PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On the Nature of the Tubes in Marsupial Enamel, and Its Bearing upon Enamel Development

J. Howard Mummery

Phil. Trans. R. Soc. Lond. B 1914 **205**, 295-313
doi: 10.1098/rstb.1914.0017

VIII. *On the Nature of the Tubes in Marsupial Enamel, and its Bearing upon Enamel Development.*

By J. Howard Mummery.

Communicated by Prof. J. Symington, *F.R.S.*

(Received January 31,—Read March 26, 1914.)

Plates 25 and 26.

The existence of tubes in the enamel of marsupials was first demonstrated by Sir John Tomes (1) in 1849, and most of our knowledge of tubular enamels is due to the researches of this author and to those of his son, Mr. Charles Tomes.

With regard to the nature of the tubes in marsupial enamel, two very different views have been held. First, that of Mr. Charles Tomes, who considers that the tubes are canals in the centre of the prisms, calcification taking place centripetally, and leading in some cases to complete obliteration of the central channel as in human enamel, in others not reaching the centre and so leaving tubes within the prisms; and secondly, the view of Prof. von Ebner and Prof. Paul, who consider that the tubes lie, not within, but between the prisms.

In the first case they would have to be considered, as Mr. Tomes says, "as entirely a product of the enamel organ" (2) and cannot properly be called dentinal tubes. In the second case they must be looked upon as of dentinal origin and derived from the mesoblast cells.

In the course of my investigations into the process of calcification in dentine and enamel (3) I showed that dry developing enamel in the kangaroo (*Macropus rufus*) when teased out in glycerine and not subjected to any other reagent, separated into delicate laminæ, these consisting of layers of the prisms in great part already calcified, and of a delicate fibrillar substance between the laminæ which appears to be derived from the processes of the formative epithelial cells, the ameloblasts (Plate 25, fig. 1). I also showed that between the laminæ, large spherical bodies were to be seen, in all respects similar in appearance to the radial type of calcospherites formed in albumen in artificial experiments, these, from their granular appearance, from their radial splitting, and from the appearance of smaller bodies by which they are surrounded, evidently undergoing disintegration (Plate 25, fig. 3).

This process of disintegration can be studied in the artificial experiments, where the appearances are precisely similar. A section of enamel of Macropus cut in the microtome without decalcification shows a profile view of the laminæ and of the calcifying bodies between them (Plate 25, fig. 2).

These appearances will, I think, lead us to the unavoidable conclusion that the

 [Published separately, August 1, 1914

prisms are the first portions of the enamel which undergo calcification, that these prisms are arranged in layers corresponding to the rows of ameloblast cells, and that the spaces between the layers are calcified subsequently and cement the tissue together. At *a* in fig. 3, Plate 25, drawn from a photograph, the regular deposit of calcific bodies in the prisms is very clearly seen and has a very different aspect from the irregular deposit between them.

These conclusions are opposed to those of Mr. Charles Tomes, who considers that "the interstitial substance and the peripheries of the prisms are the first things to be formed" (2), but are in accord with those of Mr. Leon Williams, who considers, in his study of human enamel, that the prisms are first formed as a direct product of the ameloblasts, and the cement substance calcifies later (4).

While, however, in human and other higher mammalian enamels the calcification of the cement substance follows very closely upon that of the prisms, I think it is very evident that the calcification of the cement substance in marsupials is a much slower and more imperfectly completed process.

Several other appearances in marsupial enamel which I have met with in the course of this investigation appear to throw considerable light upon the question of the nature of the tubes, and, I venture to suggest, may provide a true solution of the problem.

The first of these which I will describe was met with in the lower incisor of an adult specimen of the diprotodont marsupial *Bettongia Lesueuiri*—one of the Kangaroo rats. The specimen had been preserved in formalin and treated in bulk with chloride of gold, reduced with alkalis by the Beckwith method to avoid decalcification, and a longitudinal section prepared by grinding.

The enamel in this section shows the very abundant passage of tubes from the dentine to the enamel and their characteristic bending, and it also shows, for about one-third of the length of the tooth, numerous bud-like bodies in the enamel which form the terminations of tubes passing from the dentine.

The contents of the dentinal tubes are granular, and the fibril is faintly stained red by the gold chloride; these granules and the red stain are also seen in the enamel tubes, into which the dentinal tubes are continued, and in the terminal bud-like bodies (Plate 25, fig. 7).

As it is quite evident that the tubes which terminate in these bud-like bodies are of the same nature as those which traverse the enamel in their neighbourhood, and also evident that they are continuous with dentinal tubes, it would appear that these bodies must be the termination of tubes derived from the dentine, for, if they were the terminations of enamel tubes derived from the enamel organ, the bud-shaped ends would be directed towards the dentine and not away from it.

Their contents being identical in appearance in the two tissues would also be an argument in favour of their dentinal derivation. Some of these bodies are found quite near the surface of the enamel.

In another specimen of the same species I found precisely similar bodies in the same situation, but, being from a dry preparation, there was, of course, no staining of the tube contents.

In order to see if the indications afforded by these specimens were confirmed by other appearances in the enamel of marsupials, I made preparations of further material by several different processes and prepared both longitudinal and transverse sections by grinding.

Molar and premolar teeth of Bettongia from the same formalin-preserved specimen were subjected to the silver nitrate impregnation method of Ramon y Cajal and passed through the Weil balsam process. Owing to the brittleness of specimens preserved in formalin when prepared by the balsam process, I could not cut any transverse sections of these preparations, but was able to obtain some small pieces in longitudinal section which were very instructive.

Both enamel and dentine were beautifully stained, the fine terminal branches of the dentinal tubes being very well shown, and those tubes which passed into the enamel were filled with granules, as in the dentine.

The substance of the enamel was stained uniformly a deep yellow, the yellow brown tubes of very varying diameter being still more deeply stained. Many of the tubes are seen to run through the whole width of the enamel to the surface and are of very wide diameter in this situation, while in several places fine branches stained like the main tubes pass across them at right angles (Plate 25, fig. 8). Some tubes project from the surface of the section. Especially noticeable in these silver-stained preparations are the granular contents of the tubes continuous with those in the dentine, the uniform staining of the enamel substance, and the great variation in the size of the penetrating tubes. A measurement of these showed that some of the largest were as much as 2 μ in diameter near the dentine, the width of a prism being from 3·5 to 4 μ. On the assumption that they were central canals in the prisms they would be very much wider than is, I think, conceivable on this hypothesis. Another appearance, that of the fine cross branches also seen in specimens prepared in other ways, would be very difficult of interpretation.

Lower incisors of kangaroo and wallaby from dry skulls of young specimens were treated with alcoholic fuchsin by the method of Dr. von Beust, which consists in injecting the tooth with the stain from the pulp cavity, or allowing it to be taken up by capillary attraction (5). I obtained the best results by the injection method, the needle of a hypodermic syringe being connected with the opened end of the tooth, the solution was forced in by firm pressure on the piston for some minutes, being afterwards maintained by the use of elastic bands for from 8 to 12 hours.

Longitudinal sections of these specimens, prepared by grinding, show that the stain has penetrated the dentinal tubes and entered those of the enamel, in many places passing across to the surface. In one preparation over a considerable area the enamel is more deeply stained than the dentine. In some places the tubes

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

are seen to be crossed by fine parallel lines (Plate 25, fig. 4) which, from comparison with developing marsupial enamel, would appear to be due to staining of the organic fibrillar matrix shown in Plate 26, fig. 5.

It appears evident from the manner in which these preparations have taken the stain, that the dentine through which it has penetrated to the enamel is in some way in connection with the interprismatic substance, and that the tubes which persist as such in the enamel run in this interprismatic substance. At the junction of the two tissues the stain in these injected preparations passes not only along the tubes of the dentine into the enamel tubes, but also escapes into the interprismatic substance.

The clearest evidence in support of the view that the tubes in marsupials are interprismatic is, I think, given by the transverse sections of enamel prepared by this method.

These transverse ground sections show the unstained, or in some cases, very faintly stained prisms, isolated from one another by the brilliantly stained interprismatic substance, and as shown (Plate 25, fig. 5) the cross sections of the tubes are distinctly visible within this substance and not in the area of the prisms. In some places they appear as refractile dots, in others they are deeply stained. Only very rarely does a tube appear to be within the prism, and in the two or three instances in which this appearance occurred, I found that on careful focussing the tube could be seen to wind into the intermediate stained material. Mr. Tomes, however, shows several transverse sections of tubes within the prisms (2, Plate 16, fig. 3), while v. Ebner shows a drawing of a transverse section of the enamel of Bettongia, in which the tubes are in the interprismatic substance (8, Plate 2, fig. 14).

Developing Marsupial Teeth.

A further corroboration of the observation that the tubes run between, and not within, the prisms is furnished by sections of fœtal material. Prof. Symington kindly provided me with some specimens of fœtal Macropus (species not indicated) in a very excellent state of preservation, from which I made a great many preparations.

The great shrinking of the enamel organ, and especially of the Tomes' processes of the ameloblasts, in decalcified marsupial material, and the dragging of the cells away from the forming enamel so commonly seen in decalcified sections, led me to attempt to cut sections without decalcification. This I was able to do in somewhat advanced as well as early tooth germs, in the freezing microtome, after embedding the specimens in sugar and gum. By using a sharp knife, frequently renewed, I procured many very thin sections in which the relations of all the parts, including the whole enamel organ on the one side, and the dentinal pulp on the other, are apparently quite undisturbed and give a different aspect in several particulars from the decalcified sections.

One fact which they demonstrate is that although, as in all mammalian tooth germs, calcification commences in the dentine, it is immediately followed by the laying down by the enamel organ of a very wide area of imperfectly calcified enamel. A comparison of fig. 6 in Plate 26 with the appearances of the developing teeth of man and those of the other orders of the mammalia which I have examined shows that the area occupied by the forming enamel is many times greater in Macropus. While, therefore, the process of development in all mammalian enamel is similar, in this, which we may look upon as a primitive form of the tissue, the enamel is laid down in a fibrillar organic matrix of very great extent, and calcification is a slower process and ultimately less complete than in higher enamels, as shown by its penetration by tubes and by the free staining of the abundant cement substance.

In some of these sections cut without decalcification, the material had previously been stained in bulk with borax carmine—the tubes in the enamel were strongly stained with the carmine and were clearly relieved against the more delicately stained enamel substance (Plate 25, fig. 6, *a*, *b*).

It will be noticed that the turns of the spiral are very unequal, in some places being very close and in others widely extended. These turns are frequently considerably wider than the enamel prisms and appear quite inconsistent with the view that the tubes are within the prisms. In several places they can be seen to project from the margin of the section (Plate 25, fig. 6, *t*) and their appearance in this situation is highly suggestive of a very considerable amount of rigidity.

I think the appearances in these sections are only consistent with the fact that they are winding around the prisms in the interprismatic substance.

The most marked peculiarity noticeable in marsupial tooth germs is, as pointed out by Mr. Charles Tomes, the great development of the Tomes' processes of the ameloblasts, and in the sections now under consideration they do not show the dragging out seen in decalcified sections, where they are often reduced in many places to a mere thread. In their undisturbed relations they are seen to bridge across a distinct interval between the lower ends of the ameloblasts (or the inner ameloblastic membrane) and the forming enamel. In these preparations, which are mounted in Farrant's solution, there seems to have been very little lateral shrinking of the Tomes' processes, which are in some places nearly as wide as the ameloblasts, but no doubt some shrinking has taken place and the intervals between them are probably wider than when in a natural condition. Appearances, however, suggest that in the living state there are normally spaces between these processes and that they are not in contact with one another. An interlacing of the fibres of the Tomes' processes appears to take place at the honeycomb region, this interlacing appearing to be not of the whole process, but of bundles of fibres into which it divides.

Mr. Tomes shows in the illustrations to his paper (2) that the fibres of the processes in his decalcified specimens, drawn out into fine strings, are attached to the margins of the honeycomb, but my sections (Plate 26, figs. 1, 3, 10, and 11) show that the

compact and broad Tomes' processes divide into two or three portions, each portion composed of many fibres or strings, which pass into the honeycomb, and appear to be crossed also by processes of contiguous cells, and to be re-arranged on the farther side of it. They also can be seen (Plate 26, fig. 12) to give off in many places very fine fibres crossing the main bundles at an angle. These do not remain incorporated with the Tomes' processes, but appear to form the fine fibrillar matrix of the enamel (apart from the fibres of the prisms) which is occasionally to be detected in young enamel (Plate 25, fig. 4, and Plate 26, fig. 5).

This interlacing of the constituent portions of the processes at the honeycomb suggests that possibly one ameloblast may not be responsible for the formation of the organic foundation of only one individual enamel prism, but that the strands of which the processes are made up, and which appear to be separated at the honeycomb, are there re-united and form the basis of an enamel prism which may be made up of fibres from more than one ameloblast cell.

This would give a possible explanation of the manner in which supplementary prisms, if such exist, might be formed in the wider part of the enamel of the crown, the combination of strands from contiguous cells forming the organic foundation of intermediate prisms.

Mr. Tomes describes the Tomes' processes as being drawn out of the centre of the honeycomb, and he concludes that the marginal part of the cell or division of the honeycomb is already calcified, but appearances lead me to the conclusion that the margins of the honeycomb divisions are not calcified, but consist of a resistant organic material of a similar nature to the ameloblastic membranes. Mr. Tomes also suggests that the margins of the honeycomb divisions are formed by a material of this nature, which later disappears, but he still considers that this material undergoes early calcification, and that the Tomes' fibres can be pulled out from these so formed cells which have calcified boundaries, calcification progressing inwards from this margin.

In two of the sections cut without decalcification the honeycomb has become detached, and lies between the ameloblasts and the portions of the separated enamel. It is made up of hexagonal cell-like divisions with narrow boundaries, and shows no evidence of calcification, and it has taken the hæmatoxylin stain. We see from this that it is not an artificial product raised from the surface of the calcifying enamel by an acid, as none was used, but a definite structure.

I think, for the reasons I have given above and the demonstration that the calcification of the interprismatic substance is subsequent to that of the prisms, that the functions of the honeycomb require a different explanation.

The Tomes' fibres, extending from the ameloblasts as broad prolongations of the fibrillar cell contents, as far as the honeycomb, here spread out into more open bundles of strands which appear to become attached to the margins of the honeycomb divisions. Here they apparently interlace and become again united into fibrillar bundles which

generally, but not always (see figs. 9 and 10, Plate 26), turn at an angle to their former course and extend all across the wide area of the forming enamel, to the dentine. The honeycomb would, according to these appearances, be a kind of directing membrane, serving, like the "heddle" or guiding frame of a loom, to control the orderly arrangement of the forming prisms and the spaces between them.

In later stages of calcification there is no trace of the honeycomb, so it would appear that when a large amount of calcified enamel is already formed there would be no necessity for any directing membrane, the formed tissue being a sufficient support and guide for the ameloblastic processes.

Mr. Tomes speaks of the Tomes' processes as being soft and extensile, so that when the ameloblasts are displaced they may be drawn out into thin threads, but is it not possible that this appearance may be due to the fact that the Tomes' processes are surrounded by a jelly-like colloidal material, and that the processes when not broken off are glued together by this adhesive substance, and while probably slightly extensile their fine thread-like appearance in disturbed sections is chiefly due to the action of this agglutinating material?

It is certainly evident that the colloid material in which calcification takes place and the Tomes' processes are not one and the same thing, as Mr. Leon Williams (4) would appear to suggest when he says: "An instructive picture with reference to the nature of the Tomes' processes is shown in fig. 36" (of his paper), which he describes as "showing that the Tomes' processes are formed by drawing out strings from the albumen-like substance of calcoglobulin." I scarcely think that Mr. Leon Williams could have intended to convey this meaning, as the processes in question certainly do not consist of this substance, but are, as Mr. Tomes (2, p. 110) says, portions of the cell produced from the cell body. If not pulled away they are seen, as the last named author describes, to consist of the same strings of fibrillar matter which compose the body of the ameloblast, and which are probably in the living condition equal in width, or nearly so, to the cell itself.

In my calcified sections the Tomes' fibres appear to be evidently stiffened by the calcific deposit which can be seen as minute spherical bodies in their substance, and if the section is torn apart they usually break across, and are only pulled out in a very slight degree.

Mr. Tomes (2, p. 112) has described the Tomes' processes as passing all across the enamel area from the ameloblasts to the dentine; this is still more evident in these calcified sections, the fibres holding together better than in the decalcified tissue.

In the specimens which have been stained with hæmatoxylin in bulk, these fibres are deeply stained all the way across, and the incorporation of the Tomes' processes, their bifurcation and interweaving, can be clearly seen (Plate 26, figs. 9 and 10). The prisms are evidently not fully calcified, and in cutting them they have been somewhat separated from one another, showing their fibrous nature very distinctly.

Near the dentine, however, the building up of the enamel prisms by small calcified bodies of uniform size, as described by Mr. Leon Williams, is very evident (Plate 26, fig. 7, and Plate 25, fig. 3).

In the part of the enamel near to the forming cells these regular bodies cannot be detected, and I think it can be shown that calcification of the prisms takes place through the coalescence of smaller bodies which, deeper in the forming enamel, are fused into these regular superimposed constituents of the formed enamel prism, the organic basis of the prism being first laid down by the interwoven fibres of the Tomes' processes, which have assumed the approximate width of an enamel prism.

The fibres prolonged from the Tomes' processes are not, however, the only fibres seen in forming enamel, but other more delicate fibres are seen to cross the prisms at an angle (Plate 25, fig. 4, *b*, and Plate 26, fig. 5).

With regard to the origin of these delicate fibres, I think they are as much the product of the enamel cell as are the directing fibres of the prisms, and in very thin sections they can be seen close to the growing margin of the enamel where the Tomes' processes enter it, and they appear to be continuous with these processes, being given off from them more or less at right angles to the prisms.

The organic basis substance of marsupial enamel can also be demonstrated by decalcifying dentine and enamel in acids. Sir John Tomes (1) subjected a section of a marsupial tooth to the action of acids under the microscope. As the decalcification progressed, he noticed threads projecting from the dentine and waving about in the fluid; these he looked upon as the dentinal fibrils projecting from the tubes.

I repeated this experiment with some modifications. A thin section of enamel and dentine of Macropus, showing a very abundant passage of tubes across the boundary line of the two tissues, was treated with strong hydrochloric acid for a few minutes only, then removed, washed, and stained with toluidin blue; the dentine was coloured a pale blue, and the superficially decalcified enamel was a violet colour. The section was placed in a drop of Farrant's solution upon the glass slide, in order to hold the parts together during the treatment. Strong hydrochloric acid was added gradually to the drop of Farrant's medium, and the process of decalcification watched with a low power of the microscope, no cover-glass being used. The decalcification took place rapidly, and the specimen was not disturbed by the formation of confined bubbles. When all the calcified enamel had disappeared the tubes were found to have retained their positions and relations to one another, having not only persisted, but where they exhibited the characteristic parallel bending seen in marsupial enamel, this was as distinctly shown as in the section previous to decalcification. The stain was not entirely removed by the acid, and the contour of the enamel could be traced upon the slide, a faintly violet-tinted ground substance being left, traversed by the tubes and apparently holding them in position (Plate 26, fig. 8).

Although with the low magnification employed the actual fibrillar structure could

not be detected, I think there is no doubt it is the same material seen in calcified sections (Plate 25, fig. 5), and in the laminæ of the enamel teased out in glycerine, as well as in the specimens shown in Plate 25, fig. 4.

The fibres in the enamel first described by Dr. Andrews (6) appear to be the fibres also described (2, p. 112) and figured (2, Plate 16, fig. 9) by Mr. Tomes, and also in the drawings and photographs in the present paper (Plate 26, figs. 2, 9, and 10).

These fibres may be considered to be the directing fibres of the enamel prisms, in which the calcific matter of the prisms is deposited.

The finer fibres which I have shown to exist in young marsupial enamel (Plate 26, figs. 5 and 12) cross these directing bundles at an angle, and I think evidently constitute a delicate fibrillar foundation in which the whole enamel is laid down.

Both Dr. Andrews and Mr. Tomes appear to refer to the directing fibres only and not to these delicate fibres which cross the prisms more or less at right angles and are not collected into bundles.

The directing bundles are the direct prolongations of the Tomes' processes of the ameloblast cells, the finer fibres I am referring to are also derived from the ameloblast processes (Plate 26, fig. 12) and pass out from them more or less horizontally at the honeycomb region.

It would thus appear that the whole of the enamel in marsupials, prisms and interprismatic substance, is laid down in a delicate fibrillar organic matrix derived from the epithelial cells, just as the dentine in the higher mammalia is laid down in a similar fibrillar matrix derived from the connective tissue cells of the pulp.

As shown (in Plate 26, fig. 3) the clear bodies so often described by many observers are seen in the ameloblasts; these bodies are not calcified, but appear to consist of a colloidal material separated by the cell. Small granules and larger refractile bodies are also seen in the strands of the Tomes' processes of the ameloblasts; at the honeycomb region a more or less regular row of small refractile bodies is frequently seen, and deeper in the forming enamel the larger bodies which show a distinct reaction to polarised light (Plate 26, fig. 7).

It would appear that these small particles in the Tomes' processes coalesce beyond the honeycomb region and form the regular calcified bodies which make up the prisms, previously referred to, the large spherites being engaged in the slower process of calcification in the interprismatic substance.

Mr. Leon Williams (4) was of opinion that the calcifying process is the same both in the cement substance and the enamel prisms, or rods as he prefers to call them, the only difference being, he considered, that the rods have a substructure of organic matter. In marsupial enamel this difference certainly does not exist, there is a substructure to both rods and interprismatic substance, and while, as he says "the calcific matter forming the rod is one and the same thing with that forming the cement substance," the actual mode of deposition of this calcific matter is

somewhat different, as, in the case of the prisms, it would appear that the regular calcified bodies of which they are built up are formed by the coalescence of the small particles in the Tomes' processes and their fibrillar prolongations, and the calcification of the interprismatic substance is finally due to the disintegration and subsequent coalescence of the large bodies seen in this situation.

In considering the probable nature of the tubes in marsupial enamel the most important part of the sections to be examined is at the line of junction of the dentine and enamel. It is here that we must look for any indications of penetration of the enamel by dentinal fibrils at the commencement of calcification, and it is here we must examine carefully the deposit of the lime salts in the interprismatic substance. It appears that exactly at this junction line there is less calcification apparent than further back in the newly formed enamel: an anomaly not easy to explain, but which is of great importance to us in the present investigation.

This delayed interprismatic calcification at the dentine-enamel junction may, I think, explain to a great extent the appearances in this situation in the formed enamel of Macropus.

This area, although the first laid down, is very slowly and imperfectly calcified, and, like the interglobular areas in dentine, the imperfectly calcified part becomes surrounded eventually by more completely finished tissue. The calcification here appears to be not only delayed, but imperfect.

I do not therefore consider that the large spaces seen just within the enamel in the adult tooth are due to intrusions of dentine matrix, as held by Dr. Paul (7), but entirely to imperfectly calcified enamel matrix.

Fig. 4, Plate 26, from a photograph of a section of a developing tooth in which calcification had only just commenced, shows the newly calcified dentine at *d*, and at *c* the junction with the developing enamel, the organic foundation of which has already attained a considerable thickness. It is seen that the calcification of the enamel at the dentine line is very incomplete, there is a space in which globules of calcific matter are seen, and the narrow calcifying prisms adjoining the space are widely separated from one another.

In the calcified dentine at *d* the dentinal tubes are very fully formed and are seen to be of the same diameter at the enamel margin as at their commencement, appearing to abut abruptly on this uncalcified junction line.

In all the sections showing this stage of development the dentinal tubes have the same appearance. I am unable, therefore, to agree with Mr. Tomes when he says "the first-formed layer of dentine contains only the fine terminations of the dentinal tubes" (2).

In the adult enamel of marsupials the fine terminations are seen just short of the junction line (Plate 25, fig. 4), and stouter tubes pass across the boundary into the enamel. These stouter tubes are the only ones that are seen in these sections of developing teeth, although, no doubt, by the use of special stains the others would be

brought into view. We know also that in human dentine many tubes terminate at the enamel margin in broad ends, so much so that it suggested to Prof. von Ebner, Prof. Walkhoff, and others, that there must have been a resorption of the first deposited dentine.

In Plate 26, fig. 6, it is also seen with a low magnifying power that the calcification of the enamel is incomplete all along the junction line, as in fig. 4, and I think this fact has a strong bearing upon the question of the nature of the tubes in the enamel. The large scattered calcifying globules are seen in this position (Plate 26, fig. 7), although the calcification of the prisms, as evidenced by the regular rows of deposit in their substance, is very evident. This line of isolated globular bodies at the dentine margin is seen in many sections.

As the enamel at its junction with the dentine consists almost entirely of the organic matrix with only a very incomplete deposit of calcific matter, and as there are beyond this region very wide intervals between the calcifying enamel prisms, there is probably very little resistance to the ingrowth and penetration of the enamel by the dentinal fibril. The dentinal tubes where they meet the enamel are seen to be well defined (Plate 26, fig. 4) and are not branched. In one or two instances, by carefully focussing with high powers, I have been able to trace a few tubes across the boundary line into direct contact with the ameloblasts before any calcification has taken place in the enamel.

If, at the first formation of the dentine, the dentinal fibril passes into the organic substance of the enamel, and a connection is thus established between the cells of the dentine and the enamel matrix, it can be easily understood that the fibril would continue to penetrate this slightly resisting substance, until eventually closed round by calcification.

If these open spaces at the junction of the two tissues were due to intrusions of dentine matrix, we should expect to find them in great abundance in the first-formed enamel of Macropus, as they are so numerous in this situation in the finished tissue.

In developing teeth, however, they cannot, according to my own observations, be seen, but there is an area of uncalcified material of irregular form in contact with the dentine (Plate 26, figs. 4 and 6).

As calcification gradually proceeds at this margin, this area becomes divided into distinct circumscribed spaces, which surround the entering dentinal tubes.

The calcification does not quite reach the tube, and surrounds a hollow space in the matrix, into which the stain injected from the pulp cavity penetrates (Plate 25, fig. 4).

It is common in human teeth to find spaces in the enamel at the dentine junction, especially at the summit of the cusps of the dentine; these spaces communicate with the dentinal tubes. These appearances have been much discussed, and various opinions expressed as to their origin; it is denied by some observers that they communicate with the dentinal tubes, but it is difficult to understand how this could be doubted, and the injection method of von Beust demonstrates it as a fact.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Along with these spindles or spaces, narrow tubes in the enamel prolonged from those in the dentine are not very uncommonly found. Plate 25, fig. 11, shows both spindles and tubes in a human deciduous molar, in which the soft parts had been fixed, and the tooth injected with fuchsin and a ground longitudinal section prepared.

A comparison of this figure with fig. 10, adjoining it, which is of a premolar of the marsupial, *Phalanger orientalis*, shows a very similar condition in this situation. The enamel of Phalanger is not nearly so rich in tubes as that of many marsupials, and the mode of reduction of the tube system is very instructive, and may, I think, throw considerable light upon this question. Many tubes are seen to pass across these spindle-shaped spaces in Phalanger and emerge on the other side, passing deeply into the enamel, but I think the difference between the condition in Phalanger and in human teeth is only one of degree.

In both these specimens spaces are arranged around the dentine cusps, and, in both, tubes pass across these spaces into the enamel, but in the human tooth not so deeply. The large irregular spaces are also carried more deeply into the enamel in the marsupial, but I think it is quite evident they are similar in general form and arrangement, and it certainly suggests that they have been formed in the same way. Many of these spaces in both cases are seen to commence some little way in the enamel, which would point to the probability of their being enamel products rather than intrusions of dentine matrix.

In Plate 25, fig. 12, are also shown some of these spaces and tubes in human enamel from a temporary tooth injected with fuchsin. At *e* it will be seen that two air bubbles have been driven into one of these spaces by the syringe, showing very evidently its free communication with the tubes. In fig. 11 a very large space is seen which has not taken the stain, which I should consider to be a portion of uncalcified enamel matrix substance, cut off from the dentine by calcification. In fig. 12 tubes are seen passing along the margins of the spaces, and the interprismatic substance has taken the stain.

In many sections of human enamel I have succeeded in obtaining considerable penetration of the stain, as did Dr. von Beust, who kindly informed me of his method, and in several specimens the stain seemed to have passed more deeply into certain thin laminæ which had been in part ground away, suggesting that these are the remains of the delicate organic layers seen in the laminæ of the developing teeth teased out in glycerine previously described (Plate 25, fig. 1), and which eventually disappear when the enamel is completely calcified, for, as I have pointed out in my paper on calcification, this tendency to break up into laminæ is also shown in developing human enamel.

Mr. Tomes' observation that in many preparations the nucleus of the ameloblast has a crescentic shape, the horns of the crescent being directed towards the dentine, is fully corroborated by many of my sections, which show, I think, very distinctly that

fibres from the horns of the crescent actually do pass along the cell towards its free end, as he was inclined to think (Plate 26, fig. 3*a*.)

This appearance of the nucleus is more common where calcification is well advanced. In the early stages the oval nuclei are arranged with fair regularity at the end of the cell farthest from the dentine, but the crescentic nuclei are seen often at varying intervals in the length of the cell. It seems possible that all these delicate fibrillar strands in the ameloblasts may arise from the nuclei, but this is a point which it is very difficult to determine.

There is another appearance very common in developing enamel (Plate 26, fig. 2), that of a more or less clear outer layer of the enamel in which the prisms cannot be detected. It is also seen in finished enamel in many cases. The manner in which the fibres of the forming enamel are connected with it would suggest that it might possibly be produced by the after calcification of the substance between the Tomes' processes in the interval between the ameloblasts and the honeycomb.

Summary and Conclusions.

The points which I have endeavoured to bring forward in support of my view of the nature of the tubes in marsupial enamel are chiefly the following :—

The evidence that calcification takes place in the prisms previously to the calcification of the cement substance, already more or less completely calcified prisms being arranged in laminæ and lying in a distinct fibrillar organic matrix substance which can, in some cases, be seen in calcified enamel.

The fact that spherical calcified bodies are found upon these laminæ where they can be seen to be undergoing disintegration.

The presence in Bettongia of terminal bulb-shaped bodies, which form the terminations of dentinal tubes deep in the enamel, and the presence in the enamel tubes of the same stain and granular particles seen in the tubes in the dentine, in specimens in which the soft parts have been fixed.

The free injection of the enamel tubes with alcoholic fuchsin from the pulp cavity, the stain passing not only into the tubes, but also into portions of the matrix substance which have escaped calcification.

The great variations in size of the tubes in the enamel.

The evidence given by ground transverse sections of enamel stained by the fuchsin method, where cross sections of the tubes are seen within the stained interprismatic substance.

The demonstration in developing teeth of spiral tubes stained more deeply than the surrounding enamel, winding round the prisms, the turns of the spirals being much too wide to enable them to be interpreted as running within the prisms; and the fact that the spiral character of the tubes completely distinguishes them from the columns of prisms, which in their alterations of course are not so abrupt and do not follow the same directions.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The appearance of the Tomes' processes at the margin of the forming enamel in calcified sections, where they are seen to interlace with one another and to be stiffened in many places with calcific deposit which appears to take place regularly along their constituent fibres.

The indications that the honeycomb does not become calcified and that its function is more that of a directing membrane.

The delayed and imperfect calcification at the enamel-dentine junction; the existence of large tubes of the newly formed dentine which reach this junction line, and the slight resistance to the penetration of the fibril which would be here presented.

The evidence that the expansions around the tubes are due to final closing up of the calcification around areas of the enamel matrix in this situation, which remain uncalcified; supported by the fact that no circumscribed spaces such as exist in adult marsupial enamel are seen in this position in developing teeth.

The effects of rapid decalcification, the tubes being left in perfect continuity with the dentine and retaining their position in the matrix from which all the lime salts have been removed.

A comparison of human, partially tubular, enamel with the enamel of *Phalanger orientalis*, in which the tube system is greatly reduced.

These evidences lead me to conclude that the essential difference between marsupial and higher mammalian enamel lies in the imperfect calcification of the cement substance in the former; that this imperfect calcification allows the penetration of the dentinal fibril from the previously formed dentine; the fibril, meeting with but little resistance, passes into the enamel matrix until finally closed round by calcification, the degree in which this closing in of the fibril takes place determining the differences in the amount of penetration by tubes seen in marsupial and other mammalian enamels. The fibril appears to be surrounded in the enamel by a distinct tube wall, and its persistence after the enamel has been subjected to the action of strong acids suggests that the sheath of Neumann is also present. We should, I think, also expect that its penetration would be followed by a certain amount of peripheral calcification due to the lime-conveying fibril.

Mr. Tomes also refers to the resistance of the isolated fibres to the action of acids, comparing them in this respect to the sheath of Neumann in the dentine, but he does not consider them to be the sheaths of Neumann, as, according to the view which he holds, the dentinal tubes do not pass into the enamel.

Thus I should consider there is not an ingrowth of dentine matrix, but an ingrowth of the protoplasmic processes of the odontoblast cells. It could not occur in the presence of an early and densely calcified enamel matrix, but is not prevented by the imperfectly calcified material found in marsupials. It is not then the calcification of a mesoblastic tissue by an epiblastic one, but an ingrowth of the former into the latter, calcification taking place around it.

It will be seen that my explanation of the nature of the tubes in marsupial enamel

does not coincide with that of Mr. Charles Tomes, and I can find no evidence to support the explanation given by Prof. Paul that the tubes in marsupial enamel are due to intrusions of dentine matrix, carrying the dentinal tubes with them.

I think with Sir John Tomes, Prof. Kölliker, and Prof. von Ebner that the dentinal tubes actually pass into the enamel, and have endeavoured in the foregoing paper to explain the process by which this penetration takes place. I believe it to be due to the actual ingrowth of the dentinal fibril, which is rendered possible by the very imperfect condition of calcification at the dentine-enamel junction.

Prof. von Ebner examined the teeth from dried skulls of Macropus, Hypsiprymnus, and Petaurus, but had no opportunity of examining fœtal marsupial material.

My own conclusions have been based on the examination of fœtal marsupial material, and no previous systematic histological investigation of the developing teeth of marsupials has, I think, been described, except that of Mr. Charles Tomes.

From the investigation of dry material, Prof. von Ebner concluded that the dentinal tubes penetrate the enamel and lie between the prisms, but in most mammalian enamels the tubes in the dentine and those in the enamel are independent of one another. He compares the tubes in marsupial enamel with the isolated tubes seen in the enamel of some rodents, and with similar tubes found at the neck of the tooth in the marsupial, Petaurus.

To account for these isolated tubes he suggests that a resorption of the first-formed dentine takes place, cutting off the dentinal tubes from their connection with the enamel, this resorption taking place either through the agency of the ameloblasts or of the first-formed layer of enamel.

According to my view, that the gradual obliteration of tubes in the enamel takes place through the more complete calcification of the cement substance, the condition in Rodents and at the neck of the tooth in Petaurus would be explained differently. I should consider that these isolated tubes had been, in the first stages of development, tubes communicating with the dentine, and that the slowly proceeding calcification at the enamel-dentine junction had advanced in these animals to complete obliteration of the enamel tubes near the dentine, portions of them remaining as isolated tubes in certain parts of the enamel.

This would not be comprehensible on the view that the calcification of the enamel at the dentine junction was completed before the next layer of enamel was calcified—but as I have shown (Plate 26, figs. 4, 6, 7) the process at this margin is a slow and delayed one in marsupials and in those with abundant enamel tubes is never complete.

If, in certain cases this delayed calcification becomes more complete, portions of tubes might become isolated, as in Petaurus and some rodents. Isolated tubes in the enamel are, however, rare in mammalia, the calcification either closing in on the tubes and narrowing them, or proceeding to their complete obliteration.

The appearances in human teeth, where the dentinal tubes are often seen to be truncated at the enamel junction, have been explained by Prof. von Ebner and other

dental histologists as due also to the resorption of the earlier formed dentine. I am inclined, however, to look upon it as due to the very complete condition of calcification of the cement substance in human teeth, and the closing in by calcific deposit of what, in the early stage of development of the tooth were more or less deeply penetrating dentinal tubes.

The primitive mammalian enamel I should look upon as a tubular enamel due to the ingrowth of the dentinal fibril and all modified tubular mammalian enamels as stages in the gradual obliteration of the tubes by more complete calcification of the cement substance.

The following material was examined in this investigation :—

Teeth from the dry skull of a young specimen of *Macropus rufus*, in which the two last molar teeth were unerupted—the dentine and enamel cap of the last molar being in an early stage of development and the tooth within the crypt.

A dry lower incisor of the genus Macropus.

The teeth from a dry skull of a young specimen of Halmaturus.

Teeth from the dry skull of Dasyurus.

Teeth from a dry skull of *Bettongia Lesueuiri* (Hypsiprymninæ), and others from a fresh specimen of the same animal, preserved in formalin.

Teeth from the dry lower jaw of *Phalanger orientalis.*

Three specimens of fœtal Macropus preserved in Müller's fluid and transferred to spirit. In the youngest of these, calcification had not commenced.

BIBLIOGRAPHY.

1. John Tomes, ' Phil. Trans.,' vol. 50 (1849).
2. Charles S. Tomes, " On the Development of Marsupial and other Tubular Enamels," ' Phil. Trans.,' B, vol. 189 (1897).
3. J. H. Mummery, " On the Process of Calcification in Enamel and Dentine," ' Phil. Trans.,' B, vol. 205, p. 100 (1914).
4. Leon Williams, " On the Formation and Structure of Dental Enamel," ' Dental Cosmos ' (1896).
5. T. von Beust, " A Contribution to the Study of Immunity to Dental Caries," ' Dental Cosmos ' (June, 1912).
6. R. R. Andrews, " The Development of Enamel," ' Trans. World's Columbian Dental Congress,' vol. 1, p. 83.
7. Prof. Paul, " The Enamel Organ," ' Dental Record,' vol. 16, p. 493 (1896).
8. von Ebner, " Strittige Fragen über den Bau des Zahnschmelzes," ' Sitzungsberichte der Kaiserlichen Akademie-Wien,' vol. 99, Abtheil. III (1890).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

DESCRIPTION OF PLATES.

Lettering applicable to all the figures.

a. Ameloblasts.
e. Enamel.
d. Dentine.
c. Calcific deposit.
t. Tubes in enamel.
p. Tomes' processes.
s. Stratum intermedium.
m. Ameloblastic membrane.
o. Odontoblasts.
n. Odontogenetic zone.
pp. Dental pulp.
r. Stellate reticulum.
h. Honeycomb region.

PLATE 25.

Fig. 1.—A fragment of newly formed enamel from the enamel cap of an unerupted molar tooth of *Macropus rufus*, removed from the crypt and teased with needles in glycerine. Showing division into laminæ and globular deposit of lime salts. From a photograph. × 75.

Fig. 2.—A section of a tooth of Macropus. The laminæ formed by the layers of prisms are seen edgewise and the calcifying bodies between them. Drawn from a photograph. Calcified cut section. × 750.

Fig. 3.—The large calcospherites on the laminæ. From a similar specimen to fig. 1, more highly magnified. The small regular calcified bodies forming the prisms are clearly seen in the upper part of the drawing. Drawn from a photograph. × 1000.

Fig. 4.—From a lower incisor of Halmaturus, injected with alcoholic fuchsin (method of von Beust). A fine cross striation is seen in the dilatations at the dentine margin coloured with the stain. Ground section. × 130.

Fig. 4B.—Cross striation of a tube in the enamel, being a portion of the organic basis of the enamel which has remained uncalcified. Drawing. Ground section. × 500.

Fig. 5.—*a*, *b*, *c*. Transverse sections of enamel of *Macropus rufus*, stained as fig. 4, showing colouring of the interprismatic substance and transverse sections of the tubes lying between the prisms in the interprismatic substance. Drawn from photographs. Ground section. × 800.

Fig. 6.—A section of enamel of fœtal Macropus. Calcification well advanced—cut without previous decalcification after staining in bulk with carmine. The tubes are more deeply stained than the prismatic substance—showing turns of the spirals wider than the prisms. Fig. 6 (*b*) shows a tube projecting from the surface of the section. × 500.

Fig. 7.—Lower incisor of *Bettongia Lesueuiri*. Bulb-shaped bodies in the enamel forming the terminations of dentinal tubes—soft parts fixed in formalin. Stained with Beckwith's gold chloride. Ground section. Drawing. × 300.

Fig. 8.—Enamel of Bettongia, stained by Cajal's silver nitrate process, formalin-fixed preparation; granules in the tubes continuous with those in the dentine and showing delicate cross branches. Ground section. Drawing. ×500.

Fig. 9.—Junction of enamel and dentine in lower incisor of *Phalanger orientalis*, showing terminations of tubes from the dentine in spindle-like bodies and fine tubes passing into the enamel. Dry specimen. Ground section. Drawing. ×130.

Fig. 10.—Longitudinal section at apex of dentine cusp in premolar of *Phalanger orientalis*, showing spindle-shaped spaces and some tubes. Dry specimen. Ground section. ×130.

Fig. 11.—Human deciduous molar, soft parts preserved. Ground section in same situation as in fig. 10. Stained by the fuchsin injection method. For comparison with fig. 10. Drawing. ×130.

Fig. 12.—Separate spindles from fuchsin-injected human teeth—soft parts retained. Two small air bubbles have been driven in by the syringe—tubes are seen traversing the spindle-shaped bodies. Drawing. Ground section. ×500.

Plate 26.

Fig. 1.—The Tomes' processes at the border of the forming enamel in Macropus. Section cut without decalcification. Showing the spreading of the Tomes' processes at the honeycomb region and their incorporation with the forming enamel. Drawn from a photograph. ×1000.

Fig. 2.—Shows the fibrous nature of the forming enamel in Macropus and the clear outer layer. Calcification considerably advanced. Drawing. Section cut without decalcification. ×130.

Fig. 3.—The enamel organ and developing enamel of a tooth of Macropus cut in the microtome without previous decalcification. Delicate fibres are seen to spring from the nuclei of the ameloblasts. Drawing. Stained hæmatoxylin. ×800.

Fig. 4.—A developing tooth of Macropus, showing the thin layer of calcified dentine crossed by the dentinal tubes, the commencing formation of the enamel prisms with wide spaces between them, and the interval between the dentine and enamel showing the calcific deposit. Section not decalcified. Stain, Van Giesen. Drawn from a photograph. ×800.

Fig. 5.—The fibrillar organic matrix substance seen crossing the enamel prisms in young enamel of Macropus. Photograph. Section, not decalcified. ×500.

Fig. 6.—From a developing tooth of Macropus, showing the large amount of enamel laid down at this early stage, and the very imperfect calcification at the dentine margin. Section, not decalcified. Photograph. ×75.

Phil. Trans. B. Vol 205 Pl. 25

Fig 1.

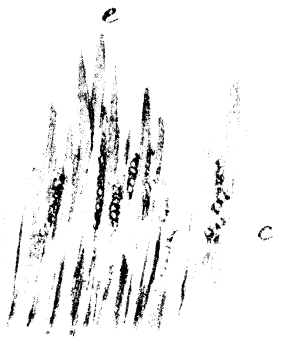


Fig 2.

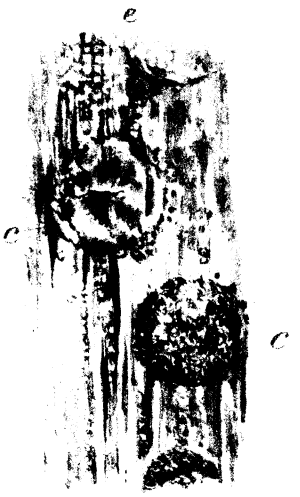


Fig 3.

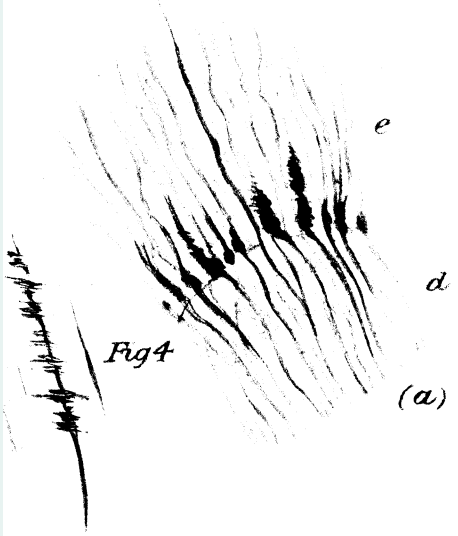


Fig 4

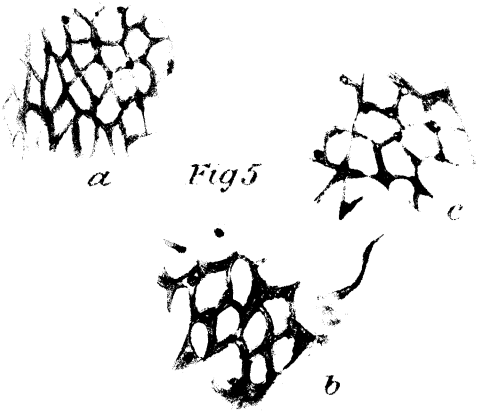


Fig 5.

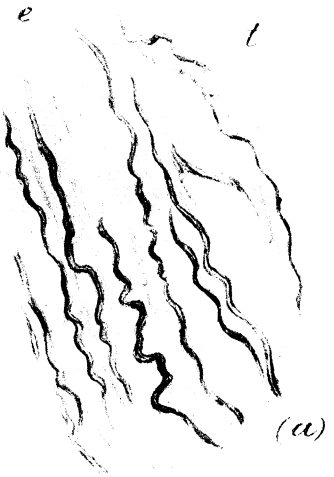


Fig 6.

(b)

Fig 7.

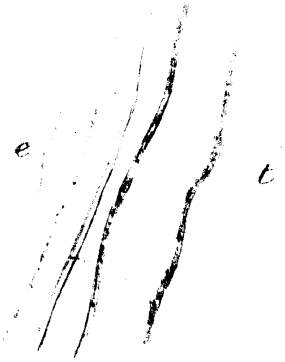


Fig 8.

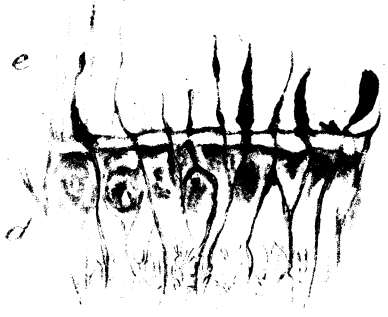


Fig 9.

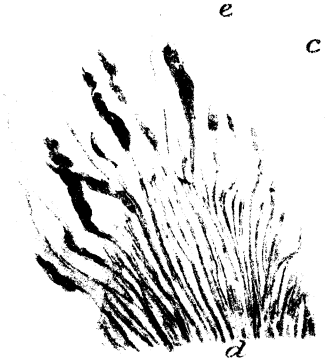


Fig 10.

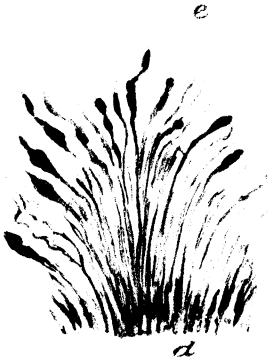


Fig 11.

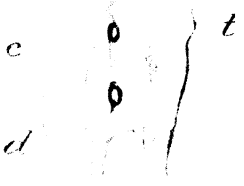
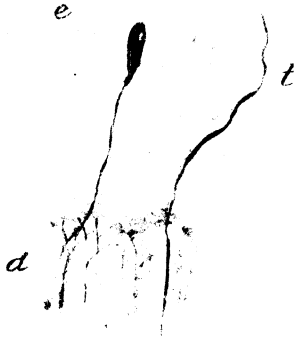


Fig 12.



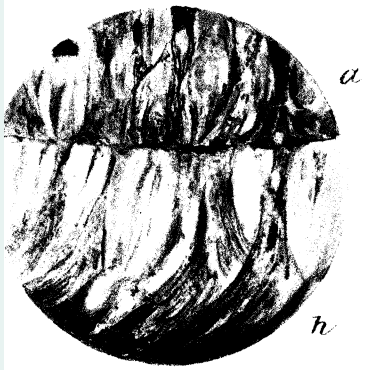


Fig. 1

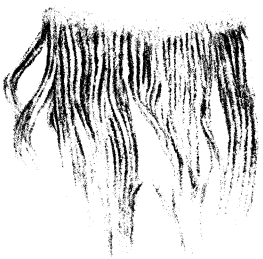


Fig. 2

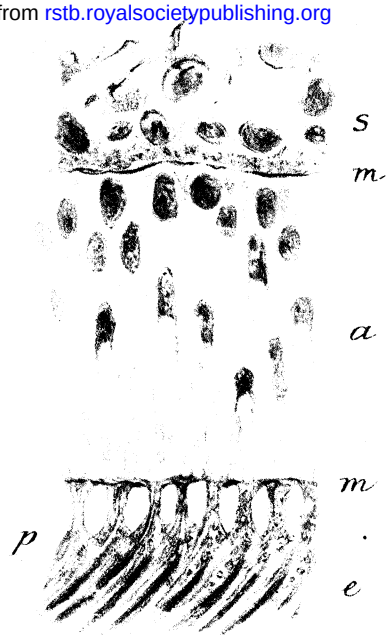


Fig. 3

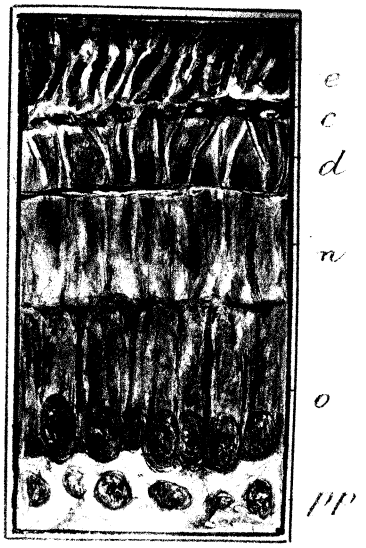


Fig. 4

Fig. 5

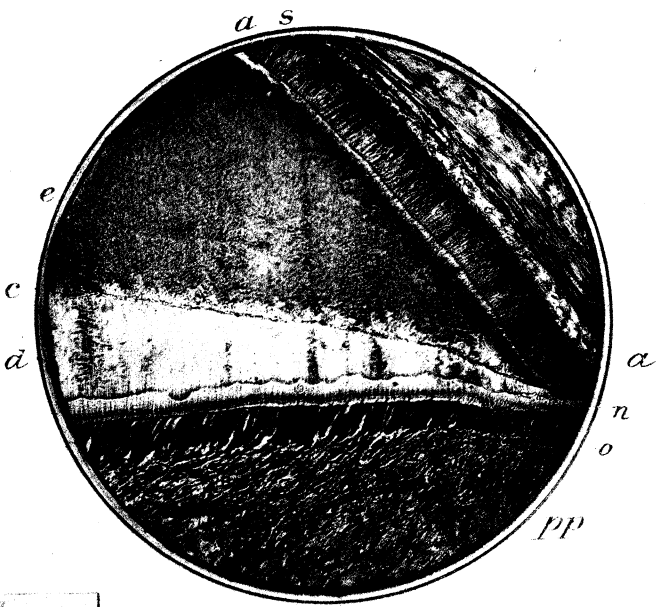


Fig. 6

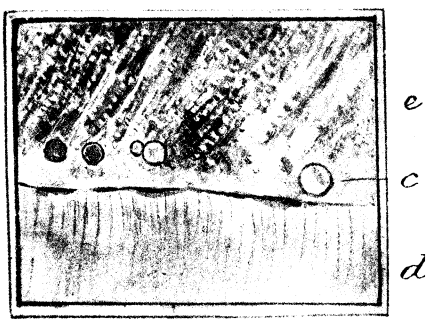


Fig. 7

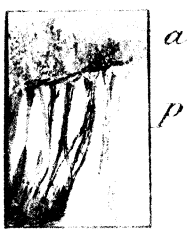


Fig. 11

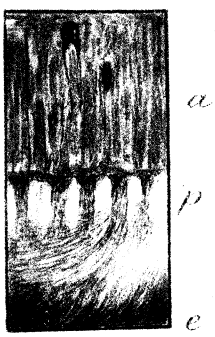


Fig. 12

Fig. 8

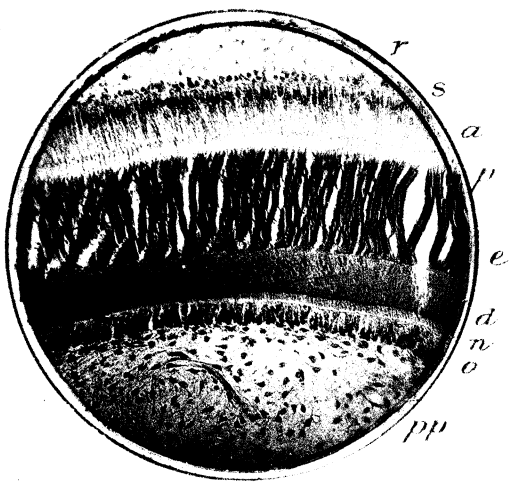


Fig. 9

Fig. 10

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fig. 7.—A similarly prepared section, showing the calcospherites at the dentine margin and the calcific deposit in the prisms. Stain, Van Giesen. Drawing from a photograph. ×500.

Fig. 8.—The decalcification experiment described in the text.

Fig. 9.—A section of a tooth of Macropus which had not been decalcified. Showing calcified dentine and the fibrous nature of the partially calcified enamel. A photograph. ×130.

Fig. 10.—Part of fig. 9 more highly magnified. A photograph. ×800.

Fig. 11.—Showing dividing of the Tomes' processes and inter-crossing in the honeycomb region. Drawing from a photograph. ×800.

Fig. 12.—The fine fibres of the Tomes' processes which can be seen in very thin sections to cross the coarser fibres at an angle. Drawing. ×600.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

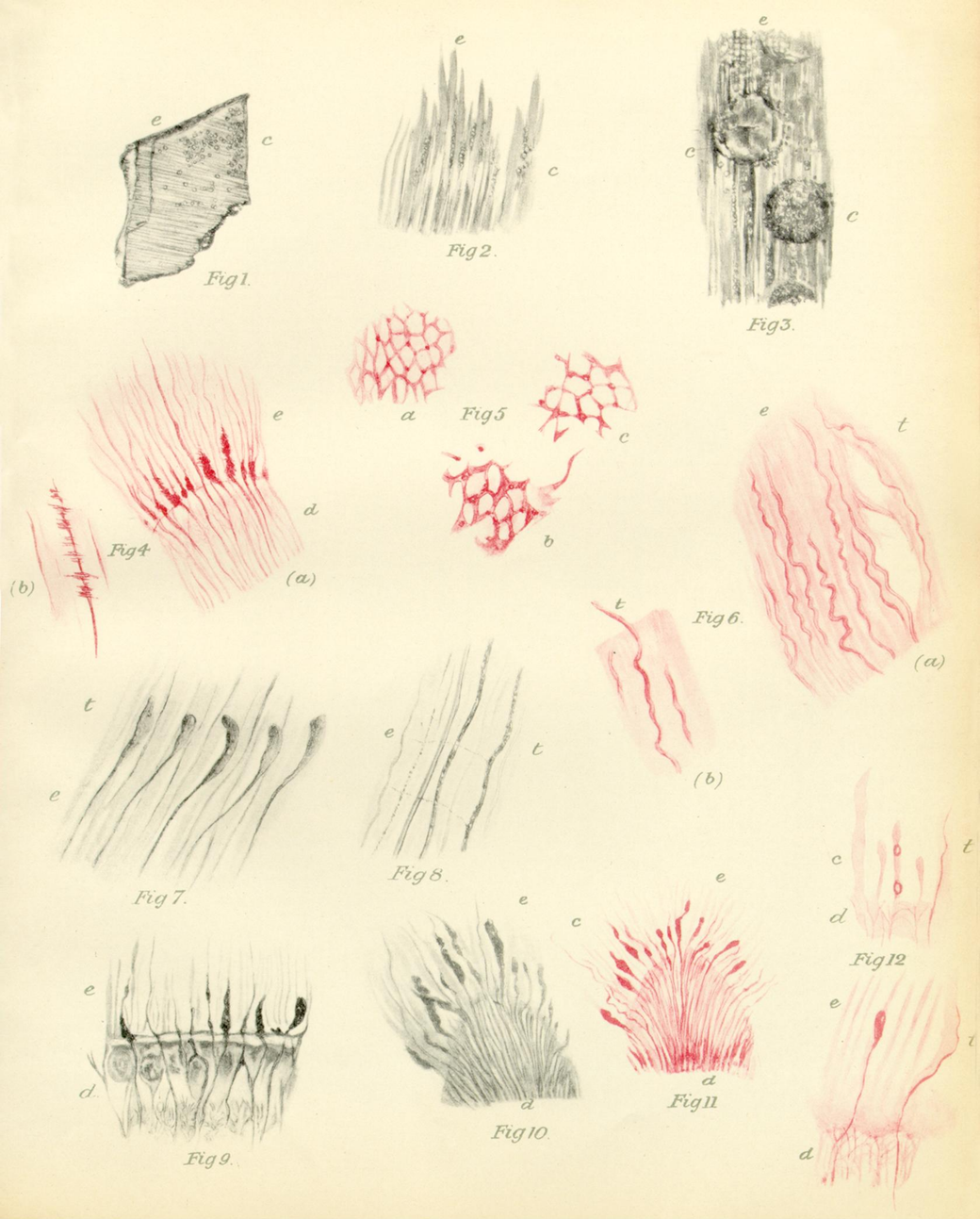


PLATE 25.

Fig. 1.—A fragment of newly formed enamel from the enamel cap of an unerupted molar tooth of *Macropus rufus*, removed from the crypt and teased with needles in glycerine. Showing division into laminæ and globular deposit of lime salts. From a photograph. × 75.

Fig. 2.—A section of a tooth of Macropus. The laminæ formed by the layers of prisms are seen edgewise and the calcifying bodies between them. Drawn from a photograph. Calcified cut section. × 750.

Fig. 3.—The large calcospherites on the laminæ. From a similar specimen to fig. 1, more highly magnified. The small regular calcified bodies forming the prisms are clearly seen in the upper part of the drawing. Drawn from a photograph. × 1000.

Fig. 4.—From a lower incisor of Halmaturus, injected with alcoholic fuchsin (method of von Beust). A fine cross striation is seen in the dilatations at the dentine margin coloured with the stain. Ground section. × 130.

Fig. 4B.—Cross striation of a tube in the enamel, being a portion of the organic basis of the enamel which has remained uncalcified. Drawing. Ground section. × 500.

Fig. 5.—*a*, *b*, *c*. Transverse sections of enamel of *Macropus rufus*, stained as fig. 4, showing colouring of the interprismatic substance and transverse sections of the tubes lying between the prisms in the interprismatic substance. Drawn from photographs. Ground section. × 800.

Fig. 6.—A section of enamel of fœtal Macropus. Calcification well advanced—cut without previous decalcification after staining in bulk with carmine. The tubes are more deeply stained than the prismatic substance—showing turns of the spirals wider than the prisms. Fig. 6 (*b*) shows a tube projecting from the surface of the section. × 500.

Fig. 7.—Lower incisor of *Bettongia Lesueuiri*. Bulb-shaped bodies in the enamel forming the terminations of dentinal tubes—soft parts fixed in formalin. Stained with Beckwith's gold chloride. Ground section. Drawing. × 300.

Fig. 8.—Enamel of Bettongia, stained by Cajal's silver nitrate process, formalin-fixed preparation; granules in the tubes continuous with those in the dentine and showing delicate cross branches. Ground section. Drawing. × 500.

Fig. 9.—Junction of enamel and dentine in lower incisor of *Phalanger orientalis*, showing terminations of tubes from the dentine in spindle-like bodies and fine tubes passing into the enamel. Dry specimen. Ground section. Drawing. × 130.

Fig. 10.—Longitudinal section at apex of dentine cusp in premolar of *Phalanger orientalis*, showing spindle-shaped spaces and some tubes. Dry specimen. Ground section. × 130.

Fig. 11.—Human deciduous molar, soft parts preserved. Ground section in same situation as in fig. 10. Stained by the fuchsin injection method. For comparison with fig. 10. Drawing. × 130.

Fig. 12.—Separate spindles from fuchsin-injected human teeth—soft parts retained. Two small air bubbles have been driven in by the syringe—tubes are seen traversing the spindle-shaped bodies. Drawing. Ground section. × 500.

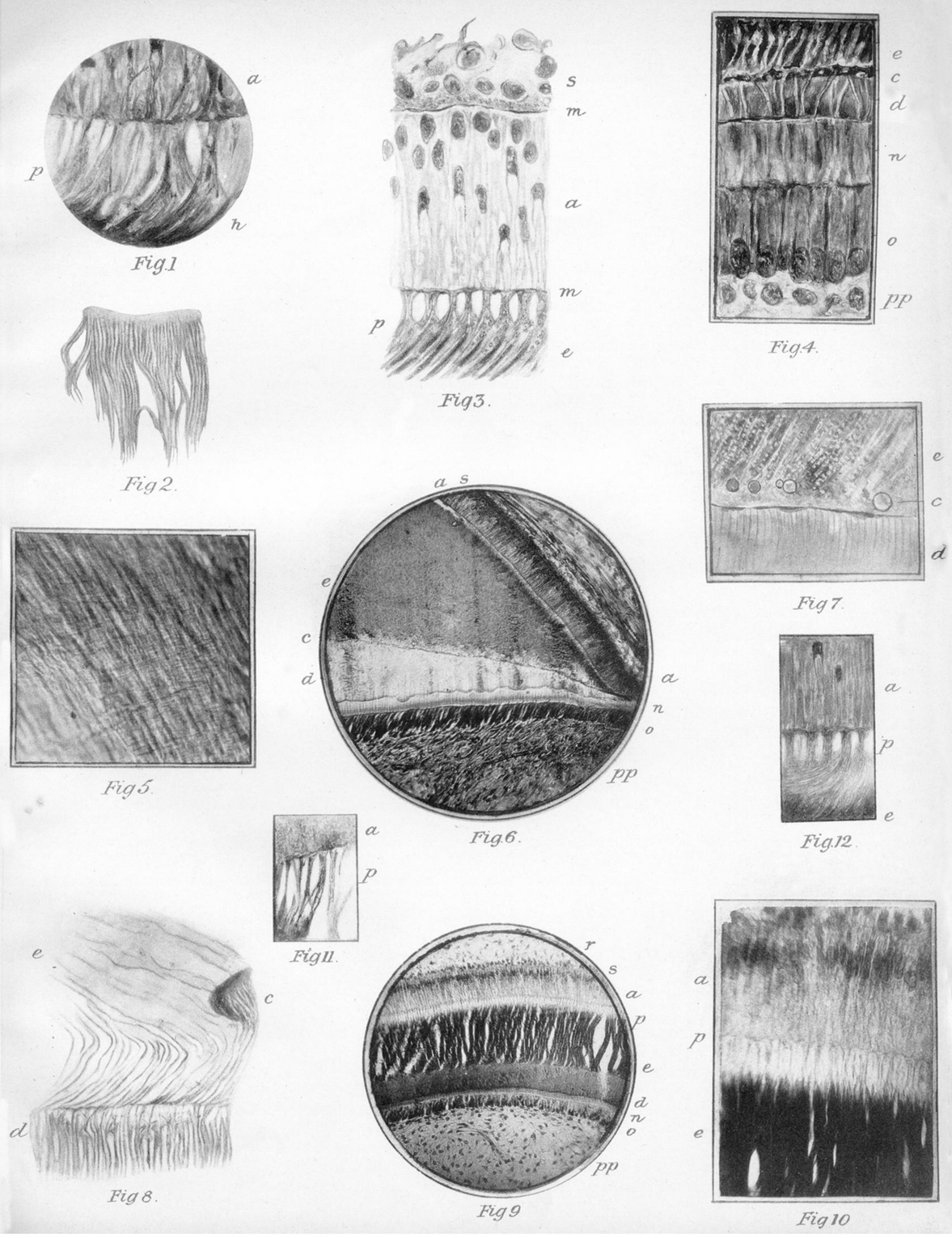


PLATE 26.

Fig. 1.—The Tomes' processes at the border of the forming enamel in Macropus. Section cut without decalcification. Showing the spreading of the Tomes' processes at the honeycomb region and their incorporation with the forming enamel. Drawn from a photograph. ×1000.

Fig. 2.—Shows the fibrous nature of the forming enamel in Macropus and the clear outer layer. Calcification considerably advanced. Drawing. Section cut without decalcification. ×130.

Fig. 3.—The enamel organ and developing enamel of a tooth of Macropus cut in the microtome without previous decalcification. Delicate fibres are seen to spring from the nuclei of the ameloblasts. Drawing. Stained hæmatoxylin. ×800.

Fig. 4.—A developing tooth of Macropus, showing the thin layer of calcified dentine crossed by the dentinal tubes, the commencing formation of the enamel prisms with wide spaces between them, and the interval between the dentine and enamel showing the calcific deposit. Section not decalcified. Stain, Van Giesen. Drawn from a photograph. ×800.

Fig. 5.—The fibrillar organic matrix substance seen crossing the enamel prisms in young enamel of Macropus. Photograph. Section, not decalcified. ×500.

Fig. 6.—From a developing tooth of Macropus, showing the large amount of enamel laid down at this early stage, and the very imperfect calcification at the dentine margin. Section, not decalcified. Photograph. ×75.

Fig. 7.—A similarly prepared section, showing the calcospherites at the dentine margin and the calcific deposit in the prisms. Stain, Van Giesen. Drawing from a photograph. ×500.

Fig. 8.—The decalcification experiment described in the text.

Fig. 9.—A section of a tooth of Macropus which had not been decalcified. Showing calcified dentine and the fibrous nature of the partially calcified enamel. A photograph. ×130.

Fig. 10.—Part of fig. 9 more highly magnified. A photograph. ×800.

Fig. 11.—Showing dividing of the Tomes' processes and inter-crossing in the honeycomb region. Drawing from a photograph. ×800.

Fig. 12.—The fine fibres of the Tomes' processes which can be seen in very thin sections to cross the coarser fibres at an angle. Drawing. ×600.