = dentine; S. = calcific scar tissue or secondary dentine. The line G represents graphically a particular point in time such that the enamel, dentine and calcific scar tissue below the line were developed after this particular moment, and the hard tissues above the line were developed before.

were formed before this particular moment. The pulp itself does not, of course, conform to this topographical division.

Where a guinea-pig has been put on a scorbutic diet the general shape of this line must be transferred in imagination to the sections of the teeth when they are examined, so that the effect of the diet on the development of new hard tissue formed after the diet commenced may not be mistaken for a change occurring in the tissue which was already formed. It is clear, therefore, that serious confusion might arise in the interpretation of transverse sections, owing to the physiological level at which the section was taken being undetermined.

The use of a cheek tooth, or, rather, of one of the folds of a cheek tooth in the present report, was rendered necessary because the incisor teeth curve in more than one plane, and cannot, therefore, be shown in their entire length in one section.

A further reason for the use of longitudinal rather than transverse sections is that from one *normal* tooth it is possible to produce a transverse section showing either a healthy virile odontoblast layer with robust fibrils entering the dentine, and the pulp cells vigorously undergoing mitosis, fig. 4*a*, Plate 57, or, alternatively, at a higher level in the pulp to show degenerate odontoblasts, which have lost their fibrils and are separated from the primary dentine, becoming embedded in secondary dentine, fig. 4*c* and 4*d*. The condition observed in fig. 4*d*, although occurring towards the biting end in a normal guinea-pig's tooth, is very much the same as is seen lower down in the pulp in subscurvy, fig. 5*a*, Plate 58. The difference, of course, is that while this condition only occurs in the senile end of a normal tooth it occurs most of the way down in the tooth of a guinea-pig on subscurvy diet, fig. 5, Plate 56. The " osteo-dentine " referred to by HÖJER and WESTIN (1925, fig. 24) as occurring in healing scurvy is only the normal secondary dentine at this level of the tooth, *cf.* fig. 4*d*, and is regularly observed in our controls.

It has been shown by one of us (FISH, 1931 and 1932) that in human teeth, and those of monkeys and dogs, when a tract of dentine dies, as it does under a superficial lesion, it constitutes an irritant to the pulp, but, instead of the necrotic dentine being exfoliated as would occur with bone, *the pulp protects itself by laying down an impermeable barrier of calcific scar tissue over the dead tract of tubules and in this way seals them off*. The calcific material so deposited is secondary dentine* and can be formed whether the odontoblasts have survived the injury and are still present or not. Often, under such lesions, not only the primary dentine but also all the associated odontoblasts die, and then a hyaline type of secondary dentine or calcific scar tissue is laid down. At

* The term " secondary dentine " has hitherto been employed to denote two entirely different types of tissue. One is the calcific scar tissue deposited by the pulp over dead primary dentine to seal it off and protect the pulp from contamination. The other is an additional layer of primary dentine which is often found at the pulp margin under a Translucent Zone (FISH, 1932, p. 56). As the " secondary dentine " referred to in this paper is entirely of the former variety it will be referred to in future as " calcific scar tissue."

other times some odontoblasts survive and lay down Tome's fibrils in the forming tissue so that it is to some extent tubular, P3, fig. 14, Plate 59. If a large mass of calcific scar tissue has to form rapidly, cells often become included in it (FISH, 1932, fig. 55).

The presence of this calcific scar tissue at the senile end of the normal guinea-pig's tooth, or of any tooth of persistent growth, can be accounted for in exactly the same way, only instead of the odontoblast dying as a result of superficial injury to the dentine, the dentine dies essentially as a result of senile degeneration of the odontoblast.

As the odontoblasts move up with the persistently growing tooth in the guinea-pig, they pass through every phase of their life-history. At the growing end of the tooth they may be seen differentiating from the embryonic mesoderm cells of the papilla, fig. 4*a*. Halfway up they are typical mammalian odontoblasts—oval cells with a pronounced fibril running into a tubule of the dentine, fig. 4*b*. At the older end of the pulp they are seen degenerating. They first lose their fibrils and become separated from the dentine by vacuoles containing exudate, fig. 4*c*. They then shrink, become angular and distorted in shape and finally break up altogether, fig. 4*d*.

When the odontoblasts lose their fibrils it may be assumed that the fibrils automatically die, and since they represent at least the most important, if not the only vital part of the dentine, it is evident that the dentine must also die. It might be predicted, therefore, from the observations recorded in the experiments referred to above (FISH, 1932), that calcific scar tissue would be laid down over the pulp ends of these tubules and seal them off from the more vital pulp tissues further down in the tooth. This actually happens and the calcific deposits so produced fill up the narrow remaining lumen of the tooth, thereby not only forming a solid biting end, but at the same time preventing mouth fluids from entering the pulp as the tooth is worn away, figs. 3 and 4*d*. There are always numerous islets of included cell debris in this calcific scar tissue.

The Effect of Scurvy on the Dentine and the Odontoblasts.

In scurvy there appears to be simply an acceleration of this process of degeneration. Instead of only the odontoblasts at the senile end of the tooth dying and their particular tubules being sealed up by the calcific scar, the younger odontoblasts all the way down the pulp also degenerate and lose their fibrils, so that the primary dentine all down the tooth also dies and is sealed off by a barrier of lime salts.

In *subscurvy* the connective tissue cells of the pulp and some of the youngest of the odontoblasts are able to remain alive for a time. They soon begin to degenerate, however, and their fibrils become detached, and a deeply staining deposit of calcium salts is then deposited over the ends of their tubules, effectively sealing them off from the pulp. Lime salts are deposited throughout the pulp, in

a well-developed collagen matrix, which encloses islets of the degenerating cells and presents the same appearance as the normal calcific scar tissue at the senile end of a healthy tooth, *cf.* figs. 4*d*, Plate 57, and 5*a*, Plate 58. Such primary dentine as is formed first at the growing end of the tooth, before the new odontoblasts begin to degenerate further, is very irregular in form.

In *full scurvy* these phenomena are modified because the pulp is more severely affected. All the odontoblasts lose their fibrils and die, so that all the primary dentine dies and is sealed off by the deeply stained barrier of lime salts, B, figs. 6*a* and 6*b*, Plate 58, but the pulp is not able to continue the reaction and lay down the expanded collagen matrix to accommodate the lime salts, as it does in subscurvy, and fill up the pulp chamber. Not only is the effect of full scurvy more severe than that of subscurvy on the more mature cells of the pulp, but the newly-developed odontoblasts, and even those in the process of development, degenerate, so that at the developing end of the tooth no primary dentine at all is formed, but simply a narrow band of amorphous lime salts where the line of odontoblasts has perished, and even this is distorted in shape, figs. 6, Plate 59, and 6*a*, Plate 58.

The failure to fill up the pulp chamber in full scurvy with well-calcified scar tissue is more serious than might at first appear. In a primate's tooth, if a tract of dentinal tubules dies, it is sealed off from the pulp by a calcific scar (secondary dentine), and this secondary dentine consists of two parts. First a deposit of lime salts forms in and over the ends of the injured tubules. This forms the actual seal or barrier, which prevents fluid exchange between the dead dentine and the live pulp ; it may be called the defensive stage of the reaction. Under cover of this barrier a boss of calcific scar tissue is deposited to complete the " healing " process, and to provide a more normal environment to the pulp at this point than the mere barrier (FISH, 1932, p. 54). This is the repair stage. In the subscurvy guinea-pig exactly the same process is repeated. First a barrier, B, fig. 5*a*, and then a boss of secondary dentine, but there is this difference, that while secondary dentine in a primate's tooth is (except possibly in the very aged) always a pathological reaction to injury of the dentine, in the guinea-pig, secondary dentine is the normal reaction to degeneration and death of the senile odontoblasts at the apex of the normal pulp. Being a reaction to physiological degeneration of the pulp and not merely to pathological death of the dentine it has a very important accessory function to perform. It not only seals off the tubules which contain the detached fibrils of the disintegrated odontoblasts, but in the second, or repair, stage it has to fill the whole lumen of the pulp chamber, replacing or encapsulating all the degenerated pulp tissue, so that when this part of the tooth erupts the younger and still functioning part of the pulp will not be opened to the mouth fluids by attrition in chewing food.

It is therefore natural, when in subscurvy the odontoblasts degenerate before their normal time, that having sealed off the tubules containing their cast-off fibrils by a barrier of calcific material, B, fig. 5*a*, the pulp should follow this operation up by filling the whole chamber with calcific scar tissue in the usual way. Otherwise, if the

attack lasted long enough, the pulp would suffer exposure as the tooth continued to grow up and was worn away.

In an acute attack of full scurvy, however, the pulp is only able to accomplish the first phase of secondary dentine production—that is, the defensive stage—and deposit the barrier of lime salts, B, fig. 6*a* and 6*b*, Plate 58, without being able to form the organized calcific scar tissue, which is required to fill up the pulp chamber, and restore function. Indeed, however long the attack of full scurvy may be drawn out, the pulp will never succeed in doing this, as is shown in fig. 6, Plate 59, where after 57 days on the scurvy-producing diet the animal eventually died without having deposited any such mass of tissue, *cf.* also fig. 9, Plate 60. It would appear that during an attack of scurvy calcium salts may be deposited but the cells cannot elaborate a matrix for their reception. If, however, the animal should recover, its first sign of recovery is to deposit this material throughout the whole of that part of the degenerate pulp which was already present at the end of the attack, *cf.* figs. 7, Plate 59, and 6*a*, Plate 58.

On the other hand, if the diet is not so severely depleted as to kill the animal, but deficient enough to prevent the proper organization of the calcific scar tissue, the tooth may actually grow up with its pulp chamber more or less open, as in fig. 9. It was upon a diet similar to that of the guinea-pig whose tooth is shown in fig. 9, that HOWE (1923) produced " caries of bone," which, presumably, was an alveolar abscess caused by exposure of the pulp to the mouth with the inevitable sequence of infection and abscess formation. If the animal in fig. 9 had been kept alive it seems certain that a similar fate would have befallen it, since the calcific deposits entirely fail to seal up the pulp chamber from the mouth and infection was already invading the pulp.

The Effect of Scurvy on the Enamel and the Ameloblasts.

The ameloblasts are also affected, although somewhat later in the course of the disease than the odontoblasts. In the guinea-pig's tooth the ameloblasts normally develop as a solid pavement of cells opposite the developing odontoblasts, fig. 4*a*. They are only functional for about one-third of the length of the tooth ; at this point a full thickness of enamel has formed and the ameloblasts thereupon degenerate and keratinize. In subscurvy the enamel continues to form, fig. 5, with, so far as can be ascertained, very little change, but in full scurvy it completely fails to do so. Not only does the enamel fail to form, but the ameloblasts which otherwise should be functional at that moment undergo permanent degeneration from which they never recover. They either disappear altogether or become completely keratinized, so that even if the animal is cured there will never be any enamel on that section of the tooth which was forming when the disease was at its height, figs. 6 and 7.

Ground sections of teeth from animals in the same group as the one used for fig. 7, Plate 59, have been prepared, and the appearance is sketched in fig. 12, Plate 61. It will be seen that the enamel appears to cease forming quite abruptly. There does not

appear to be a section of badly-formed enamel between the normal tissue and the scar which represents the growing end, while the scurvy was at its height. It must be recorded, however, that owing to the technical difficulties in preparing the ground sections it is extremely difficult to be dogmatic on this point or to be sure that the enamel formed during an attack of subscurvy is completely normal.

The Effect of Scurvy on the Jaw-Bone.

In view of the severe effect of hypovitaminosis C upon the odontoblasts and ameloblasts, its effect upon the cells associated with bone was also investigated.

In the guinea-pig, as in many other animals, there is a normal physiological " wandering " of the teeth (STEIN and WEINMAN, 1925). This is shown by absorption on one side of an interdental bony crest and the building of new bone on the other.

In fig. 10, Plate 60, can be seen the giant cells carrying out their normal task of bone absorption on one side of a crest in a normal control guinea-pig. In fig. 11, Plate 60, from a scurvy guinea-pig, the process appears to be arrested. The giant cells are breaking up, and the bone corpuscles themselves are degenerated, angular and shrunken, while the surrounding connective tissue cells have entirely lost their embryonic character and share in the general degeneration.

The Effect of Scurvy on Cementum.

The effect of scurvy on the development of cementum conforms strictly to the type of reaction observed in the other hard structures. The cementum developed before the onset of the acute stage of the disease remains unaltered, but during the actual attack the cementoblasts undergo degeneration similar to that of the osteoblasts. If sections are prepared in a bucco-lingual plane of the cheek teeth, cementum will be seen on one side of the tooth and enamel on the other. Unfortunately, the curvature of the tooth makes it impossible to produce such diagrammatic sections as those shown in figs. 4, etc. If, however, a paraffin block, such as the one used for fig. 7, from an animal which has been *cured of scurvy*, is cut bucco-lingually, a section from the series may be chosen which shows the enamel scar on one side of the tooth and the cementum scar on the other, these scars corresponding to the growing end of the tooth when the disease was at its height. Figs. 13 and 13*a*, Plate 61, show such a section. It will be seen that, at the point in the tooth where neither enamel nor primary dentine were being formed, there is also a complete absence of normal cementum. Its place is taken by an amorphous deposit of calcific scar tissue which encloses occasional cementoblasts, so that it appears exactly like the calcific scar tissue of the pulp in structure. It can, however, be shown to take origin from the periodontal tissues and not from the pulp, because it is often deposited over a small area of the new primary dentine which has developed after the cure of the disease had become established, B, fig. 13, Plate 61,

and C, C1, fig. 14, Plate 59). The appearance of this cementum scar on the surface of the new normal dentine shows not only that it is periodontal in origin, but also that it was formed during the period of cure and not when the disease was acute.

Two points about this cementum scar tissue deserve special mention. One is that it resembles human lacunar cementum, although the guinea-pig never produces lacunar cementum in normal circumstances. The other point is that this type of cementum scar can be seen in the teeth of rabbits which have been injured experimentally in the growing area of the root and allowed to heal, just as the calcific scar tissue of the pulp (secondary dentine) can also be produced by experimental trauma, fig. 14, Plate 59. It would appear from this that the lacunar cementum of human teeth may be a response to irritation of some kind ; for if it were a normal human tissue it would be surprising to find it produced as a scar tissue in rabbits and guinea-pigs which never produce it in normal circumstances. Further work is being carried out on this subject.

It appears that this scar tissue is, to some extent, an irritant to the normal tissues, since, in an animal which was killed 31 days after the attack of scurvy had been relieved by administration of Vitamin C, the scar tissue was being absorbed in places, and this has constantly been observed. In fig. 13, Plate 61, there is no absorption, as only ten days had elapsed since the attack when the animal was killed.

The " Scar Tissue " Theory.

Deficiency of vitamin C in the diet of guinea-pigs, therefore, appears to affect the vitality of all cells which are specially concerned with the laying down of the hard tissues. Primarily there is simply premature degeneration and death of the odontoblasts and ameloblasts associated with degeneration of the cementoblasts, osteoclasts, osteoblasts and bone corpuscles. In the most severe cases there is degeneration of the general connective tissue of the dental pulp with congestion of its vessels.

All the other changes which are so striking in the histological picture of the condition are merely secondary, and represent the inevitable result of the premature dissociation of the odontoblasts from their fibrils or of the premature degeneration of the ameloblasts or cementoblasts. The massive calcific deposits in the pulp in subscurvy, with their prolific cell inclusions, are simply calcific scar tissue, the natural result of a hasty attempt to seal off dead primary dentine which became a source of irritation to the pulp when its dentinal fibrils were cast off by the degenerating odontoblasts. The narrow amorphous deposit of lime salts in full scurvy is just the less successful attempt in the same direction of a pulp which is still more degenerated and so less able to react. The absence of enamel or cementum at the growing end of the tooth in full scurvy is the result of the degeneration of the ameloblasts and cementoblasts. After the attack is cured the enamel is represented by a keratinous scar, the dentine and cementum by a calcific scar.

Previous workers on this subject have sought much more complicated explanations.

Everyone appears to agree that both the ameloblasts and the odontoblasts degenerate, and also that the first sign of degeneration in the odontoblasts is the loss of their fibrils. It does not, however, appear to have been considered that when the odontoblasts lose their fibrils the effect on the dentine is fatal, and that the reaction of the tooth to the sudden death of all, or most, of the primary dentine of which it is formed will be the predominating feature of the subsequent activity of the pulp, hampered as it is by its own degeneration.

This interpretation which we have placed on the findings appears to agree with all the observed phenomena, and has the advantage that it is simple and is in accordance with pulp, dentine, enamel and cementum reactions in other mammals (human, monkey and dog), and forms a close parallel with pathological reactions in other tissues.

The " Pulp Bone " or " Osteo-dentine " Theory.

In contradistinction to this interpretation, the tissue which we have called calcific scar tissue or secondary dentine has been described by all other workers as an entirely new tissue which they call " Pulp bone " or " Osteo-dentine." This difference is fundamental and it seems important to determine whether vitamin C deficiency does, as we think, cause degeneration of cells and particularly of the special cells associated with the production of the hard tissues of the body, or whether, as other writers maintain, it promotes the development and hyperfunction and virtual metaplasia of some of these cells together with the degeneration of others. Amongst the writers who take the latter view are TOVERUD (1923) (who, however, compares " the osteo-dentine," as he calls it, with the normal tissue sealing off the end of a tooth), REYHER, WALKHOFF and WALKHOFF (1928), WILTON (1931), KOTANYI (1927), and ROBBS and co-workers (1921). One of the most extensive accounts, given by HÖJER and WESTIN (1925), is typical of the complex pictures described by all these writers. Amongst the reactions they postulate are :—

(1) absorption of bone in the jaw ;
(2) deposition of new bone in the jaw ;
(3) disappearance of odontoblasts ;
(4) amorphous calcification of pre-dentine ;
(5) absorption of dentine ;
(6) formation of bone in pulp (pulp bone) ;
(7) atrophy and resorption of pulp bone, dentine and pulp.

The reactions indicated in this summary appear to be incompatible with each other, calcification and absorption occurring in the same tissue at the same time and *degenerating odontoblasts* producing new bone.

For instance, it is difficult to picture " the dentine becoming porous through a process which starts from the protoplasm contents of Tome's canals " and also accept the same author's statement that the odontoblasts have already shed their fibrils which *are* the

protoplasmic content of Tome's canals. We have never observed that the dentine becomes " porous," and consider that most of the incompatibility apparent in the above summary is due to its authors having used transverse sections from different physiological levels of the teeth, and also to the fact that nothing was then known of the reaction of a tooth to death of the primary dentine—in fact, such an occurrence had not then been recognized.

The bone absorption and deposition in the jaw, referred to by these writers, are presumably the normal physiological phenomena of " tooth wandering " which are always observed in young guinea-pigs, but the processes appear in our preparations to be held up during scurvy owing to cellular degeneration, figs. 10 and 11. The " amorphous calcification of predentine " is what we have described as calcific deposits sealing off the dead primary dentine, figs. 5*a* and 6*b*. It remains, therefore, only to consider whether the tissue found in the pulp during subscurvy, or when the animal is recovering from an attack of acute scurvy, is, as we suggest, calcific scar tissue or whether it is a new deposit of bone in the pulp, as previous writers have thought.

It is suggested by these workers that a degenerating pulp in which the odontoblasts have admittedly lost their fibrils and all the cells are distorted, has suddenly developed osteoblasts and laid down large masses of bone. This is said to occur although the ordinary bone cells of the jaw are themselves degenerating and finding it hard to survive, and despite the fact that even a healthy pulp is incapable of forming bone. Indeed, it is difficult to see what use a dental pulp would have for a bony skeleton in any circumstances ; so useless indeed would the tissue be that it would have to be regarded as virtually a neoplasm.

The only evidence that the new tissue is bone is its superficial resemblance to bony trabeculæ, though in ground sections this resemblance entirely disappears, fig. 12. It is also suggested that the degenerating cells included in it resemble osteoblasts. There are, however, no Haversian systems to be seen and no embryonic mesoderm cells which might be developing into osteoblasts—on the contrary, all the pulp cells, so far from undergoing mitosis, are admittedly degenerating, and it is difficult to see how any of them could be mistaken for osteoblasts. The cells are, in fact, so degenerated that it would seem impossible from their appearance alone to say what they had been. Even if they did *resemble* osteoblasts the case would not be proved, for it has been shown (FISH, 1932, fig. 35, A) that such cells often appear associated with the development of secondary dentine in the ordinary reactions of teeth in monkeys and other animals—but only in an otherwise healthy pulp.

The " Pulp bone " theory presents a bewildering picture quite as inexplicable on general biological grounds as it is unprecedented in any other form of pulp irritation, and it leaves entirely out of account any reaction to the death of the primary dentine.

It is significant that this tissue, whether it be calcific scar tissue or pulp bone, develops not only in the pulp chambers of animals suffering from subscurvy, but also in the pulp chambers of animals which have had acute full scurvy *after* a cure has commenced and

the animal has regained its health. In fig. 6*a* a tooth is shown from a guinea-pig which had been on a vitamin C free diet for 15 days and was then killed. In fig. 7 is seen a tooth from a guinea-pig which had been on a similar diet for 20 days and was, therefore, presumably in a similar condition to the one in fig. 6*a* at the end of that period, but which instead of having been killed at once was put back on a curative diet for 10 days and then killed. The part of the tooth in fig. 7 from P to N represents that which was the growing end during the scurvy period, and the part N to G has developed in the 10 days since the scurvy and is a normal growing end of a tooth, but a change has also taken place in the pulp above the point N during these 10 days.

In fig. 6*a* (scurvy), the pulp, though degenerated, contains no " Pulp bone " ; there is only the thin defensive barrier of calcific deposit over the dead primary dentine. In fig. 7 (cured scurvy), however, there is the same thin barrier of calcific deposit above the point N, but there is also a mass of the well-formed subscurvy type of calcific scar tissue or " Pulp bone." Now by analogy with fig. 6*a*, the thin black barrier was no doubt there when the cure started, but the massive subscurvy type of calcific deposit was not, so that it must have developed during the cure period.

If, therefore, this tissue were really pulp bone, then pulp bone is not diagnostic of subscurvy since it has developed in the pulp of this tooth at a time when it was so completely recovered from scurvy that it could form the large section of perfectly developed tooth N to G in fig. 7. On the other hand, if the tissue is simply better formed secondary dentine, or calcific scar tissue, then it was to be expected that the moment the tooth was cured and recovered sufficiently this calcific scar tissue would appear in all the completeness of its full development in that part of the pulp in which all the odontoblasts were dead, and which had, up to the moment of cure, been too depressed to do more than deposit a meagre precipitate of lime salts over the surrounding dead dentine.

A crucial experiment was performed to test the accuracy of our view that the new tissue laid down was calcific scar tissue and not pulp bone. It was argued that if the effect of scurvy was simply to produce degeneration of the odontoblasts, cementoblasts, ameloblasts and bone cells and if these massive deposits of calcified tissue represented the normal physiological attempt of nature to remedy the damage when the animal recovered from the attack of scurvy, then it should be possible to produce an exactly comparable picture by damaging these same cells mechanically and allowing repair to take place.

The rabbit has teeth structurally almost indistinguishable from those of the guinea-pig except in point of size. This animal was therefore used for technical convenience. The rabbit was anæsthetized and the skin over the lower border of the horizontal ramus of the mandible was incised. The edge of the bone was snipped away and a fine probe inserted up into the pulp chambers of several of the cheek teeth. The wound was closed aseptically and allowed to heal.

Eight weeks later the animal was killed and the teeth were sectioned. Fig. 14 shows the result. The whole of the damaged pulp tissue has been replaced by calcific scar tissue,

which completely seals off the dead primary dentine. This tooth is therefore in exactly the same condition as the tooth in fig. 7, which was affected by scurvy and subsequently repaired.

Similarly, there is a mass of calcific scar tissue where cementum should have been, and this is precisely the same as the cementum scar in cured scurvy, figs. 13 and 13*a*. It was felt that this experiment provided conclusive evidence of the accuracy of the scar tissue theory and also shed considerable light on the pathological reactions of dentine and cementum in general.

The Cure of Scurvy.

On our theory the whole histological picture in fig. 7 immediately becomes self-explanatory. In the part N to G a considerable deposit of normal primary dentine has formed during the ten days of cure, yet no trace of new primary dentine has developed in the part above point N during this period. In other teeth examined, even when the cure has lasted three times as long, there was still no new primary dentine above this point. It is, therefore, reasonable to conclude that since there is no new tubular dentine in this part of the tooth, none of the odontoblasts survived the scurvy period. The health of the general pulp tissue, however, rapidly improved when the cure started and it was able to lay down the extensive collagen matrix for the calcific scar tissue which fills up the whole pulp chamber. Superimposed, therefore, on the original barrier of defence, seen as a thin black line B, laid down during the scurvy period, is the ordinary repair type of calcific scar tissue S, such as is always seen sealing off the senile end of a normal tooth, or throughout the pulp of the tooth of a subscurvy guinea-pig.

Fig. 8, Plate 60, corroborates this view, for, in this instance, the scurvy periods never lasted long enough to destroy the odontoblasts completely, so that although they lost their fibrils and the primary dentine died and was covered with a narrow calcific deposit, the odontoblasts regenerated (FISH, 1932, fig. 34, B) when the cure was started and normal tubular dentine was once more laid down. This shows that, as one would expect, there is a possibility of rescuing the odontoblasts between the moment when they begin to degenerate and lose their fibrils and their final dissolution. In the tooth illustrated by fig. 7 the scurvy had lasted too long for them to survive.

The Association of Scurvy and Caries in Guinea-pigs.

Fig. 7, perhaps, also explains the suggestion of HOWE (1923) that caries develops during vitamin C deficiency in guinea-pigs. The whole molar tooth normally only takes 40 days to grow out from developing end to occlusal surface ; consequently no part is exposed in the mouth for more than a few days, so that it appeared very unlikely that true caries could develop. On the other hand, in these animals which have recovered from scurvy there is a zone in the tooth where there is no enamel, N to P, fig. 7, and no primary

dentine, so that the keratinous epithelium lies in contact with the calcific scar tissue of the pulp. When this zone erupts, as it will if the animal is kept alive and healthy, it would become stained and, being porous and friable, might simulate dental caries.

Fig. 9 shows another possible explanation of *apparent* caries. In this case, by alternating a scurvy and a curative diet for short periods each, the pulp has become so degenerated that the pulp chamber is very inadequately filled up with calcific scar tissue, even up to the biting end of the tooth. As the tooth is ground away, therefore, in chewing, large cavities appear in the occlusal surface, but they are not likely to have time to become carious before they are ground away.

The Effect of Scurvy on the Rate of Growth of the Teeth in Guinea-pigs.

The rapid growth of the teeth in guinea-pigs has certain disadvantages from an experimental point of view, since, in a long experiment, the part of the tooth which shows the effect of the earlier diets will have grown out and been worn away before the experiment terminates.

We were able to confirm at least on general lines the observation of DALLDORF and ZALL (1930) that full scurvy has a most marked effect on the rate at which the tooth grows out, as, indeed, it does on the growth of the guinea-pig in general. If the length of tooth from X to the growing end in fig. 6, which took 40 days to develop, be compared with the corresponding length from N to the growing end in fig. 7, it will be realized that only about one-quarter of the normal growth takes place when the animal is suffering from avitaminosis C, since in the latter case the piece of tooth referred to took only about 10 days to develop.

SUMMARY.

The teeth and jaws of guinea-pigs suffering from acute full scurvy, chronic subscurvy, developing scurvy, curing scurvy, and scurvy complicated by other dietary deficiencies, together with various controls, have been examined. A new interpretation is given of the dental abnormalities seen in vitamin C deficiency, the "Pulp bone" theory being rejected, and the difference between the appearance of the tooth in scurvy and subscurvy accounted for. A theory is advanced as to the mode of action of vitamin C, which is regarded as needed primarily for maintaining the functional activity of certain types of cells, including odontoblasts, osteoblasts, ameloblasts, etc.; other effects in vitamin C deficiency being physiological sequelæ of this primary action. Attention is directed also to the practical importance of the disorders in the enamel and cementum, overlooked by past workers.

These conclusions are based on the following observations :—

(1) In a preliminary discussion it is pointed out that it is necessary to examine longitudinal serial sections, since the tooth of the guinea-pig exhibits persistent

growth, any given normal tooth showing ameloblasts, cementoblasts, odontoblasts and other cells in an embryonic, mature or senile condition at different levels at the same time. Failure to recognize this has led other workers to misleading conclusions.

(2) When odontoblasts degenerate, either physiologically or pathologically, they shed their fibrils. In either case this incurs the death of the primary dentine. The dead dentine is then normally sealed off by a calcific scar tissue, which also replaces the pulp, filling up the pulp chamber. This scar tissue encloses islets of cellular *debris* and corresponds to secondary dentine in human teeth.

(3) The effect of scurvy on the teeth and bone of the guinea-pig is primarily a premature degeneration of the odontoblasts, ameloblasts, cementoblasts, bone corpuscles, osteoblasts and osteoclasts. All other effects are secondary. No apparent morphological change takes place in the *hard* tissues already formed.

(*a*) *Dentine.*—In *subscurvy* partial degeneration of the odontoblasts, leads at first to the formation of some irregular dentine, and later as the odontoblasts become more degenerated the pulp chamber becomes entirely filled with a calcific scar tissue, corresponding with normal secondary dentine and enclosing many of the degenerated and dead cells. In *full scurvy* on the other hand there is more complete loss of normal function. Premature degeneration and death of all the odontoblasts causes death of all the primary dentine (which thereupon becomes sealed off by a narrow amorphous layer of lime salts), and no new primary dentine or calcific scar tissue is able to form (*cf.* subscurvy). If, however, the the attack of scurvy is then *cured* calcific scar tissue is able to form, and replaces the degenerated pulp.

(*b*) *Enamel.*—Premature degeneration of the ameloblasts causes complete cessation of enamel formation during an attack of full scurvy. If the attack is cured the section of enamel which should have formed is represented by a keratinous scar, deposited on the calcific scar, which marks the failure of the primary dentine to develop.

(*c*) *Cementum.*—Premature degeneration of the cementoblasts prevents the formation of primary cementum at the growing end of the tooth during full scurvy. After an attack is cured a calcific scar, with cell inclusions, indistinguishable in type from that calcific scar tissue which fills the pulp chamber, is deposited at this point.

(*d*) *Bone.*—The normal absorption and deposition of bone which accompanies physiological tooth movement is brought to a standstill by the degeneration of all the cells associated with it.

(4) The deposition of calcific scar tissue, such as is found in the pulp sealing off dead dentine, or replacing cementum after an attack of scurvy, can be brought about

experimentally by simple mechanical injury to teeth of persistent growth in normal animals. For this, amongst other reasons, the suggestion of previous workers that the scar tissue is a neoplastic growth of pulp bone, specific to scurvy, is rejected.

(5) The technique of giving alternating periods of scurvy with periods of cure and using longitudinal sections, has proved of value in elucidating these reactions, the successive portions of the growing tooth corresponding with each of the separate experimental periods being readily distinguished.

(6) The failure of normal enamel formation, to which we find vitamin C deficiency gives rise, may be of significance in the causation of human caries. The purely structural defects resulting from vitamin C deficiency in the guinea-pig may in themselves bear a superficial resemblance to caries, and may account for the claim of HOWE to have produced experimental caries in this species.

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DESCRIPTION OF PLATES.

Where a number followed by a small letter is printed in italics at the side of a figure the number and letter refer to an enlarged section.

FIG. 2, Plate 58—NORMAL.—Photomicrograph × 10 of a transverse section of two of the cheek teeth of a guinea-pig to show their folded shape and the distribution of the dentine (D), enamel (absent) (E), cementum (C), pulp (P), epithelium (T) and alveolar bone (A). Figs. 3, 4, 5, etc., are photomicrographs of longitudinal sections of a single fold of such teeth.

FIG. 3, Plate 56—NORMAL.—Photomicrograph × 23 of a longitudinal section of one fold of a cheek tooth of a normal control guinea-pig.

Diet: Basal diet plus unlimited cabbage. Killed after 165 days.

This figure is included in order to display the whole length of the tooth from growing end to biting surface; but as the dentine is dead from the point 4*d* to the biting surface B and no further biological change can take place in it, this part of the tooth has been omitted from figs. 4, 5, 6, 7 and 14 for convenience in reproduction. This senile part of the tooth, however, always serves as a record of the vitamin C intake at the time it was being formed and when this record is significant, as in figs. 8 and 9, it is shown at appropriate magnification. The index letters are referred to in the description of figs. 4, 4*a*, 4*b*, 4*c* and 4*d*.

FIGS. 4, 4*a*, 4*b*, 4*c* and 4*d*, Plates 56 and 57—NORMAL.—Higher magnifications of fig. 3. Fig. 4 is × 40, figs. 4*a*, 4*b*, 4*c* and 4*d* are × 690.

At the growing end the embryonic mesoblast cells of the dental papilla become massed together into a definite layer PO opposite a similar layer of epiblast cells PA from the epithelial downgrowth. These are the pre-odontoblasts and pre-ameloblasts. Further up in the tooth odontoblasts O and ameloblasts A are associated with active dentine and enamel formation. From the growing end, up to the point AM the enamel leaves behind it an insoluble matrix after decalcification, while, beyond the point AM, where the ameloblasts begin to degenerate, this entirely dissolves away.

The odontoblasts O gradually assume a more adult appearance as they approach the higher levels of the tooth (*cf.* figs. 4*a* and 4*b*). At OD the primary dentine has attained its maximum thickness and the odontoblasts have begun to undergo senile degeneration and have lost their fibrils (*see* fig. 4*c*). Vacuoles V appear between the primary dentine and the odontoblast layer, and the cells themselves have become shrunken, distorted in shape and stained heavily with hæmatoxylin. Strands of collagen matrix CM (fig. 4*d*) are laid down and calcify, forming scar tissue—secondary dentine—which seals off the dead dentinal tubules and obliterates the pulp canal of the tooth, enclosing islets of degenerated pulp tissue I which lie embedded in it unchanged until they reach the biting surface B, fig. 3, and become bitten away.

FIGS. 5 and 5*a*, Plates 56 and 58—SUBSCURVY.—Photomicrographs of longitudinal section of one fold of a cheek tooth of a guinea-pig on a diet restricted as to vitamin C. Fig. 5 × 40, fig. 5*a* × 690.

Diet.—Basal diet plus 1 gm. of cabbage *per diem*. Died after 116 days.

The ameloblasts appear to degenerate somewhat earlier than in a normal tooth. The odontoblasts degenerate at OD very much earlier than in the normal tooth, and the upper part of the pulp chamber is filled with hastily formed calcific scar tissue SD1. (This massive deposit is the "pulp bone" of earlier writers.) Primary dentine P is still forming at the growing end although it is somewhat irregular in shape (*cf.* figs. 4 and 6).

The blood vessels of the pulp are congested, and the whole tissue is somewhat degenerated.

The upper half of the tooth, and practically the whole of the primary dentine, were completely formed before the diet took effect, and these are therefore normal.

Fig. 5*a* corresponds, in its relative position in the tooth, with fig. 4*b*. There is a change in type of the primary dentine at C which corresponds with the moment when the odontoblasts began to degenerate, and the most recent part of the primary dentine NP is not so well formed. As the odontoblasts degenerated more completely and shed their fibrils the dismembered fibrils were sealed off in their tubules at B by a deeply stained calcific deposit. The disintegrating odontoblasts OD and other degenerate cells are encapsulated in the rapidly forming calcific scar tissue SD.

FIG. 6, Plate 59—FULL SCURVY.—Photomicrograph × 40 of longitudinal section of one fold of a cheek tooth of a guinea-pig deprived entirely of vitamin C.

Diet.—Basal diet without cabbage. Died after 57 days.

In this tooth both the ameloblasts and odontoblasts were degenerating as soon as they were formed, before either primary dentine or enamel could develop. At the growing end a layer L of odontoblasts and ameloblasts was developed, but became folded and crumpled instead of producing dentine and enamel and pushing the tooth upwards.

The odontoblasts lose their fibrils and the cells break up and disappear. The primary dentine P, which had already formed when the scurvy set in and was dependent upon these dead odontoblasts, also dies therefore, but, being a hard tissue, remains *morphologically* unaltered; it is, nevertheless, sealed off by a barrier B of very heavily stained calcific tissue. This is the first stage in the formation of a calcific scar by the pulp, but the process does not proceed further as in subscurvy (*cf.* figs. 5*a* and 6*b*). This is probably because the general connective tissue of the pulp is more degenerated than in subscurvy, and more vessels are thrombosed.

The most significant differences between full scurvy and subscurvy are, therefore, that in full scurvy neither enamel nor primary dentine is forming at all at the growing end, nor does calcific scar tissue development in the pulp proceed beyond the deposition of a barrier of lime salts.

FIGS. 6*a* and 6*b*, Plate 58—DEVELOPING SCURVY.—Photomicrographs from one fold of a cheek tooth of a guinea-pig kept on a diet devoid of vitamin C for 15 days and then killed. Acute scurvy is just developing. In animals which were killed after the diet had continued for only 10 days, no appreciable effect was apparent. Fig. 6*a* is × 45, fig. 6*b* is × 690.

Diet.—Basal diet without cabbage. Killed after 15 days.

The development of primary dentine and also of enamel has ceased completely at the point P. The ameloblasts A and odontoblasts O have all degenerated, even those which have only just developed. The primary dentine which had already been laid down before the scurvy developed has died and is already sealed off by the barrier of lime salts B. This first stage of calcific scar tissue formation continues down to the growing end, marking the situation where dentine and enamel, in normal circumstances, should have been laid down. No attempt is being made by the pulp in this animal or in the one illustrated in fig. 6, or in any other suffering from full scurvy which we have examined, to continue the formation of calcific scar tissue and fill up the whole pulp chamber, as occurs in subscurvy. (*Note*—A comparison of figs. 4*b*, 5*a* and 6*b* shows in fig. 4*b* normal odontoblasts, in fig. 5*a* odontoblasts degenerating, but a pulp still capable of barrier formation and also of producing an extensive deposit of calcific scar tissue, while in fig. 6*b* are seen degenerating odontoblasts in a pulp which can precipitate lime salts over the dead dentine but cannot lay down the collagen matrix necessary for the formation of typical calcific scar tissue. Later in the disease (fig. 6) the odontoblasts have died and are represented by mere cell debris.)

FIG. 7, Plate 59—CURE OF SCURVY.—Photomicrograph × 40 of one fold of a cheek tooth of a guinea-pig which was placed on a scurvy-producing diet for 20 days and, when full scurvy had developed, was cured by being put back on normal diet for 10 days and was then killed.

Diet.—Basal diet without cabbage 20 days. Basal diet plus unlimited cabbage 10 days.

It may be assumed that when the cure was started ten days before the animal was killed, the tooth would have presented an appearance like that in fig. 6*a*. It follows that the normal growing end from N to G has developed since, and that all the calcific scar tissue S in the pulp has also been laid down during this period of cure. The narrow black calcific barrier B which lines the pulp is identical with that in fig. 6*a*, so that no doubt it was formed during the development of scurvy.

The part between P and N represents the section of new tooth developed during the last 5 or 10 days of the scurvy period, since primary dentine ceased to form at point P and recommenced at point N In this part of the tooth neither primary dentine nor enamel were laid down and the tooth consists in this section of a mass of calcific scar tissue replacing dentine covered by a layer of degenerated and apparently keratinized enamel epithelium. Above this section for a considerable distance the primary dentine is extremely thin, and evidently the odontoblasts in this area were entirely destroyed by the disease, since no further tubular dentine developed therein even after the scurvy had been cured.

FIG. 8, Plate 60—INTERMITTENT ATTACKS OF SCURVY.—Photomicrograph × 70 of part of a fold of a cheek tooth of a guinea-pig suffering from repeated short periods of deficiency.

Diet.—Basal diet alone, 6 days. Basal diet plus 5 gm. cabbage, 3 days. Basal diet alone, 11 days. Basal diet plus 5 gm. cabbage, 11 days. Basal diet alone, 10 days. Basal diet plus 5 gm. cabbage, 6 days. Basal diet alone, 16 days. Died.

The appearance of the tip of this tooth shows that a brief attack of scurvy developed when only the outer part of the dentine D had formed. The odontoblasts accordingly began to degenerate and shed their fibrils. This caused the death of the dentine D, which was sealed off by the barrier B. Before the odontoblasts completely died, however, the animal was given vitamin C which enabled the odontoblasts to revive and lay down a further portion of normal tubular dentine D 1 (*cf.* fig. 14, P2). This phenomenon was only very rarely observed. Generally the further supply of vitamin C was not administered in time to resuscitate the odontoblasts, and therefore scar tissue formed, or alternatively it was given too early to necessitate the laying down of the calcific barrier B.

FIG. 9, Plate 60—"COMPLICATED" SCURVY, SHOWING PRODUCTION OF CAVITY.—Photomicrograph × 78 of part of one fold of a cheek tooth of a guinea-pig suffering from scurvy complicated by other dietary deficiencies.

Diet.—Unlimited "Sussex" rolled oats, 1 gm. skimmed milk powder and 0·3 gm. cabbage per day (supplemented at periods with extra cabbage and whole milk).

The section showed that an attack of severe scurvy had lasted for some considerable time, during which no fresh primary dentine had been formed. Corresponding to this period is a barrier of lime salts S at the pulp margin all the way up to the biting edge. It appears, too, that the normal sealing of the senile end of the pulp chamber by calcific scar tissue was prevented on this diet, and the pulp remains open to the mouth. The pulp is already showing signs of infection F which might easily have spread down to the growing end, if the animal had lived, and produced an alveolar abscess (the bone caries of HOWE, 1923). There is a large cavity C at the biting edge, due to absence of the usual calcific scar tissue, which might be mistaken for caries.

FIG. 10, Plate 60—NORMAL BONE.—Photomicrograph × 500 of the bony septum between two of the cheek teeth of a control guinea-pig on normal diet. The physiological wandering of the tooth is indicated by bony absorption with giant cells G in their lacunæ. The connective tissue and bone cells are all normal. Active mitosis is occurring at M.

FIG. 11, Plate 60—FULL SCURVY—BONE.—Photomicrograph × 500 of the absorbing edge of an alveolar bony septum between two cheek teeth of a guinea-pig which died during an attack of acute scurvy.

Diet.—Basal diet for 20 days.

The absorption which had been going on (as in fig. 10) has ceased. The giant cells G are degenerated. The connective tissue cells C have lost their embryonic character, mitosis has ceased and they are shrunken and distorted. The bone corpuscles also are degenerating and the bone itself is more deeply stained than usual.

FIG. 12, Plate 61—CURE OF SCURVY—ENAMEL.—Drawing of a ground section of a tooth from a guinea-pig on the same diet as the animal used for fig. 7. E = enamel, EP = epithelium, D = primary dentine, S = calcific scar tissue. It will be seen that the enamel terminates abruptly when the attack of scurvy develops. There is no appreciable intermediate area of badly formed enamel.

FIGS. 13 AND 13*a*, Plate 61—CURE OF SCURVY—CEMENTUM.—Photomicrographs × 100 and × 690 of a cheek tooth of a guinea-pig on the same diet as in fig. 7. The part from A to B, fig. 13, was the growing end of the tooth during the attack of scurvy, and shows that no primary dentine and no cementum was formed at that time. The animal was killed 10 days after the cure was started, and a calcific scar is seen forming in place of the normal cementum. This calcific tissue is enclosing cells C, which are clearly cementoblasts, fig. 13*a*. The cementum formed before the attack, CB, and the cementum formed after, CA, on the new primary dentine, NPD, is normal. In fig. 13*a*, C1 is a typical cell of the cementoblast layer; C2 is a similar cell becoming enclosed, and C3 shows the cell completely enclosed. The cementum scar is continuous with the calcific scar tissue filling the pulp chamber.

FIG. 14, Plate 59—REACTIONS TO EXPERIMENTAL TRAUMA CORRESPONDING TO THOSE OBSERVED IN SCURVY.—Photomicrograph × 40 of one fold of a rabbit's cheek tooth. The lower border of the mandible was snipped away and a probe introduced into the pulp chamber through the growing end of the tooth in order to produce degeneration and death of the cellular elements of the pulp. Some of the cementoblasts were also injured. The wound healed by first intention and eight weeks later the animal was killed.

The point A represents the growing end of the tooth at the time of injury, P1 the primary dentine which was already formed, S, S1 and S2 are masses of calcific scar tissue filling up the pulp chamber where the pulp tissue was injured by the probe. These masses correspond exactly with the tissue S in fig. 7 which replaces the degenerated pulp after an attack of scurvy has been cured. P2 is a later deposit of normal tubular dentine deposited by a part of the pulp which escaped gross injury by the probe (*cf.* fig. 8). P3 is a deposit of tubular dentine laid down since the operation. Its faulty structure shows that the odontoblasts which formed it had suffered some degree of injury at that time.

C and C1 are masses of scar tissue replacing the cementum and showing cell inclusion at C1. These deposits again correspond exactly with those observed in an animal which has been cured of an attack of scurvy, *cf.* figs. 13 and 13*a*.

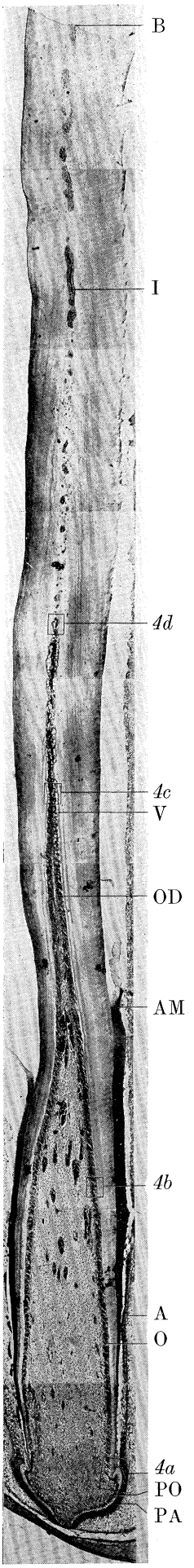

Fig. 3.—Normal.

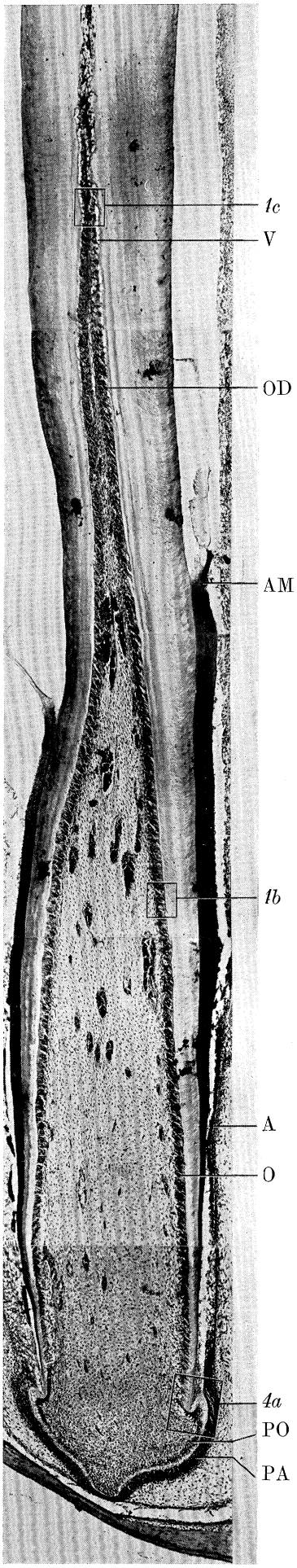

Fig. 4.—Normal (further enlarged).

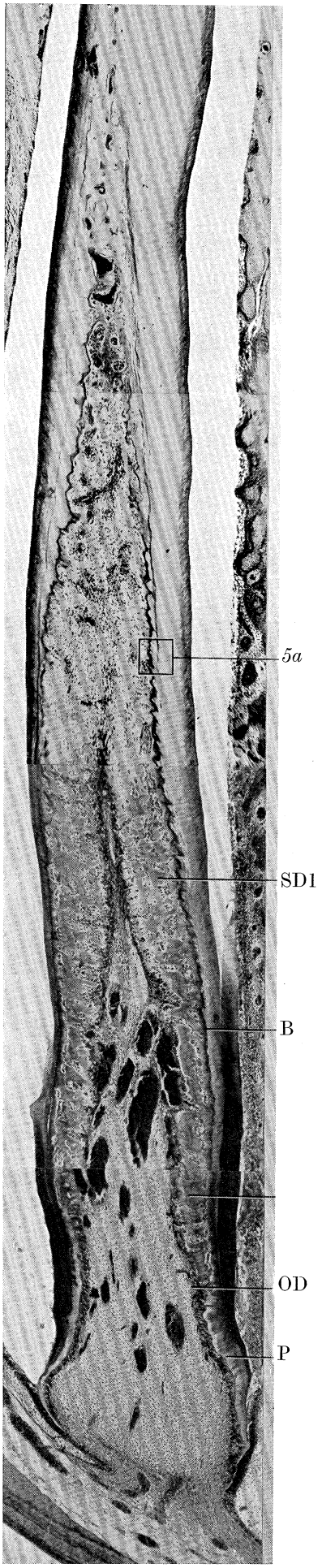

Fig. 5.—Chronic Subscurvy.

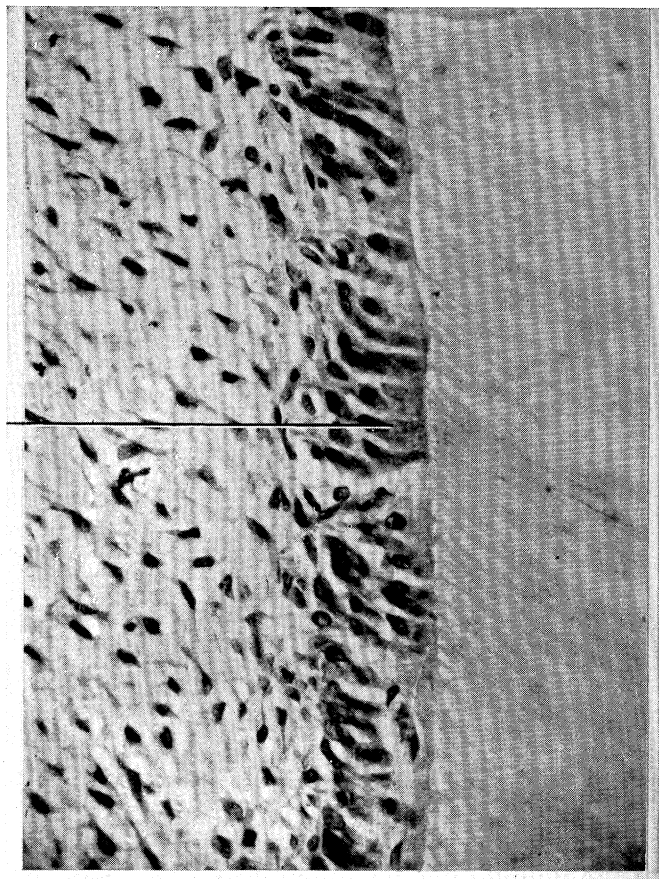

FIG. 4*b*.

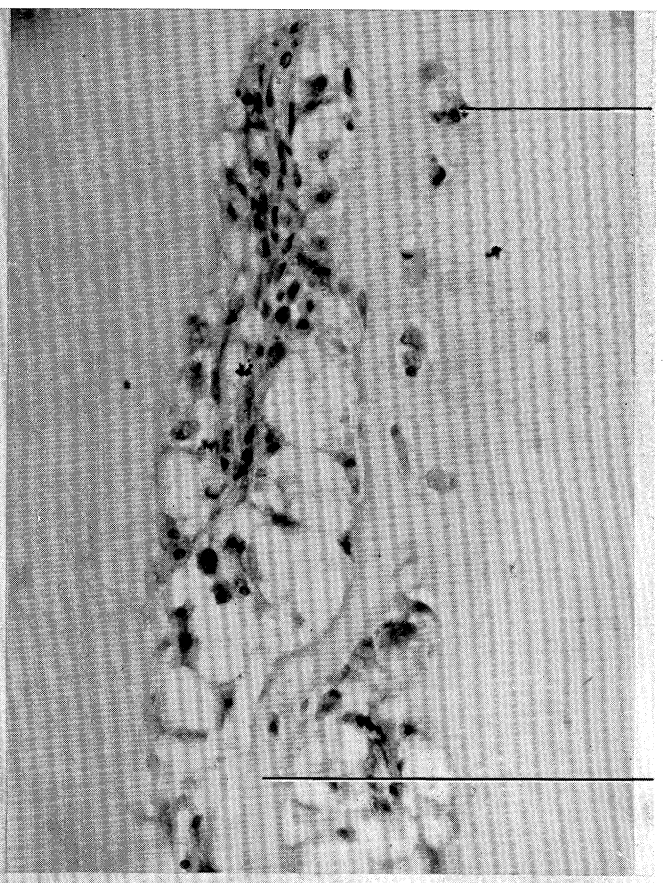

FIG. 4*d*.

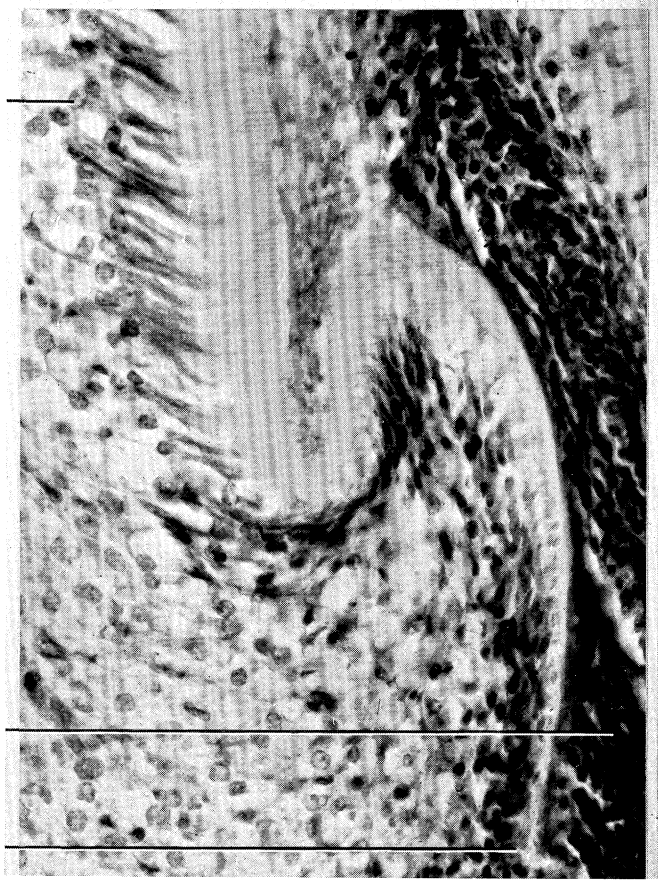

FIG. 4*a*.

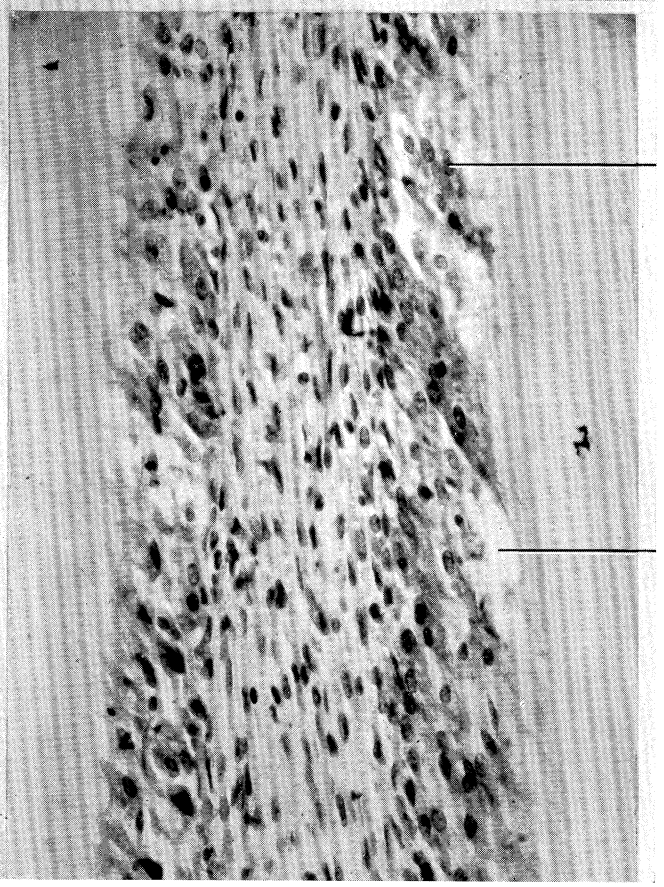

FIG. 4*c*.

Figs. 4*a*–4*c* enlarged portions of fig. 4.

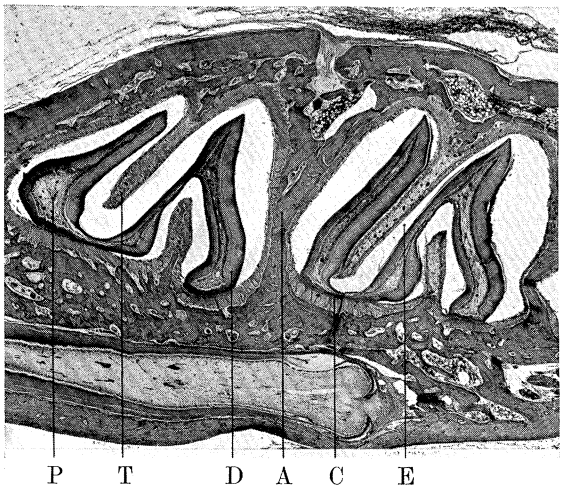

FIG. 2.—Normal (transverse section).

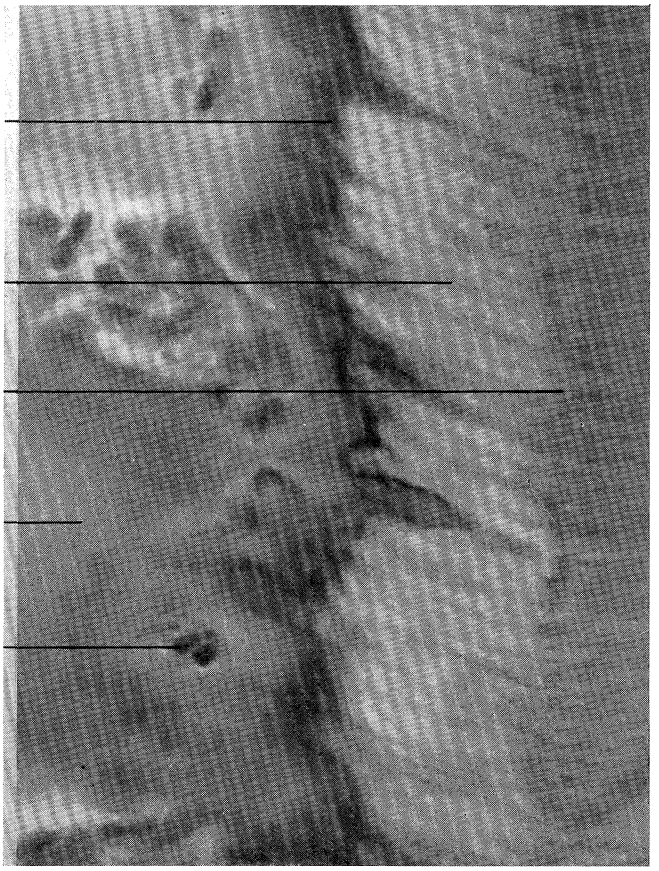

FIG. 5*a*.—Enlarged portion of fig. 5.

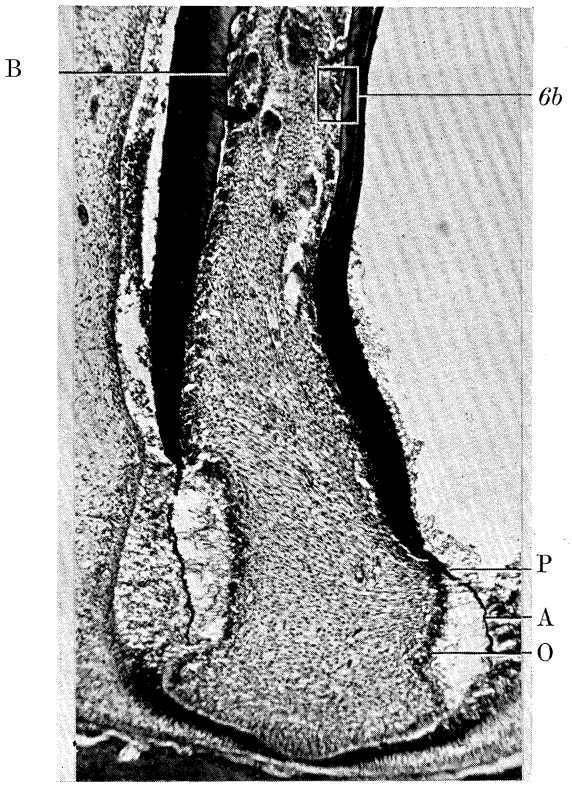

FIG. 6*a*.—Start of Acute Scurvy.

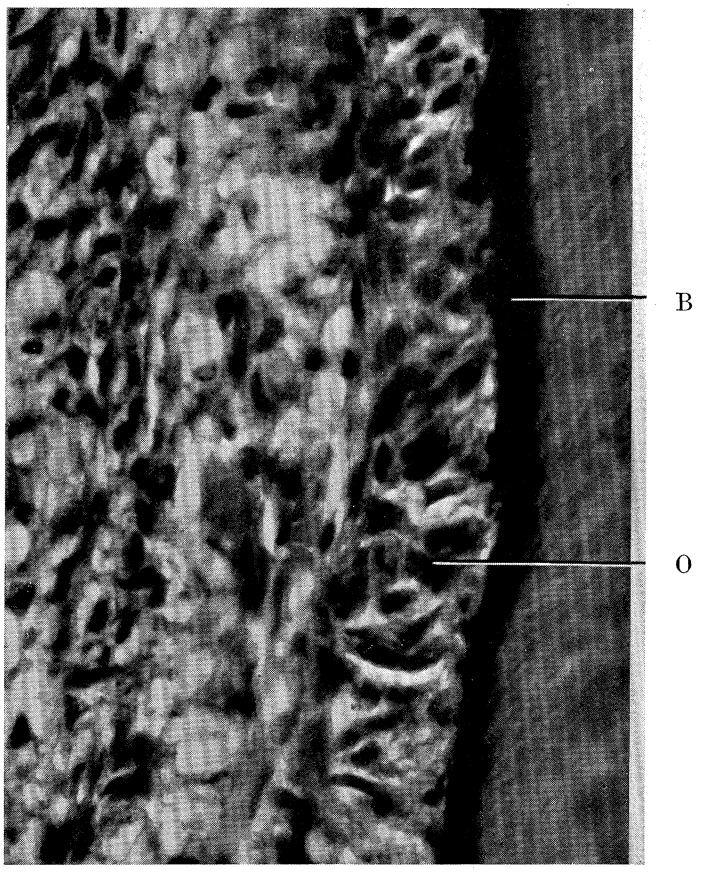

FIG. 6*b*.—Enlarged portion of fig. 6*a*.

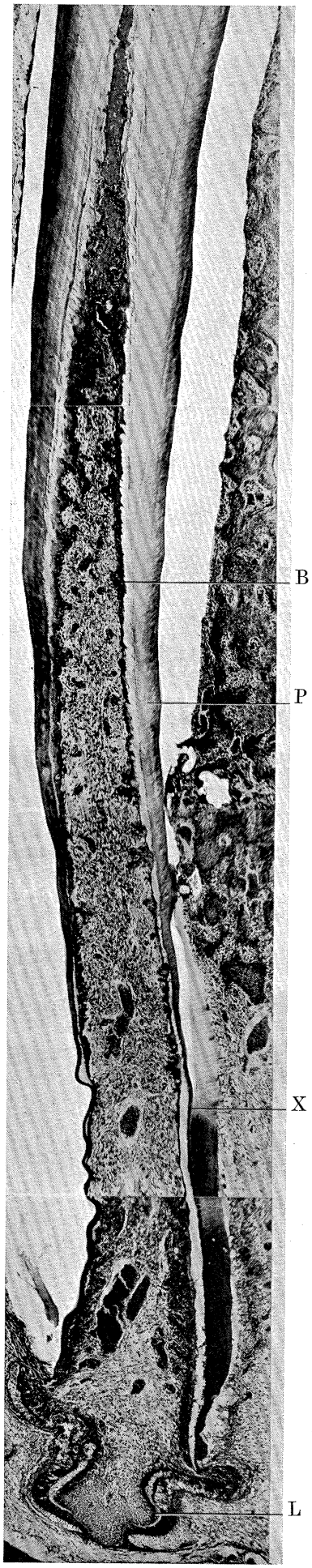

FIG. 6.—Acute Scurvy.

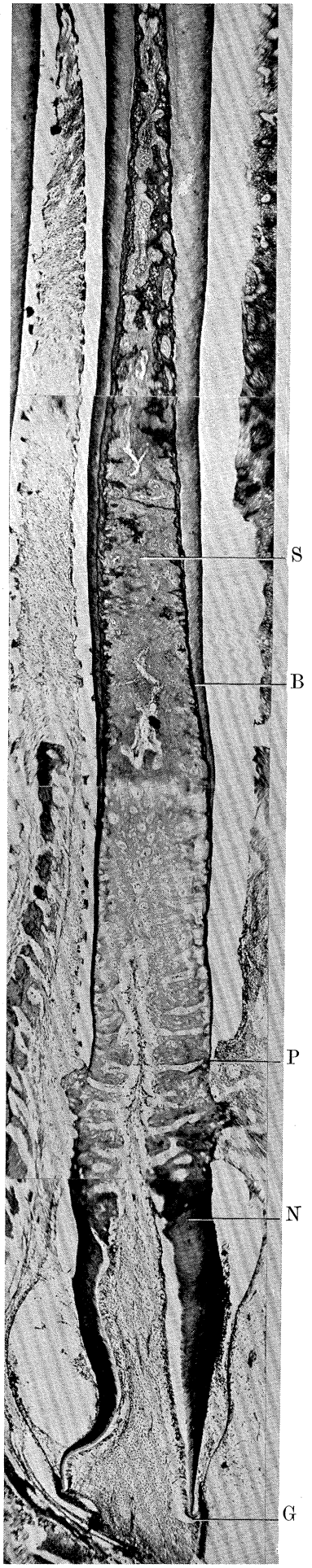

FIG. 7.—Scurvy and Cure.

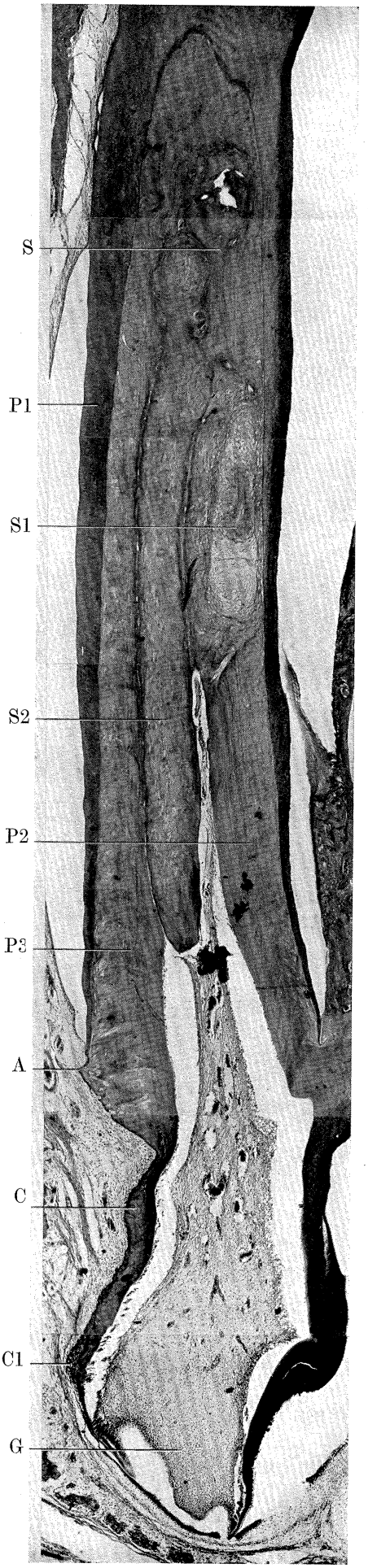

FIG. 14.—Experimental Trauma.

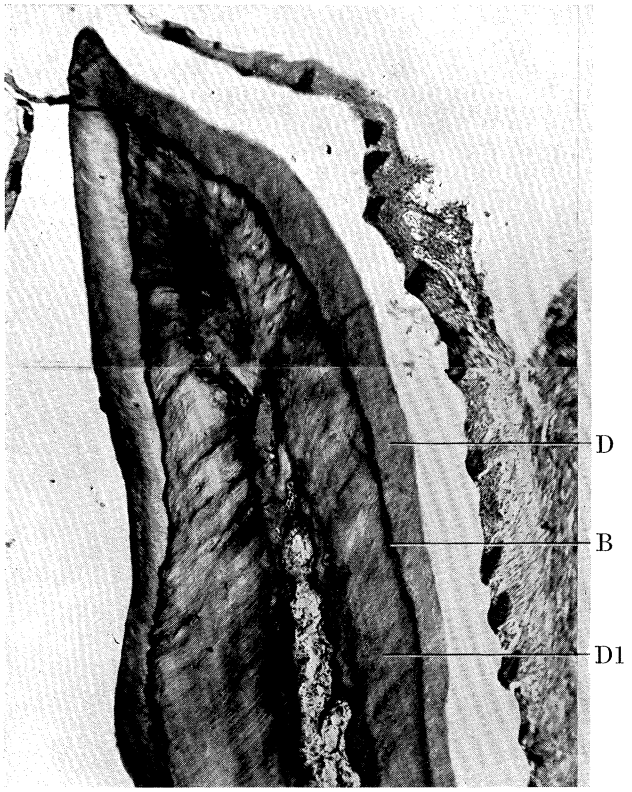

FIG. 8.—Intermittent Scurvy.

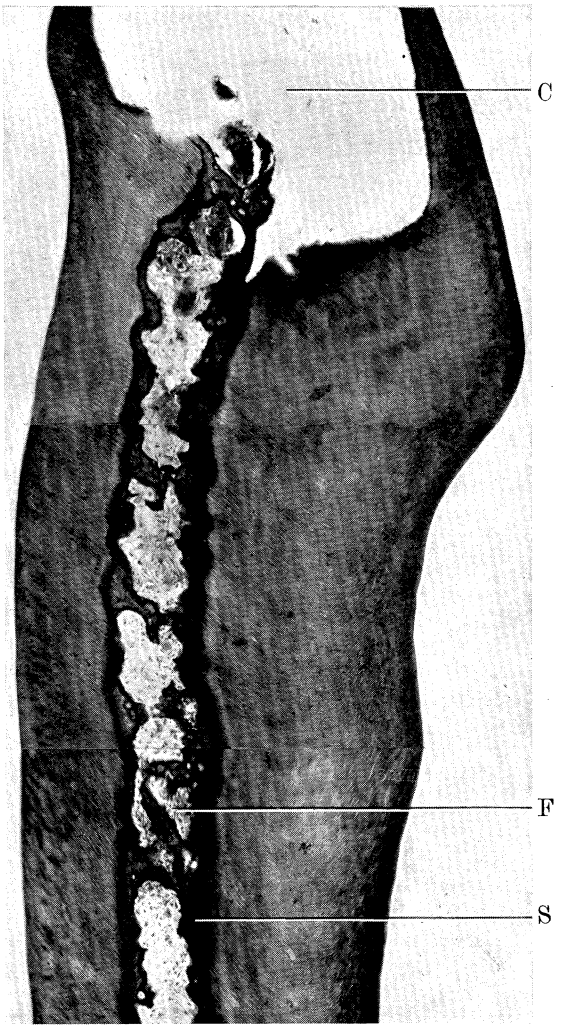

FIG. 9.—Complex Deficiency.

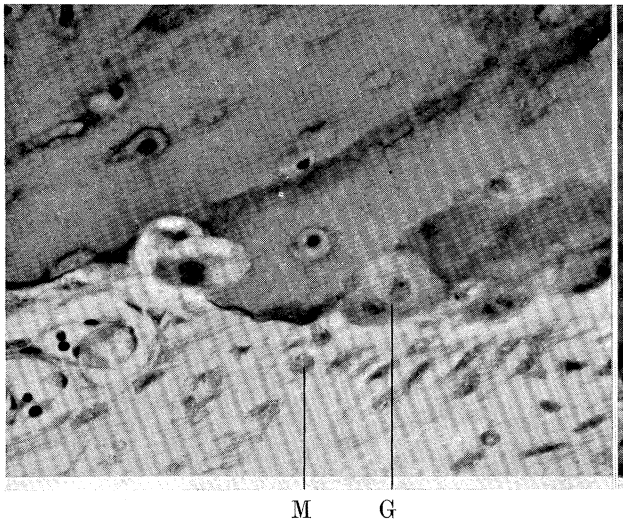

FIG. 10.—Jaw Bone, Normal.

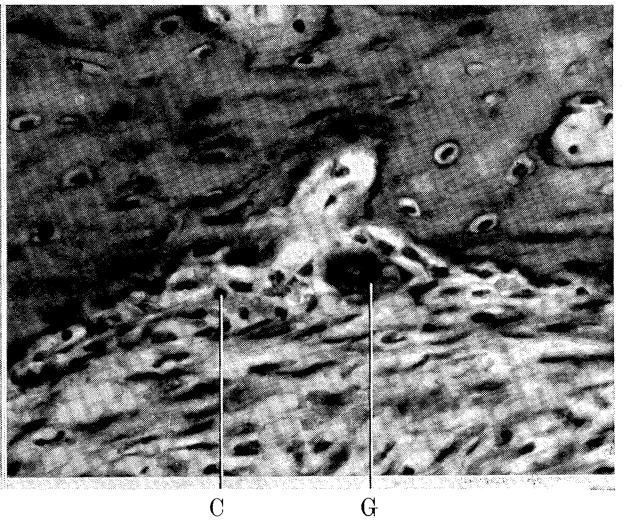

FIG. 11.—Jaw Bone, Acute Scurvy.

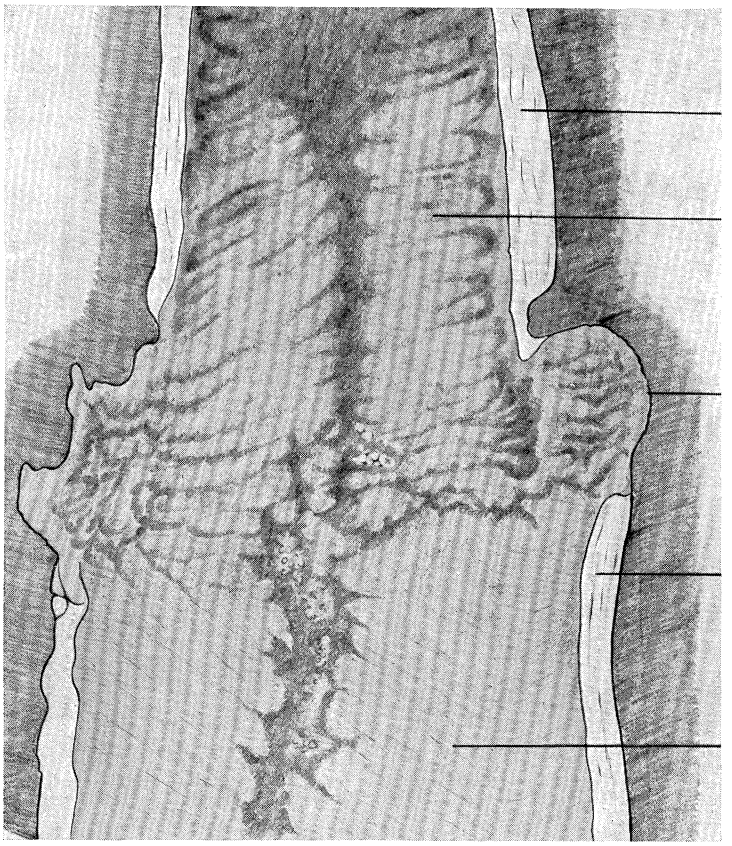

FIG. 12.—Cure of Scurvy, showing Enamel.

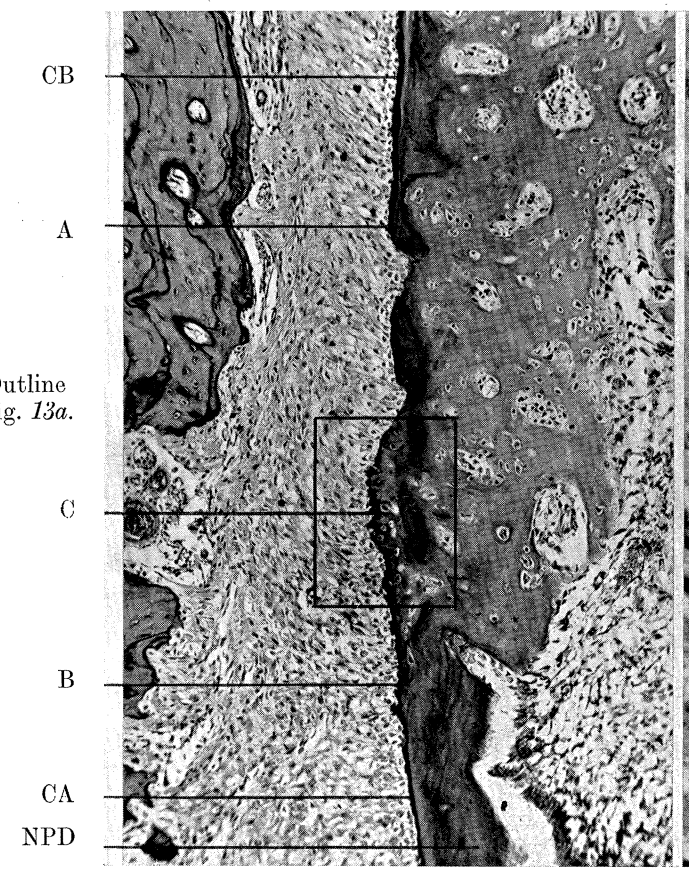

FIG. 13.—Cure of Scurvy, showing Cementum.

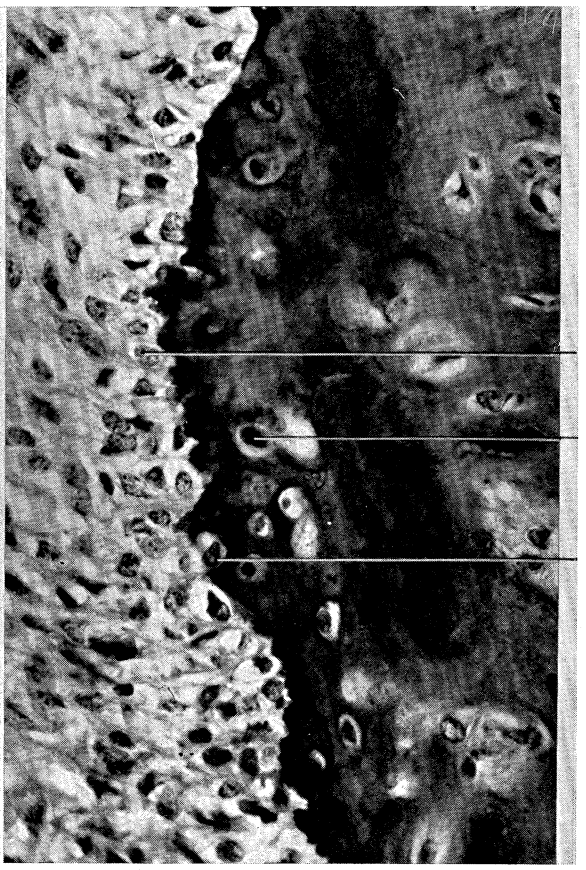

FIG. 13*a*.—Enlarged portion of fig. 13.

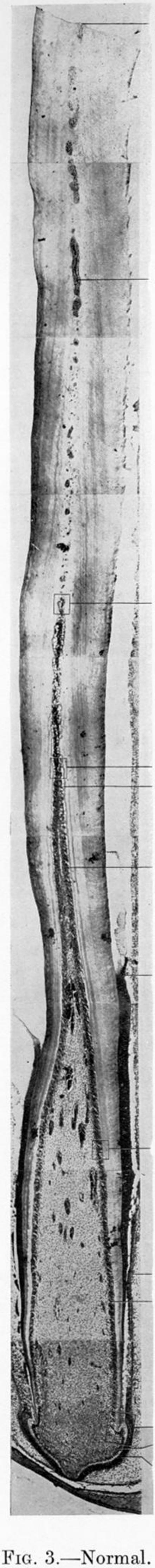

FIG. 3.—Normal.

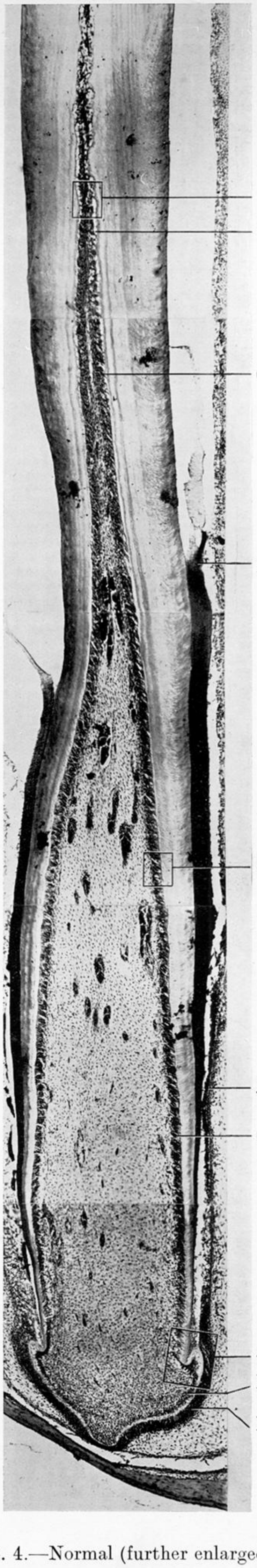

FIG. 4.—Normal (further enlarged).

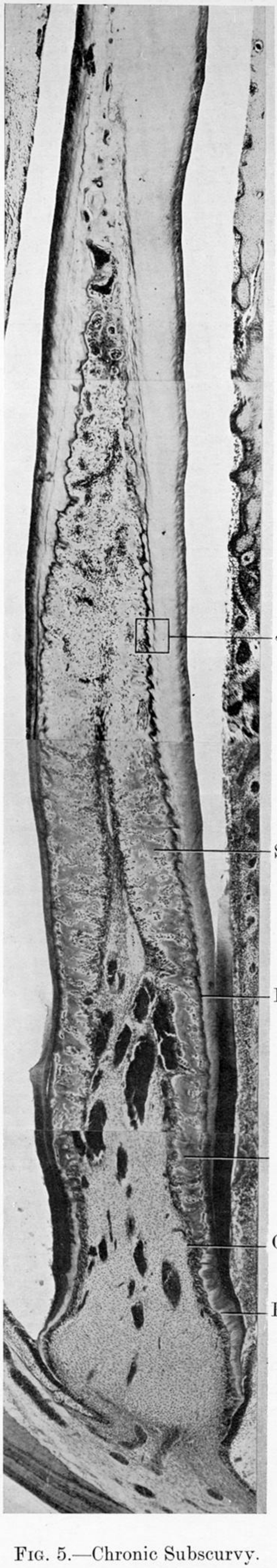

FIG. 5.—Chronic Subscurvy.

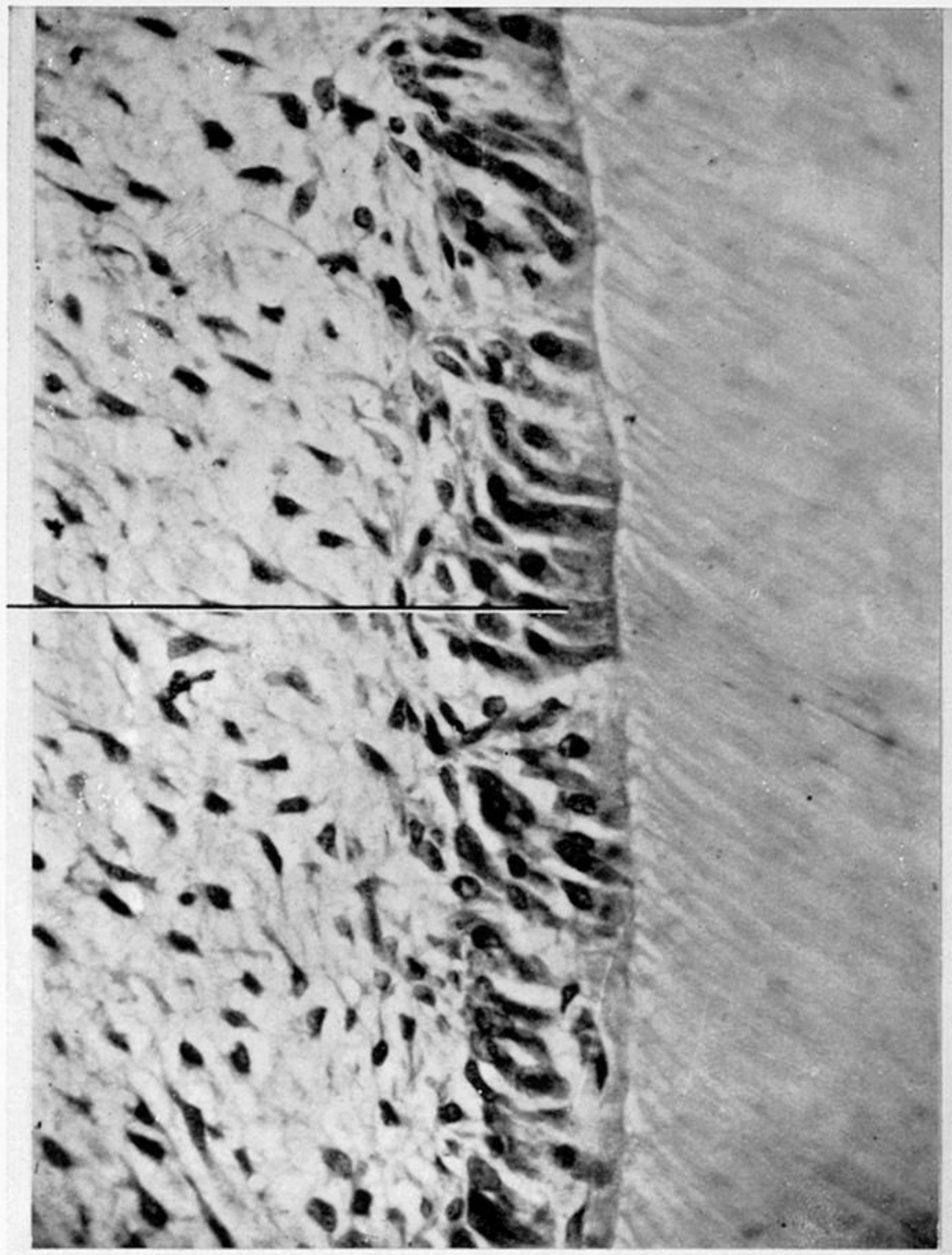

FIG. 4*b*.

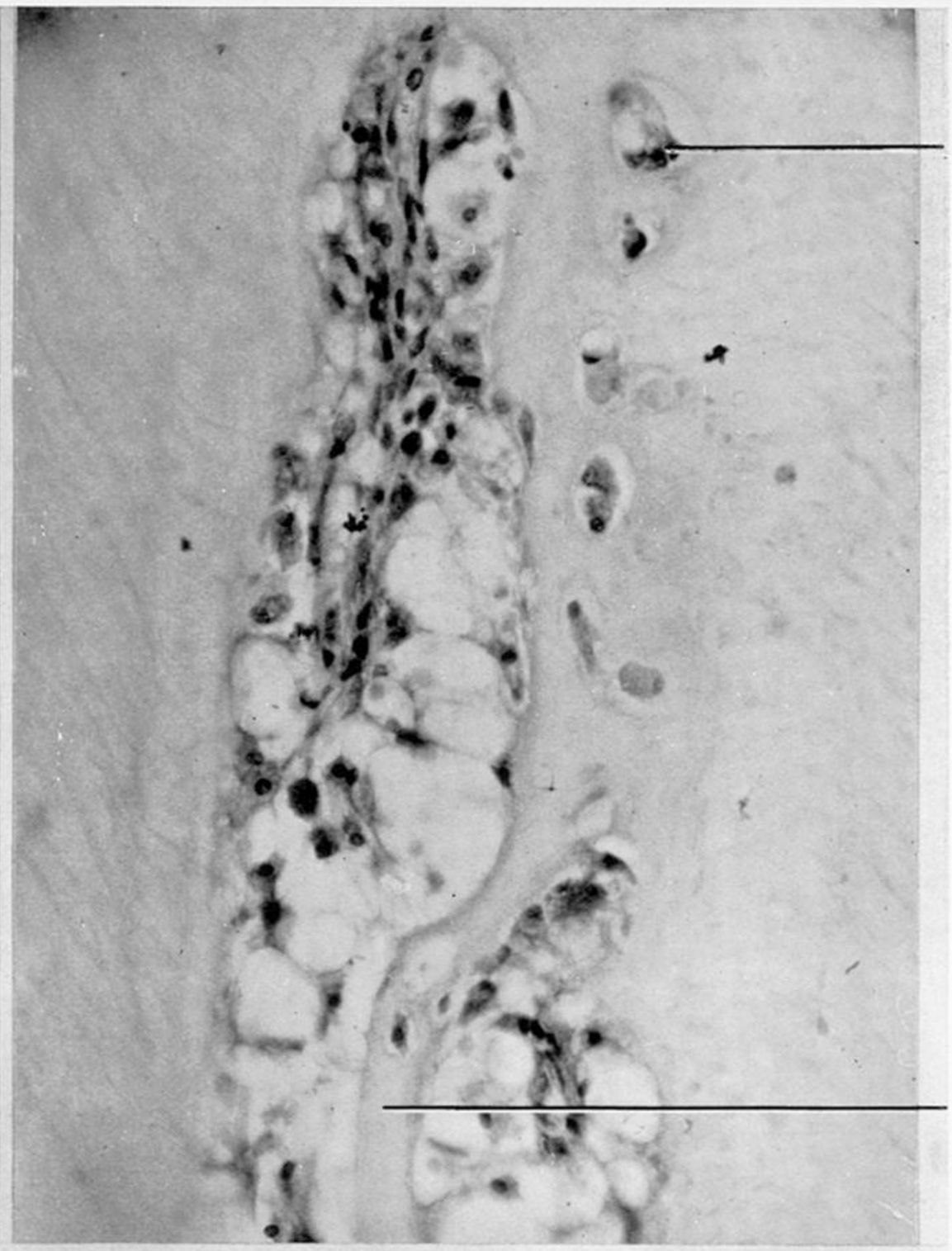

FIG. 4*d*.

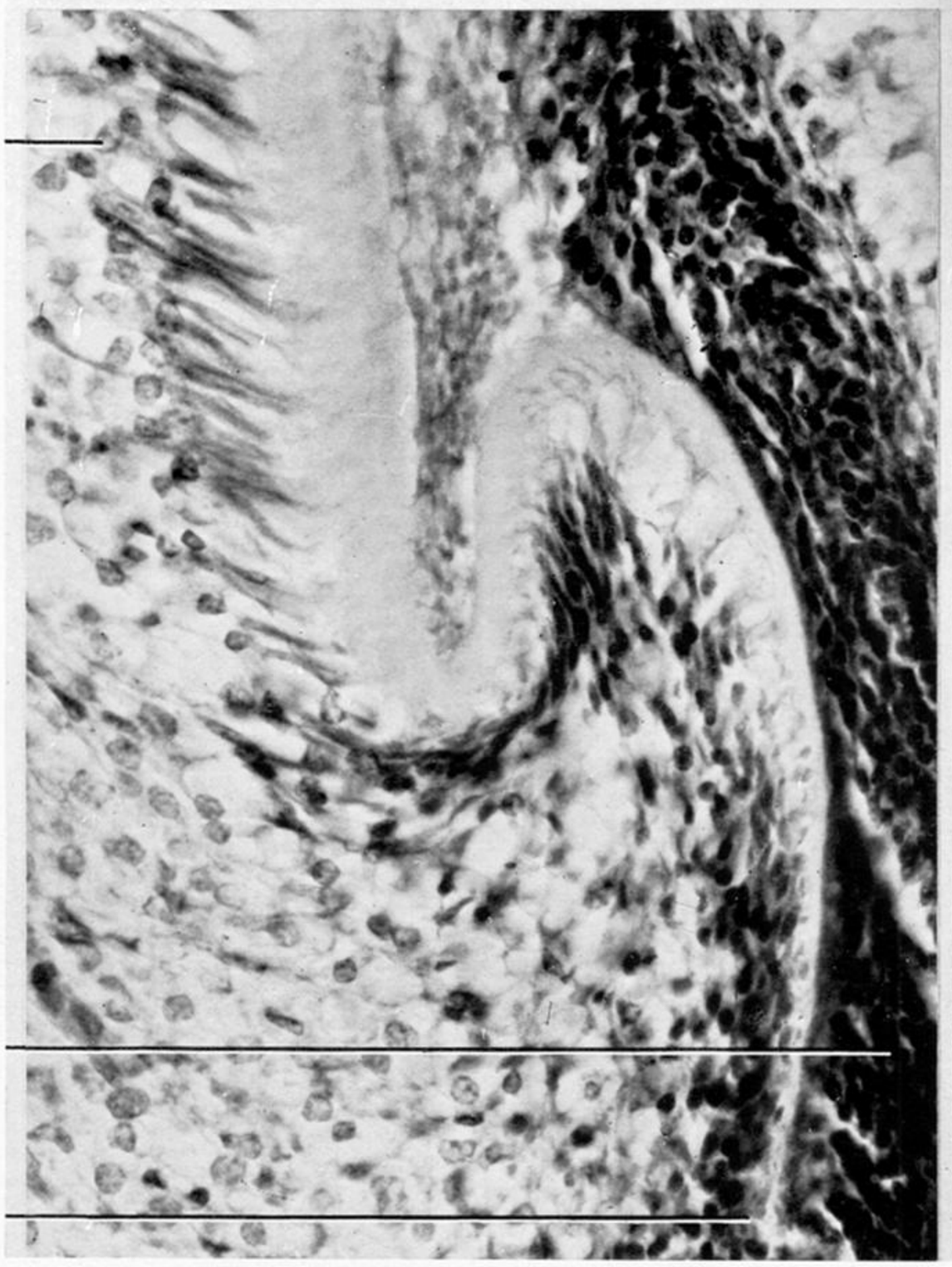

FIG. 4*a*.

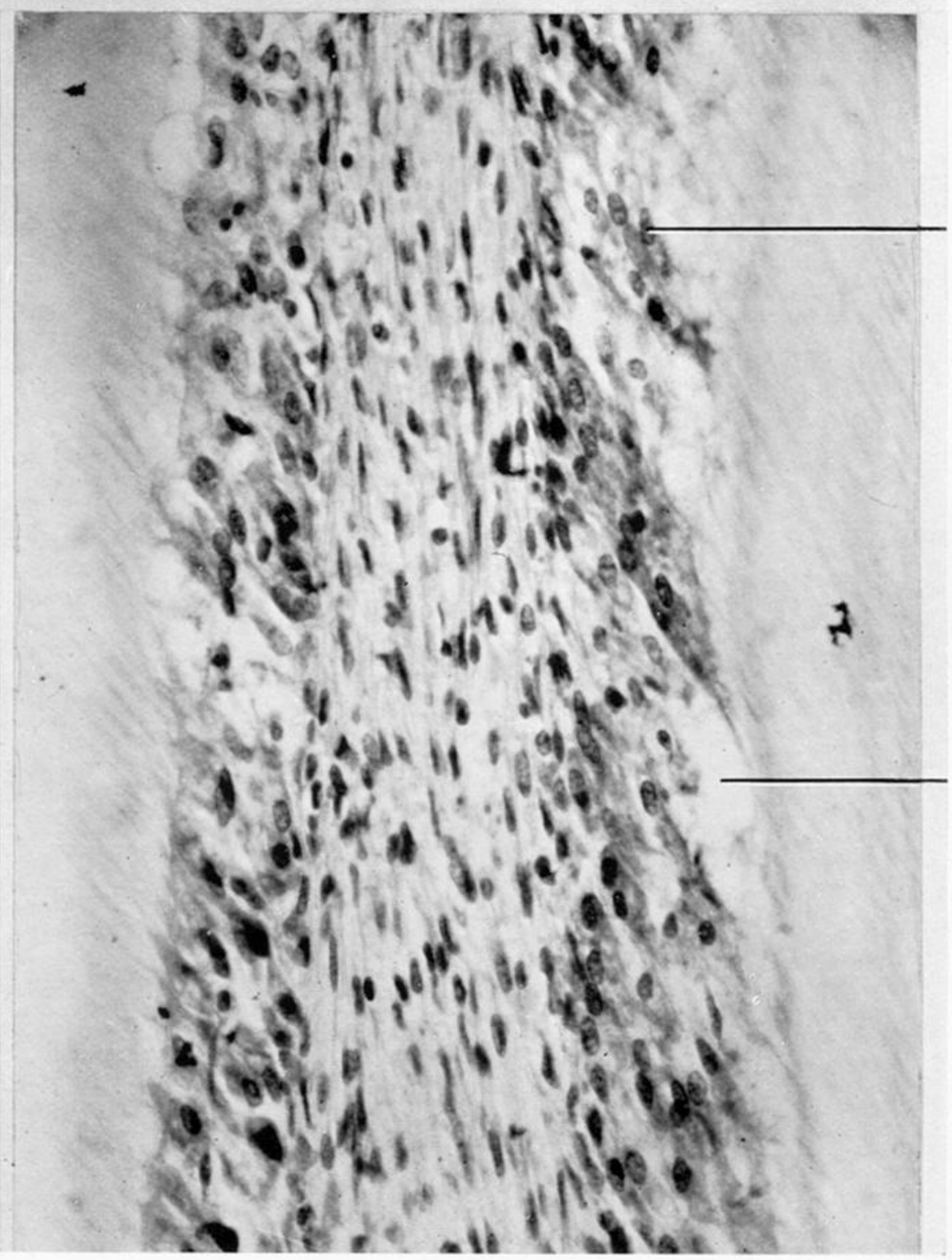

FIG. 4*c*.

Figs. 4*a*–4*c* enlarged portions of fig. 4.

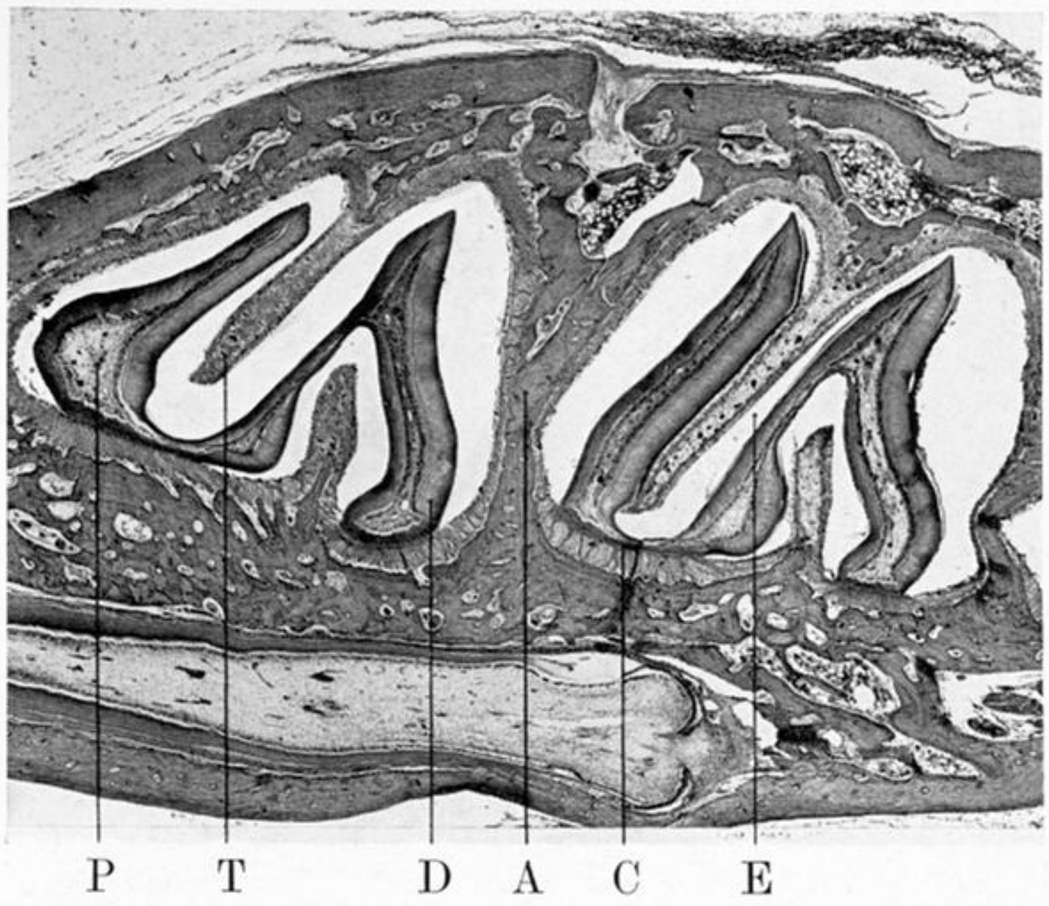

FIG. 2.—Normal (transverse section).

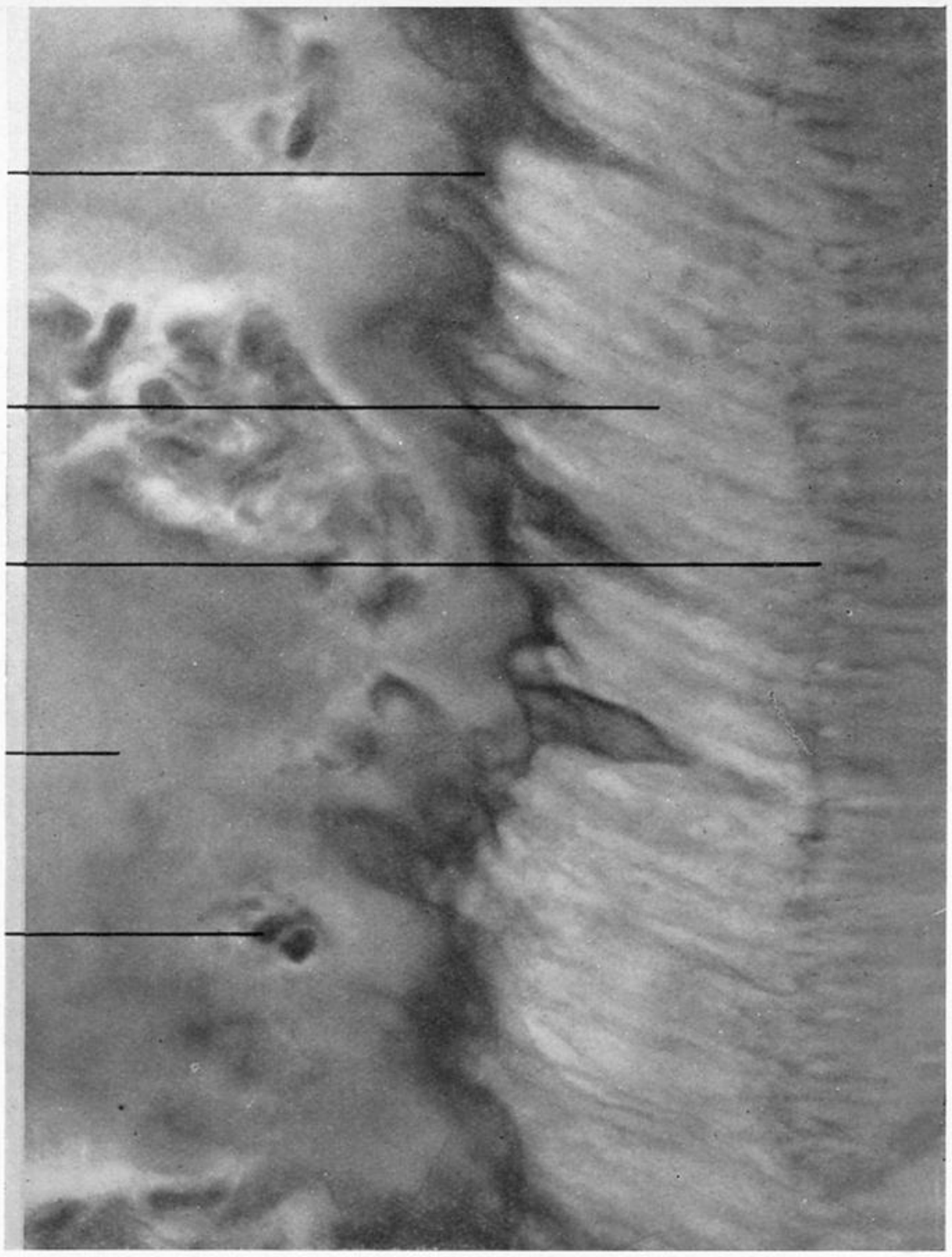

FIG. 5*a*.—Enlarged portion of fig. 5.

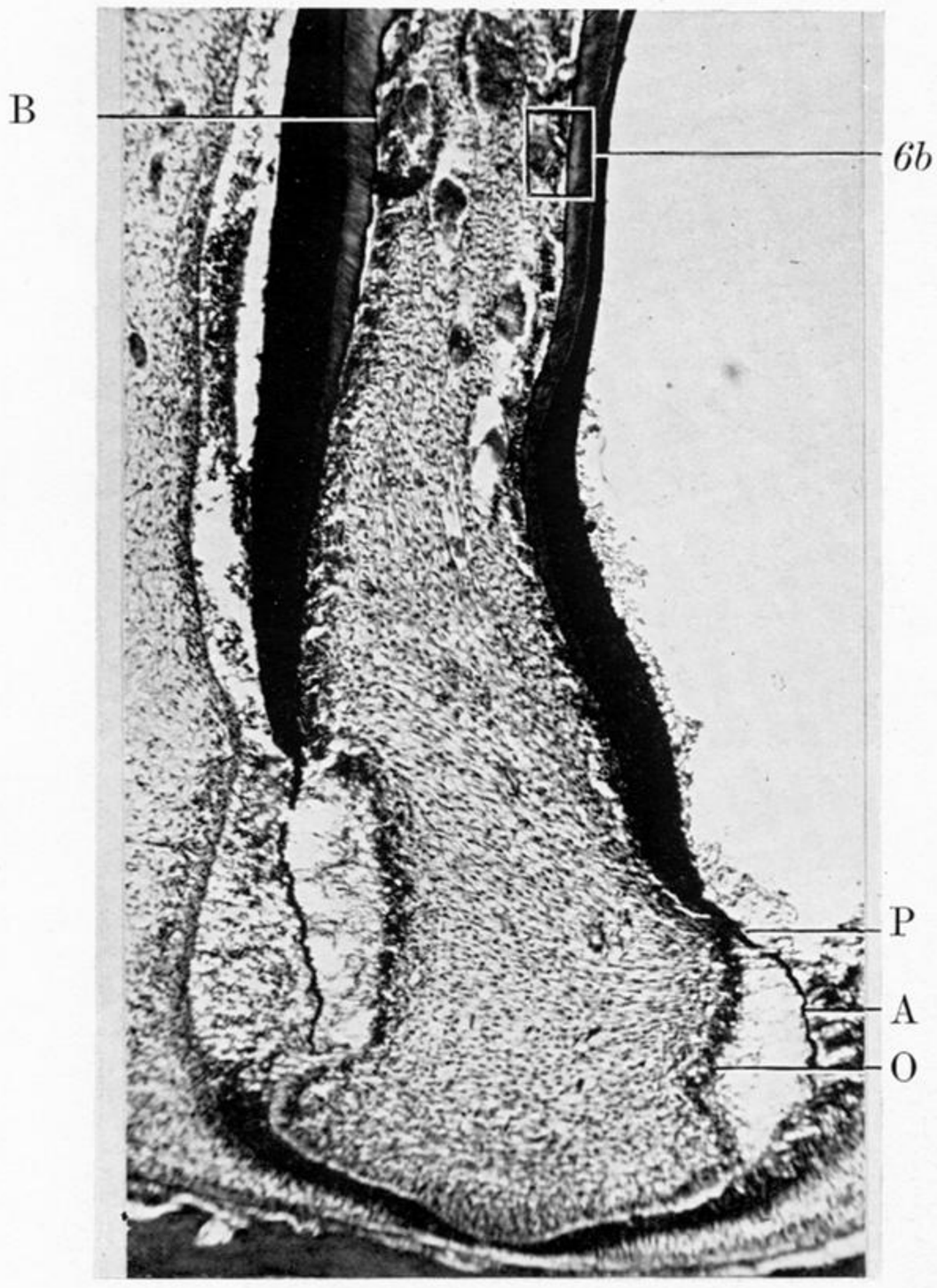

FIG. 6*a*.—Start of Acute Scurvy.

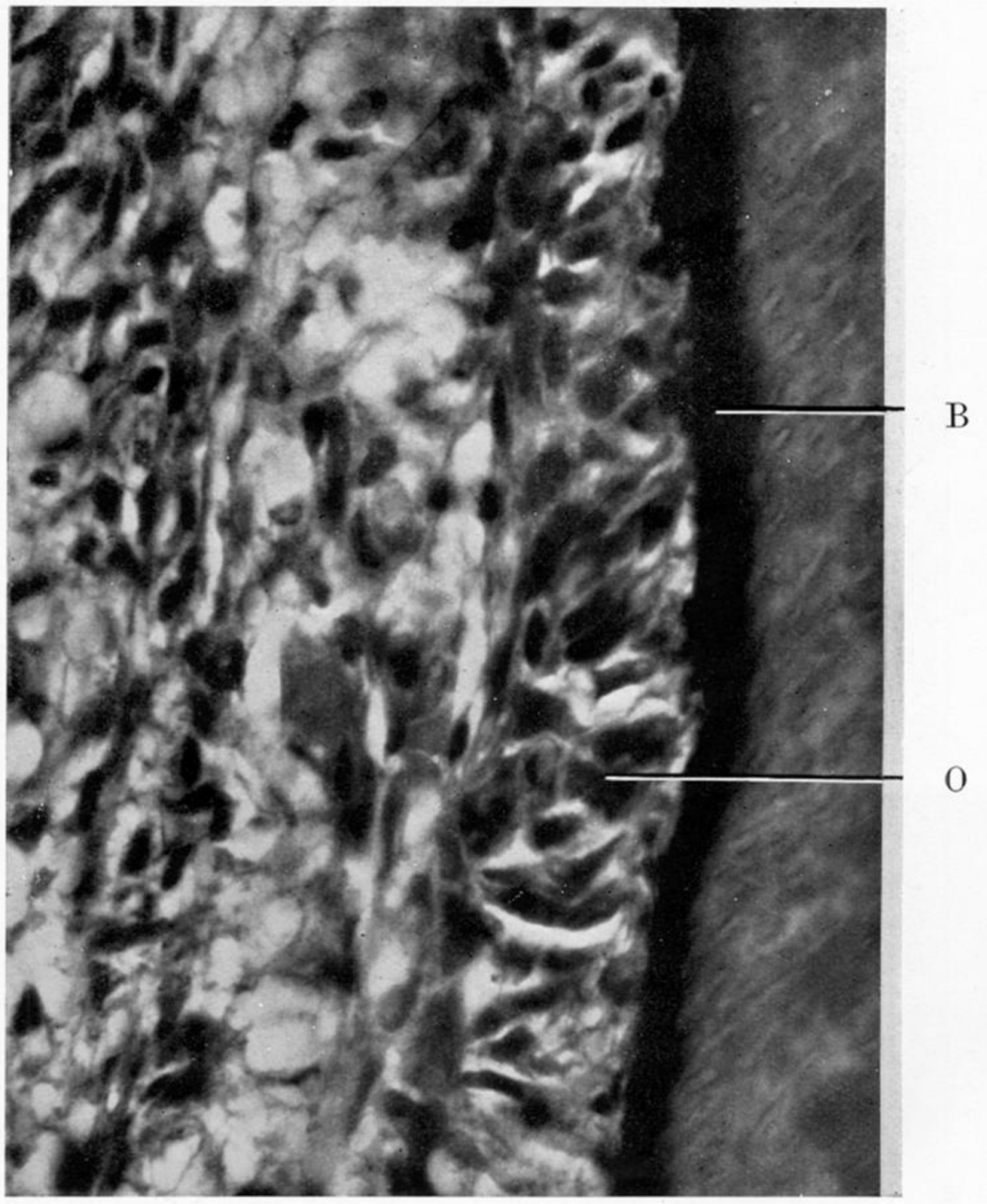

FIG. 6*b*.—Enlarged portion of fig. 6*a*.

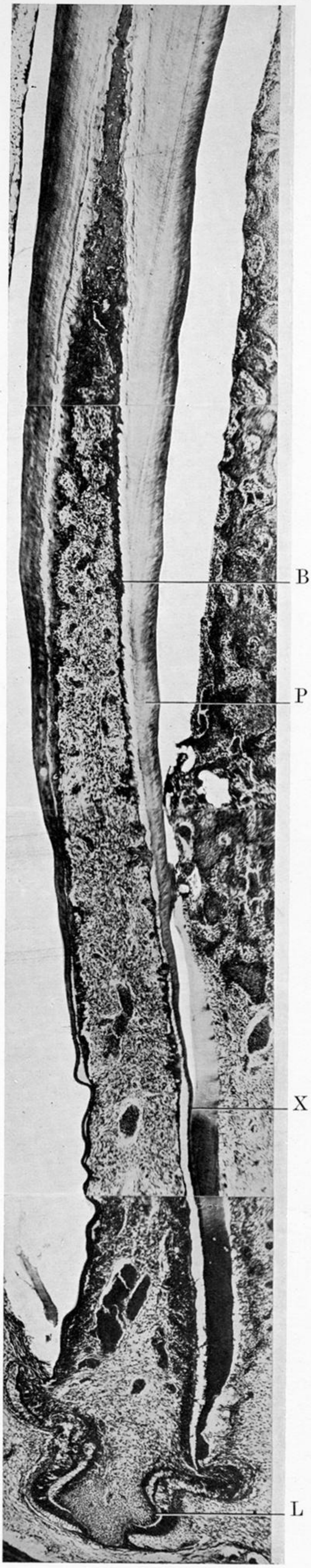

FIG. 6.—Acute Scurvy.

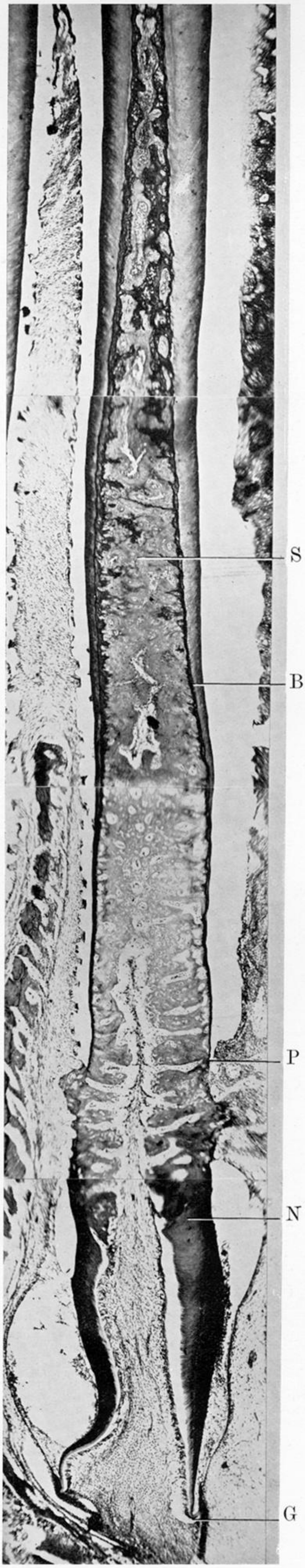

FIG. 7.—Scurvy and Cure.

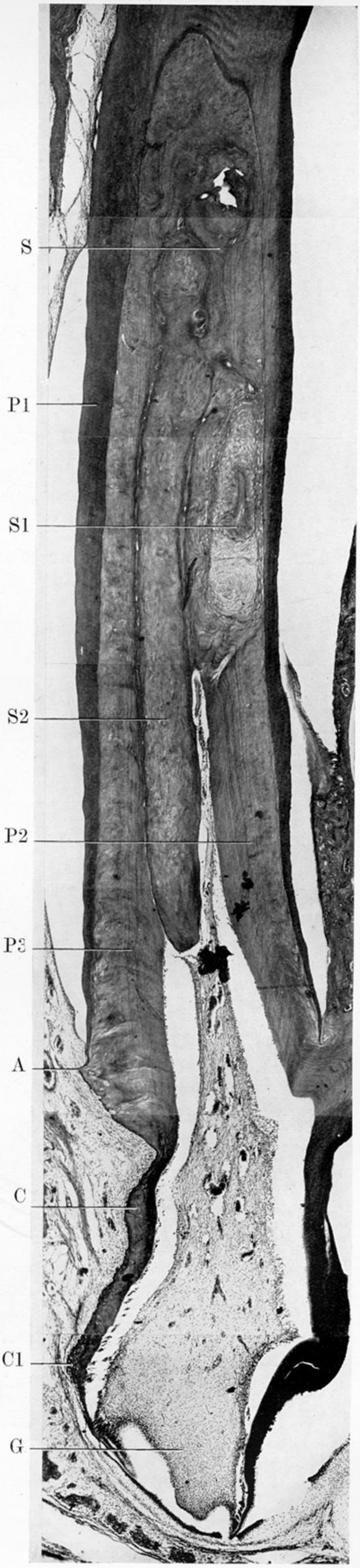

FIG. 14.—Experimental Trauma.

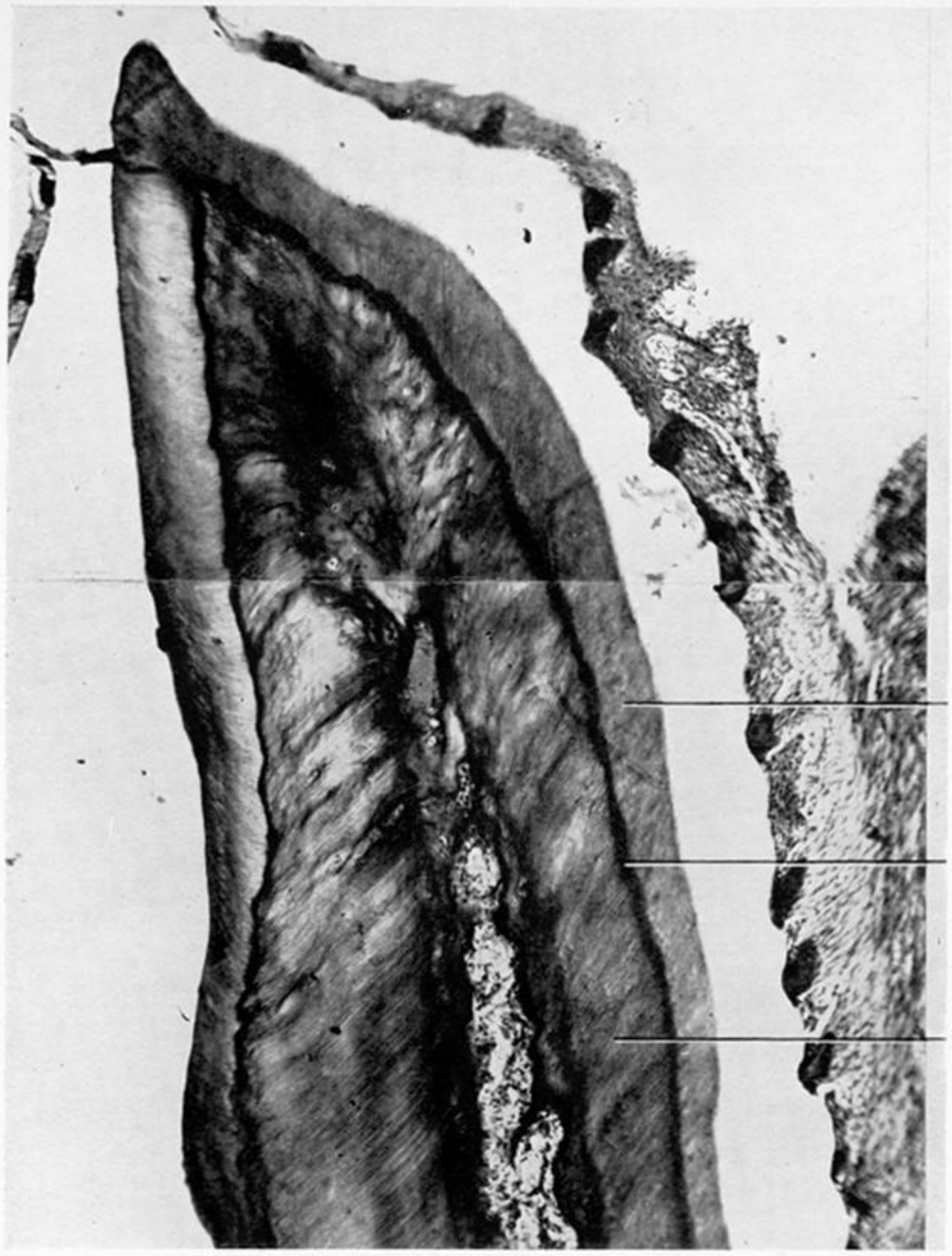

FIG. 8.—Intermittent Scurvy.

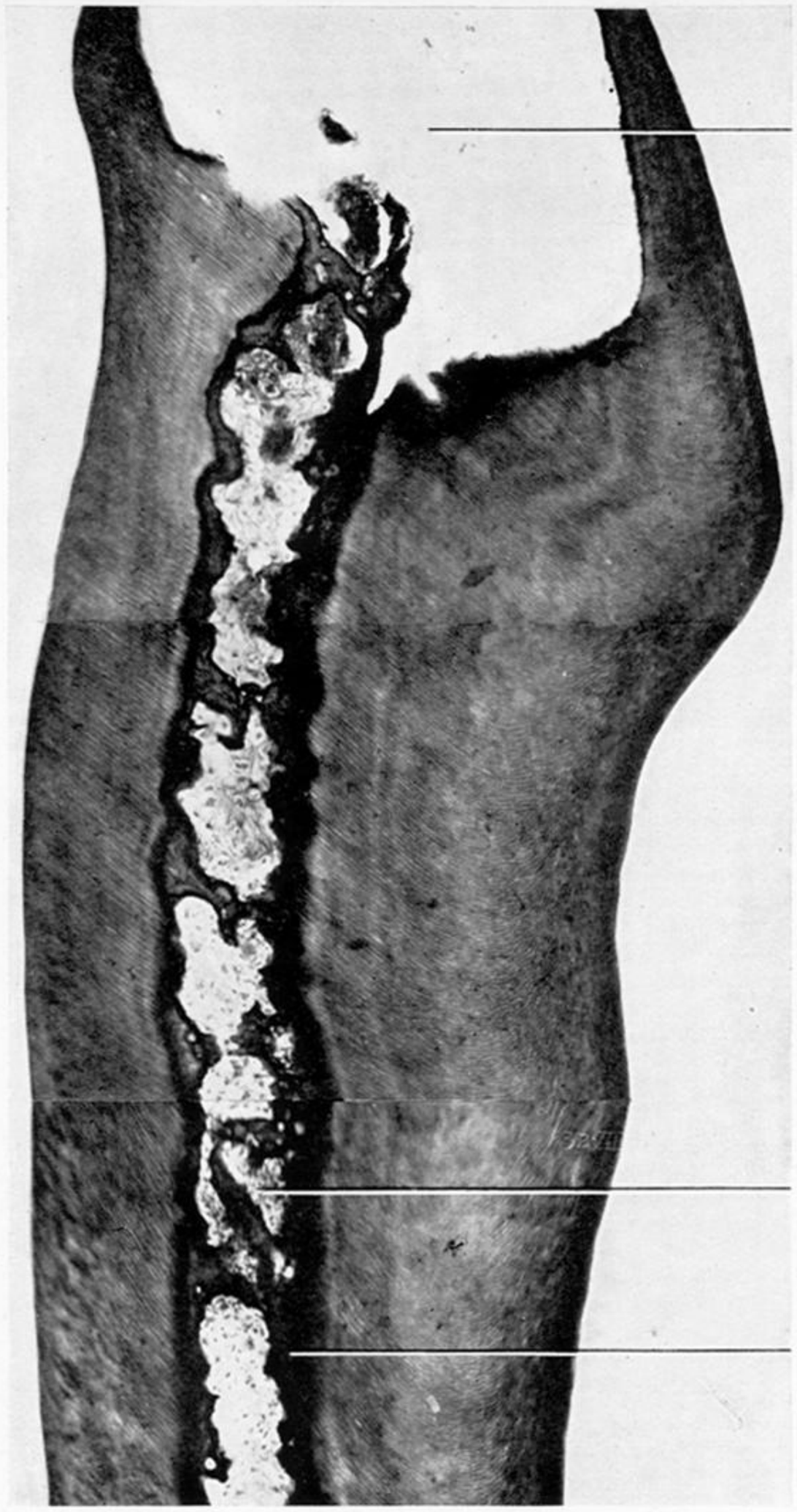

FIG. 9.—Complex Deficiency.

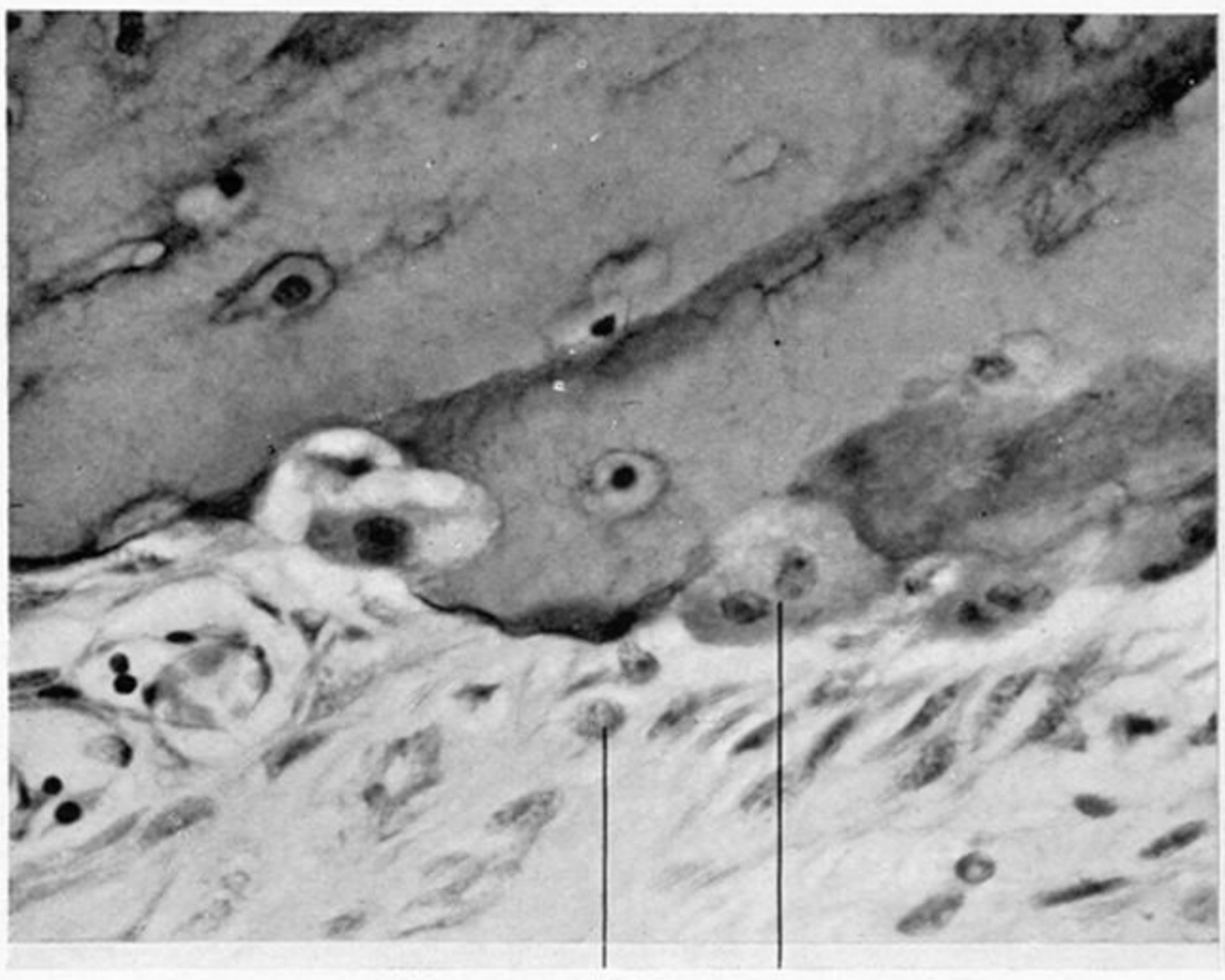

FIG. 10.—Jaw Bone, Normal.

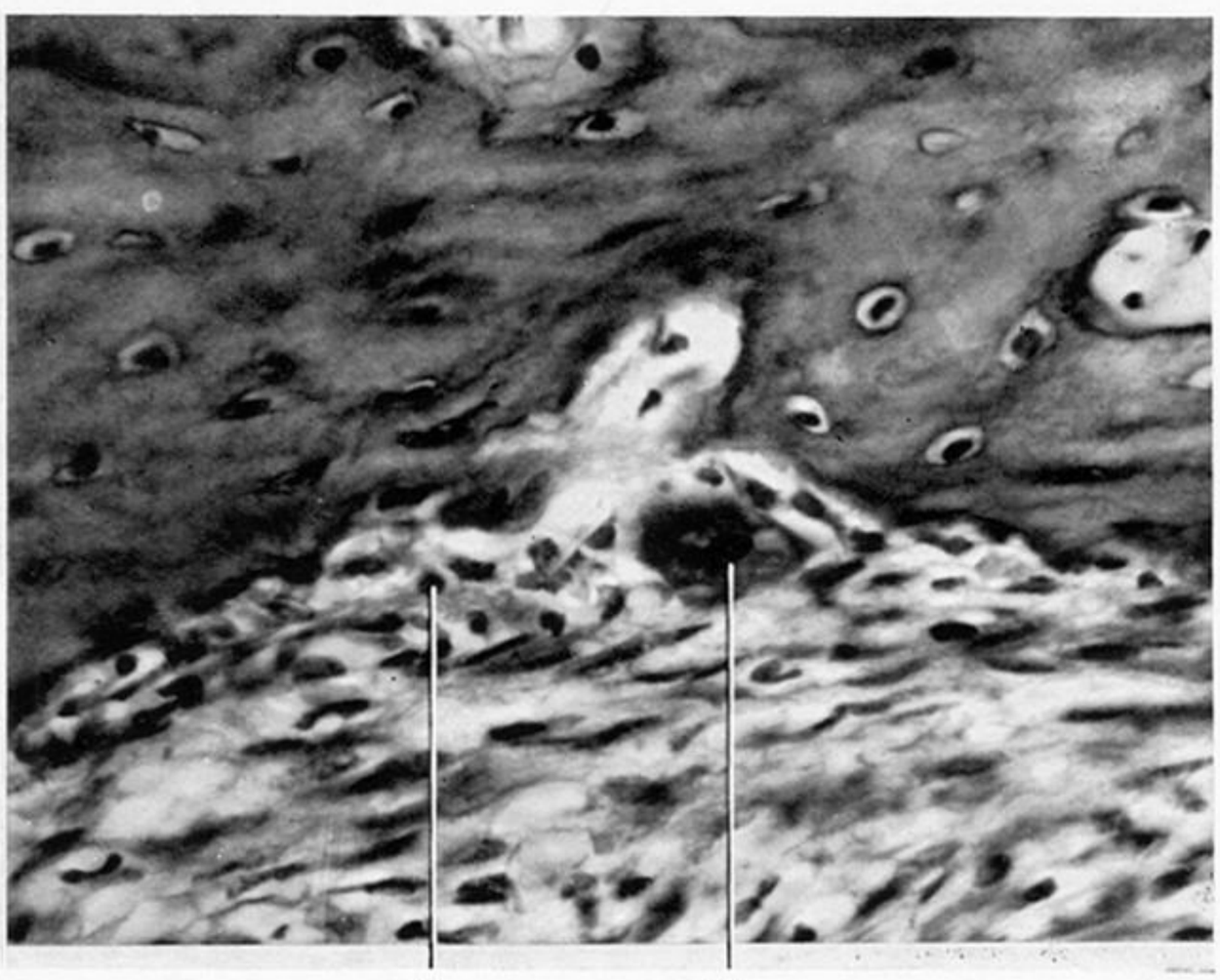

FIG. 11.—Jaw Bone, Acute Scurvy.

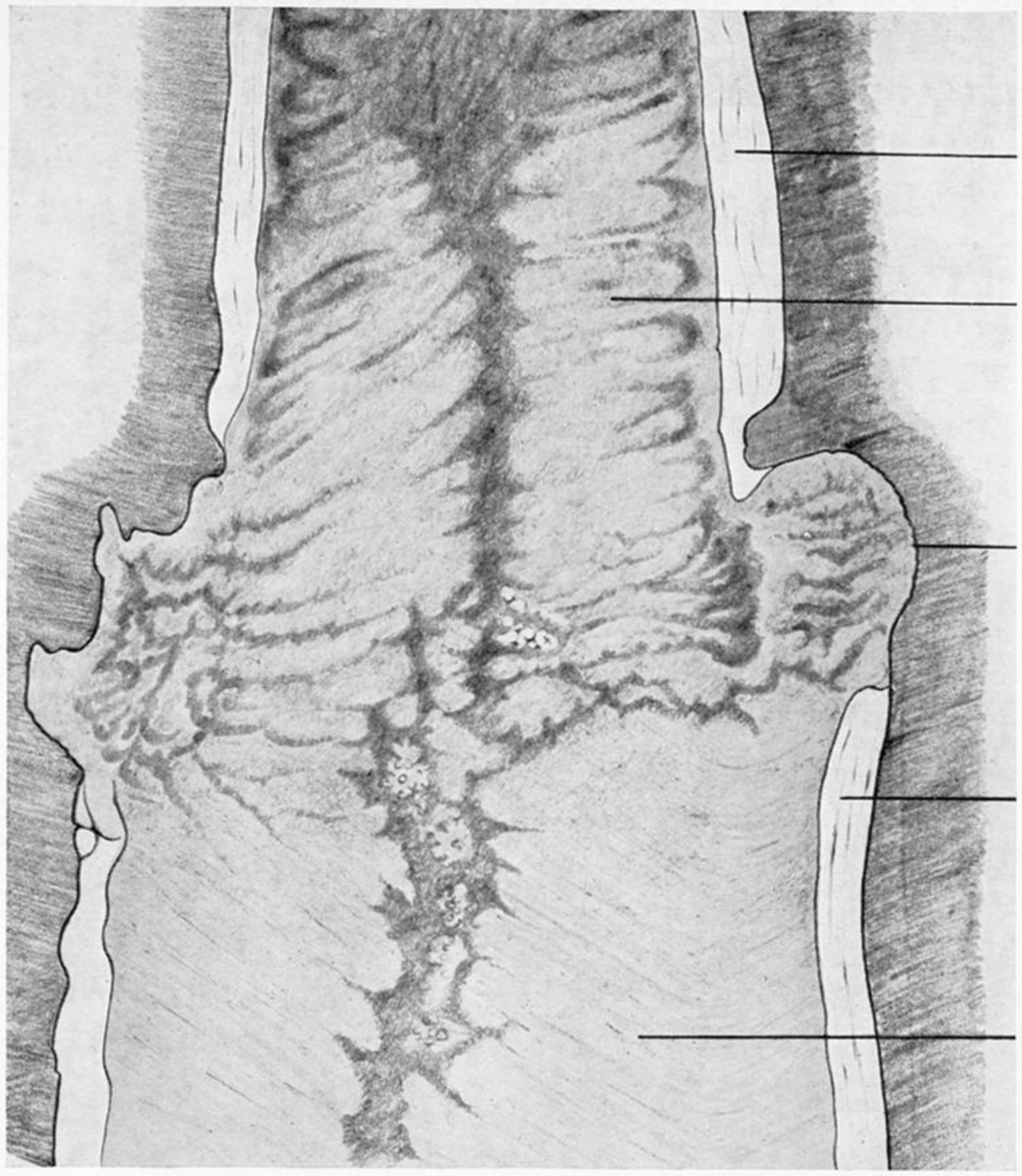

FIG. 12.—Cure of Scurvy, showing Enamel.

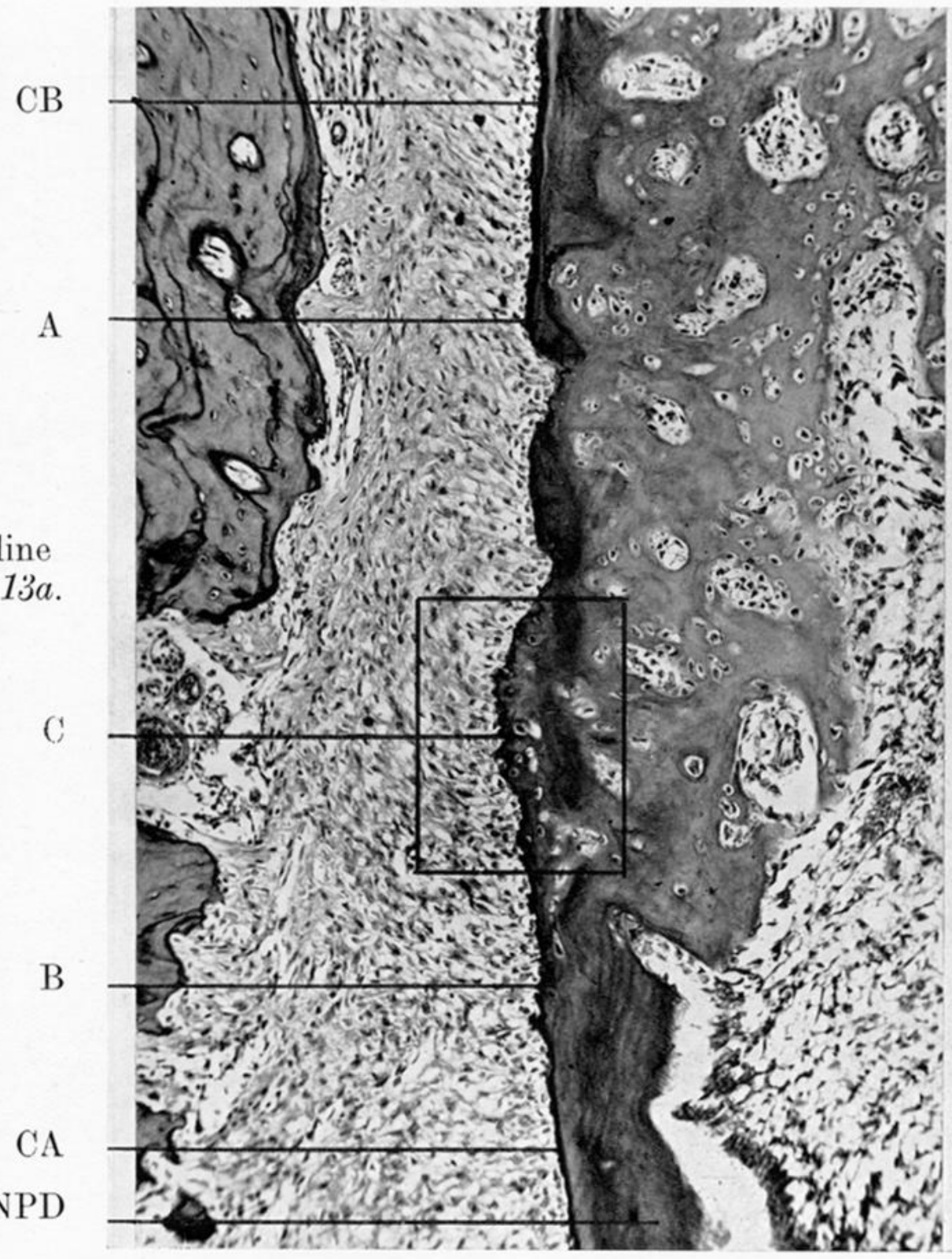

FIG. 13.—Cure of Scurvy, showing Cementum.

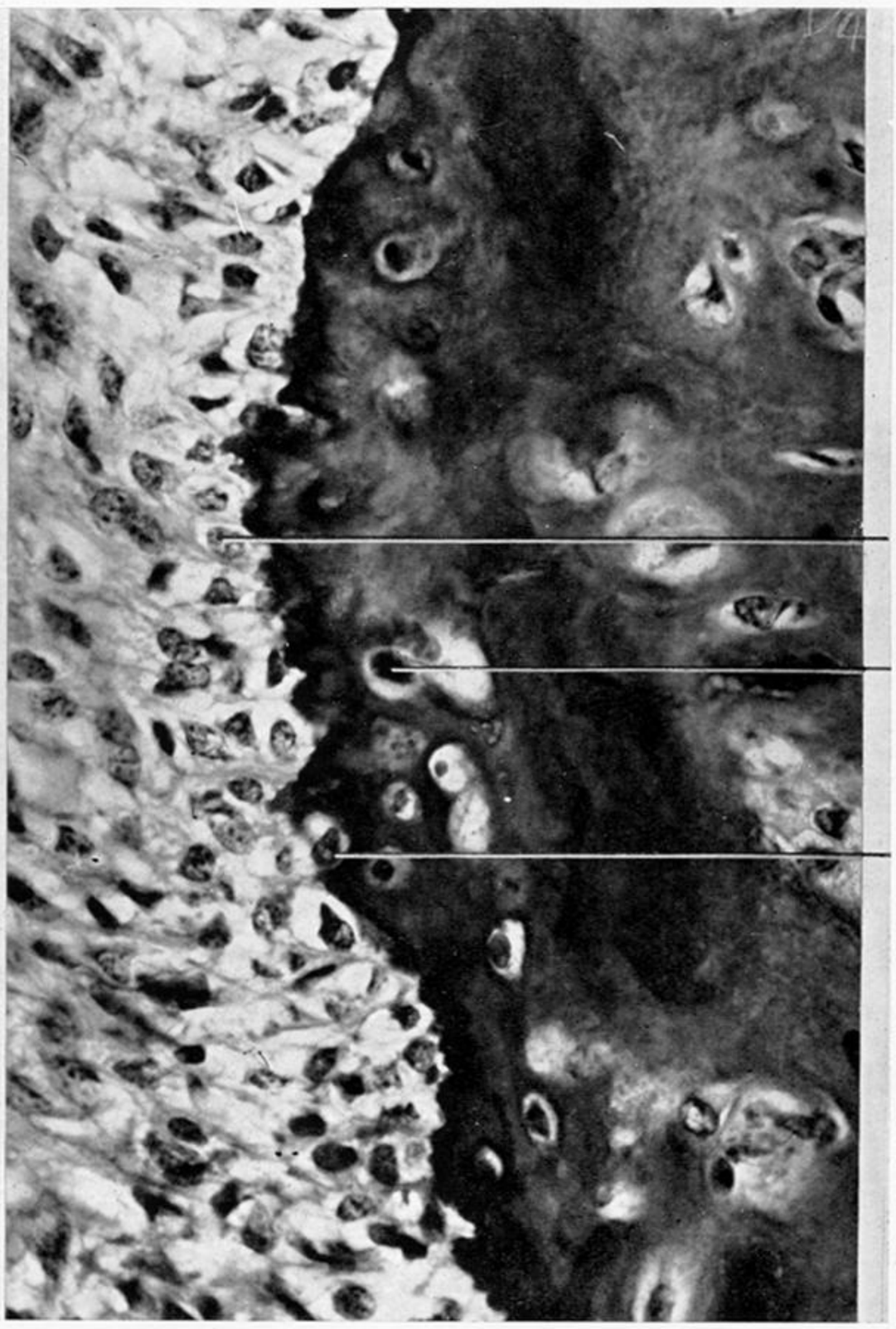

FIG. 13*a*.—Enlarged portion of fig. 13.