PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Cytomorphosis of the Marsupial Enamel-Organ and Its Significance in Relation to the Structure of the Completed Enamel

J. Thornton Carter

Phil. Trans. R. Soc. Lond. B 1918 **208**, 271-305
doi: 10.1098/rstb.1918.0008

VIII. *The Cytomorphosis of the Marsupial Enamel-Organ and its Significance in Relation to the Structure of the Completed Enamel.*

By J. Thornton Carter.

Communicated by Prof. J. P. Hill, *F.R.S.*

(Received May 22,—Read December 7, 1916.)

(From the Department of Zoology, University of London, University College.)

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Introduction.

In Marsupials the enamel of the teeth differs from that of most other Mammals in the presence of structures, continuous with tubules of the dentine, the point of junction often being somewhat enlarged. These structures pass outwards, usually pursuing a sinuous spiral course, and terminate a little before reaching the surface of the enamel, so that the outer zone of this tissue usually appears as a clear, structureless layer. On applying a strong acid the enamel is dissolved, and these structures alone remain attached to the dentine, from which, however, they readily part: they are resistant also to the action of alkalis, and from their behaviour to stains it is evident that they are organic.

The structures in question were first described in 1847 by Sir John Tomes (1) as continuations of the tubes present in the dentine, and from that time onwards it has been usual to employ the word "tube" in describing them. C. Tomes (2), however, whilst retaining the word, is in doubt as to its correctness, and writes "whether, like

 [Published, December 20, 1917.

the dentinal tubes, they consist of a resistant sheath containing a softer fibril, or whether they wholly consist of the resistant material, has not yet been shown."

Mummery (4) regards them as true tubes, and gives a description of their contents as granular, but also a little later in the same paper he alludes to them as appearing on transverse section as "refractile dots in some places, in others they are deeply stained."

In my sections of developing enamel where these structures are cut across, they appear most often as homogeneous dots, rarely as though the axial area was of a slightly different consistency from the periphery, and in a very few cases they are annular. In sections prepared by grinding dry teeth the tubular appearance is somewhat more frequent. I have never observed any appearances to suggest that these structures invest a protoplasmic process, and the difference in the staining properties in peripheral and central regions is one of degree only, suggesting a difference in physical consistency due to surface phenomena.

The developmental facts presented later in this paper negative the interpretation of the tubular form as the normal one, and I consider the term "fibril" affords a much more accurate description of these structures, both as regards their form and their mode of origin, and have employed the word to designate these structures throughout this paper.

These fibrils are most richly developed in the teeth of Macropodidæ, where, according to Tomes, they exist in the proportion of about one to every four enamel prisms. They are not equally distributed throughout the enamel, but are most frequent where the enamel is thickest, and do not extend quite to the surface, the outer portion of the enamel being devoid of them. In other families they are less abundant, while in the Wombat, alone of Marsupials, they are entirely absent.

Historical.

For many years these enamel "fibrils" were considered to be dentinal tubes included in the enamel, but in 1897 C. Tomes published (2) the results of certain investigations on Marsupial enamel, and stated his conclusion that they were due to an arrest of calcification during the formation of the prisms.

According to his hypothesis, calcification begins at the periphery of each prism, and by its arrest leaves an uncalcified central area, which is occupied by a prolongation of the cytoplasm of an ameloblast.

Mummery, in a recent paper (4, p. 308), has reverted towards the earlier view of a dentinal origin. His conclusions, quoting his own words, are that "there is an essential difference between Marsupial and Higher Mammalian enamel, and that the essential difference lies in the imperfect calcification of the cement substance of the former; that the imperfect calcification allows the penetration of the dentinal fibril from the previously formed dentine. 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. . . . . Thus I should consider there is not an ingrowth of dentine matrix, but an ingrowth of the protoplasmic processes of the odontoblast cells. A little later (4, p. 309), he writes: "I think with Sir JOHN TOMES, Prof. KÖLLIKER, and Prof. VON EBNER that the dentinal tubes actually pass into the enamel."

These statements of MUMMERY appear contradictory, for, though he clearly states that there is no ingrowth of the dentine matrix, but only of the dentinal fibril, yet he expresses his concurrence with the view that the dentinal tubes actually pass into the enamel, and that their behaviour to acids suggests that the sheath of Neumann is present in the enamel. His own description of the sheath of Neumann is that it is "not the outer portion of the fibril, but is an integral portion of the dentine lining the tube" (5, p. 108). The theories of both TOMES and MUMMERY would therefore assume for the "fibrils" a fortuitous origin due to incomplete calcification; the former by arrest in calcification in a prism, the latter by imperfect calcification in the interprismatic substance. It is necessary therefore to enquire into their respective views as to formation and structure of enamel.

C. S. TOMES (2, p. 114) accounts for the ultimate structure of the enamel by considering that "each ameloblast gives origin to an axial prolongation of its own interior plasm. The ameloblast is not itself actually calcified, as was supposed by many observers, myself included, but this fibrillar prolongation of its plasm does calcify; hence it seems probable that a single ameloblast gives rise to the whole length of an enamel prism, itself receding as the enamel grows thicker. In each individual fibre, calcification goes on from without inwards, leaving, during the formation of the greater part of the thickness of the enamel, a central tract soft and uncalcified. Ultimately, however, as the exterior of the enamel is approached, the axial canal becomes smaller and smaller, and finally thins out to nothing, so that a solid prism is the result. This occurs earlier in the process in some Marsupials than in others. The tracts of calcification belonging to each fibre do not fuse completely with their neighbours, but a small amount of interstitial calcified material is poured out between them."

It is not easy to deduce whether TOMES believes that the enamel prism is formed by secretion from the ameloblast or by conversion of the peripheral portion of its cytoplasm, for he first speaks of each ameloblast giving origin to an axial prolongation of its own interior plasm, by which one would infer that he alluded to the cytoplasm of the ameloblast—as much a part of the cell as the nucleus itself—and in the succeeding paragraph states that the ameloblast is not itself actually calcified, but the fibrillar prolongation of its plasm does calcify.*

* C. S. TOMES in a subsequent paper ('Phil. Trans.,' B, vol. 193) writes: "The deposition of lime salts at the points most distant from the vessels . . . . is not, however, unusual . . . . and *it is what happens in the calcification of a mammalian ameloblast which undergoes calcification at its distal extremity*" (the italics are mine).

Mummery's (6) view of enamel calcification has been summarised by himself as follows :—

"Enamel is laid down as an organic framework by the Tomes' processes of the ameloblast cells, which, as these cells recede, stretch across the forming enamel for its whole width, finer fibres passing in a transverse direction, and so forming with the prism-directing fibres a kind of network. The calcifying substance taken up from the blood by the cells of the enamel organ is dialysed through the inner ameloblastic membrane and deposited in the form of minute globules within the Tomes' processes (as held by Leon Williams). These are regular bodies, and are built up like a row of bricks to form the prisms."

Thus both Tomes and Mummery believe that a protoplasmic process, an extension of the cytoplasm of the ameloblast, stretches throughout the entire thickness of enamel during its formation, and that this process eventually becomes calcified to form the enamel prism.

These conceptions of enamel formation involve the acceptance of the view that the enamel organ secretes two distinct products—the one of which goes to form the prism whilst the other is responsible for the intervening substance—a point of view first enunciated by Leon Williams (7).

C. S. Tomes (2, p. 114) states his own view to be that "the tracts of calcification belonging to each fibre do not fuse completely with their neighbours, but a small amount of interstitial calcified material is poured out between them."

It is obvious that Tomes believes, with Leon Williams and Mummery, in the existence of a distinct product which lies between the calcifying areas.

Though Tomes' theory of enamel development and of the origin of the fibrils depends on the existence of a living fibril derived from the ameloblast, he does not make any mention of differences in the appearance in these cells, such as one might reasonably expect to find if, whilst some cells extended throughout the whole thickness of the enamel, increasing many fold the total amount of cytoplasm in the cell, others did not so extend, their prolongations having undergone obliteration by calcification.

The illustrations attached to this paper show conclusively that no such difference exists.

Neither Tomes nor Mummery offers any explanation to account for the spiral nature so often assumed by the fibrils.

Since enamel is doubtless formed from a secretion of the enamel cells, it is essential before one can safely elaborate any theory as to enamel formation, or of the origin of any structures found within the enamel, that there should be a clear knowledge of the structure and life-history of the cells responsible.

Some years ago, not being aware of any detailed account of the life-cycle of the cells of the enamel organ, I decided to undertake the task, and to follow out the changes passed through from the first inflection to the close of their functional career. This was done in various Vertebrates, and when I began preparations for extending

the research to Mammalia, Prof. Hill suggested that I should commence by investigating Marsupial enamel organs, and gave me unlimited access to his collection of Marsupial material. I obtained complete series of sections of fœtal heads, varying in length from 1·8 cm. to 5·2 cm., and comprising such various species as *Macropus ruficollis*, *Trichosurus vulpecula*, *Dasyurus viverrinus*, and *Didelphys aurita*, besides the jaws of several young and adult specimens. The excellent preservation and fine fixation of the specimens can be judged from the drawings accompanying this paper.

Particularly valuable for investigating the appearances seen in the forming enamel was a complete series of longitudinal sections of one of the lower incisors of a large pouch fœtus of *Macropus ruficollis* (erroneously described in successive editions of Tomes' 'Dental Anatomy' (3, p. 578) as growing "from persistent pulps"). The tip of the tooth was just emerging from the soft tissues, and in order to render the cutting of sections more easy, Prof. Hill kindly dissected out the tooth in its sac. In these sections one is able to see all stages of the ameloblasts in the one and the same section.

The embryos were imbedded in paraffin and cut in complete series of sections from 6 μ to 10 μ in thickness. They were then stained in iron hæmatoxylin, followed by a plasma stain. In the case of isolated teeth, the thickness of the sections and the stain employed were varied according to the structure to be examined.

The life-cycle of the ameloblasts in Marsupials may be divided into 14 stages, of which the latter nine, coinciding with the elaboration and secretion of the enamel-forming substance, represent the active functional period in the life of the cell, and the more marked morphological changes passed through in these stages will be briefly described.

It is with these later stages that this paper is mainly concerned and the earlier stages, those passed through from the first inflection of the dental lamina to a period just previous to functional activity, are not dealt with here, for they throw no light on the subject of this paper. They are, however, important in view of the fact that the interpretation of Marsupial dentition involves the identification of tooth-germs which do not proceed to calcification. Differentiation in the cell affords a means of indentification of great value when endeavouring to interpret such admittedly unsatisfactory evidence as the aggregation of cells to form "buds." Through the further kindness of Prof. Hill, I have undertaken an investigation into this obscure field with the hope that the employment of cytological criteria may throw some light on a subject the solution of which would be of such value to zoology.

Cytology of the Ameloblasts.

Stage I (Plate 7, figs. 1 and 2).

Immediately before the appearance of a thin layer of dentine on the surface of the tooth pulp, the ameloblasts are seen to constitute a layer of cells continuous over the

whole surface of the dentinal papilla; they are elongated in shape, having large oval nuclei of regular outline, densely crowded together, and the chromatin evenly distributed throughout the karyoplasm in the form of minute fragments. The nucleolus of small size is usually situate about the centre of the nucleus, and is not invested by chromatin; at least in sections stained in iron hæmatoxylin, the acidophile nucleolus stands out quite clearly before differentiation is completed.

The lateral surfaces of the ameloblasts are not in contact but are seen to be separated by a transparent homogeneous acidophile intercellular material. At this stage I have been unable to demonstrate the presence of intercellular cell-bridges connecting these surfaces, which are such a marked feature of later stages. The cytoplasm is finely granular, showing no sign of an alveolar or fibrillar structure. As a rule no sharp line of demarcation exists between the ameloblasts and the cells of the stratum intermedium (though occasionally when a little shrinkage has taken place a clear zone may be seen between these cells), whilst mitosis is frequent in both.

Stage II (fig. 3).

With the appearance of a thin layer of dentine, the ameloblasts increase somewhat in length and the outline of the individual cells becomes much more clearly defined, the nuclei lying sometimes about the centre of the cells but more often they are placed nearer to the base. The chromatin is no longer dispersed throughout the karyoplasm, as in the previous stage, but has begun to be aggregated into spherules forming chromatin knots.

The columnar form of the ameloblasts is not due solely to pressure of contiguous cells (as would seem to be the case in the cells of the stellate reticulum illustrated in fig. 2 (*s.r.*), where the nuclei have a markedly oval form, which they lose at a later stage when, with the increase in size of the enamel organ, they come to lie farther apart and become almost round in shape), but is an assumption of polarity due to a physiological differentiation in the cytoplasm, inasmuch as there is a slight appearance of fibrillation in the cell, most marked at the basal end. The cytoplasm, however, is of a finely granular structure and the granules are evidently not identical in their nature, since those in the basal area of the cell and about the nucleus are basophile whilst towards the secreting surface they are mostly acidophile in reaction.*

It is worthy of note that when this stage of differentiation is reached the power of cell division seems to be lost, for although mitoses are abundant in the preceding stage, they now cease.

* The behaviour of these various granules to stains forms a valuable test of the effectiveness of fixation.

I have never had an opportunity of experimenting with the actual fixation of Marsupial material, but in 1907 I carried out some investigations on fixation of enamel cells in the Pike. This animal was chosen because of the ease of obtaining it alive in various stages of growth and keeping it in the

Stage III (fig. 4).

At this stage the cells have become much longer, and the nucleus lies sometimes near to the base, in others about the centre of the cell, as shown in fig. 4. The basophile granules assume a linear arrangement, and give to the cell a finely fibrillar appearance, whilst in that area of the cytoplasm between the nucleus and the dentine, the acidophile granules appear to coalesce, and give a fine alveolar appearance to this portion of the cell; the meshes of the alveoli also are basophile, but less so than the fibrils in the neighbourhood of the nucleus. The outline of the cell is clearly defined, and lying between it and the dentine there are well defined processes, which are strongly acidophile.

Stage IV (fig. 5).

From this period onwards the rate of secretion is very rapid; the ameloblasts elongate until they measure over 100 μ in *Macropus ruficollis*, and, coincidentally, they lose their distinct columnar outline, owing to the accumulation within them of metaplasmic material, which appears to arise from coalescence of the acidophile

laboratory until needed, and also because its developing teeth do not lie in bony crypts, but are placed in a flap of soft tissue lying on the lingual side of the jaw, from whence it may be rapidly detached.

A number of tubes, containing an equal quantity of the same fixative, were prepared in advance, and into these small pieces of the soft tissue containing the tooth germs were dropped at intervals of one minute. After an equal period of immersion, these were removed, imbedded in paraffin without decalcification, sectionised, and stained with iron-hæmatoxylin with a counter stain of Bordeaux R.

The cytoplasm of ameloblasts in the earlier stages of functional activity exhibited a finely granular structure.

No apparent difference in nuclear structure was shown with an interval of several minutes in fixation, but the cytoplasm gave evidence of marked change unless fixed within three minutes of death, this change manifesting itself in the behaviour of the granules to the stains.

If fixed within three minutes of death, the granules in the neighbourhood of the nucleus and the base of the cell stained deep black with the iron-hæmatoxylin, whilst the area of the cell towards the dentine was charged with granules which had taken the plasma stain, but interspersed amongst them were a number of granules with a basophile reaction.

When fixation was deferred beyond three minutes after death, a marked and progressive change took place, for the granules no longer showed a selective discrete behaviour to the stains, and a more or less diffuse acidophile reaction was exhibited, which extended almost to the nucleus.

This change suggests that certain substances which enter into the composition of the granules had broken down and become diffused throughout the cytoplasm.

Thus in ameloblasts *post-mortem* change occurs with great rapidity, and methods of fixation which may be effective in many tissues are not sufficiently precise for these cells.

This is not strange when one realises that in a secreting cell there is a wide differentiation between various areas of the cytoplasm, and that the physico-chemical conditions existing therein are undergoing constant and active change.

Jennings has written of unicellular organisms:—"It is of the very greatest importance for the understanding of the behaviour of organisms to look upon them chiefly as something dynamic—as processes rather than as structures." This applies with equal force to the study of the cell.

droplets or granules seen in the cytoplasm in the earlier stages, distorting the outline of the cells, and rendering it impossible to distinguish separate cell bodies.

The remaining cytoplasm becomes fibrillar and intensely basophile, and gives the appearance of an irregular meshwork, this being due to the washing out from the interspaces of the metaplasmic material.

The nucleus, whilst remaining oval in shape, diminishes greatly in size, measuring in length 6–8 μ, and in width 4–6 μ. It stains very deeply, and this appears to be due not only to the aggregation and condensation of the chromatin into masses, but also to an actual change in the nature of the chromatin itself, for in sections stained with iron-hæmatoxylin it is much more difficult to extract the stain, the chromatin remaining densely black long after nuclei of the earlier and later stages have become quite colourless.

The nucleus also recedes towards the base of the cell until, in many cases, it appears actually to reach its limits.

Coincidentally with this stage, the nuclei of the cells of the stratum intermedium often increase in size to a diameter of about 12 μ, and the chromatin is dispersed throughout the karyoplasm.

Stage V (figs. 6 and 7).

The principal feature of this stage is an abrupt decrease in the length of the ameloblast cell, though it still retains the fibrillar appearance and basophile reaction of its cytomitoplasm, with indistinct cell outlines laterally.

The nuclei have usually lost their spherical or oval shape, and assumed an irregular form, apparently in response to a change in the physical conditions of its environs rather than to any amœboid action of the nucleus itself, for this change of nuclear shape is invariably associated with the close proximity of distinct masses of metaplasmic substance.

Sometimes one sees the nucleus more or less spherical in contour at its distal portion, and at its other end partly embracing a metaplasmic globule, but I have never seen any evidence in ameloblasts of a true branching of the nucleus, as has been described in certain other secreting cells (16).

The fibrils constituting the cytomitoplasm apparently terminate in the nuclear membrane. For a long time I was of opinion that they passed through the membrane (which is usually very indistinct at this stage, though the nucleus preserves a well defined appearance), and terminated in chromatin masses lying in the karyoplasm; but adopting the method of staining deeply with iron-hæmatoxylin and effecting differentiation in weak iron-alum under a water-immersion lens, I have come to the conclusion that, in ameloblasts, there is no evidence of such penetration, but that the fibrils are purely cytoplasmic, and formed by certain of the basophile granules seen in earlier stages assuming a linear arrangement.

At this stage, the nuclei of those cells of the stratum intermedium, which are

directly connected with the bases of the ameloblasts by means of cell-bridges, undergo a marked change, resulting in their degeneration and ultimate disappearance (fig. 9). The first sign of the change appears to be that the perinucleolar chromatin, the deeply basophile material which usually surrounds the nucleolus (*nlo.*), disappears, so that the acidophile nucleolus is surrounded by a clear annular zone (fig. 9, 3), and eventually the whole of the nuclear chromatin passes outward and becomes aggregated on the nuclear membrane, where it is usually not evenly distributed along the whole circumference, but a small surface has little deposited thereon (fig. 9, 5). This condensation of the chromatin most frequently, but by no means always, takes place on the surface towards the ameloblasts.

The achromatic network becomes clearly visible (fig. 9, 4). This is succeeded by an appearance of opacity in the nucleus, except as regards the chromatin, as though the karyoplasm, cleared of its chromatin, had undergone coagulation (fig. 9, 5). At this stage it often shrinks under the action of reagents, this shrinkage being most marked at that portion of the periphery where there is little or no chromatin. The cytoplasm retains its original form, and the shrunken nucleus is seen to lie within a well defined contour (fig. 9, 6). Gradually the nucleus disappears, first the altered karyoplasm and achromatic structures, and finally the chromatin.

Coincidently with these nuclear changes, the cytoplasm (*cyt.*) becomes very coarsely alveolar, or, in other words, large vacuoles of a fluid or semi-fluid nature are formed, which appear to coalesce, and eventually the more solid interspaces disappear.

Stage VI (fig. 8).

The change in the cells of the stratum intermedium just described is associated with a recovery in the structure and form of the ameloblasts. The first stage in this process is marked by the fibrils of the cytomitoplasm losing their irregular distribution and becoming arranged in fairly straight lines through the long axis of the cell, at one end terminating in the nuclear membrane, and at the other ending in well defined deeply basophile granules lying just beneath the inner cell-membrane.

The length between the two ends of the cell has increased somewhat from Stage V, but the nuclei are still comparatively small, and the chromatin is usually aggregated into one or two large masses. The nuclear membrane is indistinct.

Stage VII (fig. 10).

In this stage the cells have begun to reassume a definite columnar form, and the outlines of individual cells once more appear. This change is associated with a disappearance of the coarsely fibrillar cytomitoplasm, which seems to resolve itself into granules similar to those from which it had its origin, though a faintly fibrillar appearance persists in the cytoplasm.

The nuclei have increased in size, and are now spherical or oval in form, with the

chromatin in a more dispersed state than in Stage VI. They appear to lie in a neutrophile area or zone, which is not sharply circumscribed, but gradually merges into the basophile fibrillar cytoplasm.

Stage VIII (figs. 11 and 12).

This stage is marked by the ameloblasts having once more become well defined cells, separated from their neighbours by intercellular areas traversed by cell-bridges.

As in the preceding stage the nucleus appears to be partially or completely surrounded by a clear zone or space, now, however sharply circumscribed: this appearance is not sporadic, but is always to be seen in the ameloblasts at this stage, whatever fixative may have been employed. For some time I was inclined to regard it as a condition caused by unequal shrinkage under the action of the various reagents employed in fixing or staining, but later found that in sections stained with iron-hæmatoxylin, by differentiating slowly in a weak solution of the iron-alum, and watching the process by means of a water-immersion objective, this clear space can be seen to be traversed by delicate fibrils, which stain faintly and appear similar to the achromatic contents of the nucleus. I have examined a great number of cells of this stage, and have little doubt that this neutrophile area is a normal condition and associated with the rejuvenation of the cell.

In Stage VII, the cytoplasm was seen to be composed of fine fibrils interspersed among granules. This has now given place to an alveolar structure (well shown in fig. 13) extending throughout the whole length of the cell; the walls of the alveoli (which appear to be complete cavities) are basophile, but their contents attract the acid stain.

In preceding stages, the ameloblasts have been characterised by uniform length, but now they vary, though preserving a well-defined regular margin towards the forming enamel (figs. 11 and 12). At this stage, it is a frequent occurrence to find cells of the stratum intermedium inserting themselves between the ameloblasts, and appearing eventually to become functional as such (fig. 12).

Stage IX (fig. 14).

This stage marks the end of the functional life of the ameloblasts. The cytoplasm, which in the preceding stage was alveolar, has again become faintly fibrillar, and the intercellular areas, which were so conspicuous, have disappeared, though the outlines of individual cells are clearly defined. There is a rapid decrease in length, associated with an increase in breadth, a flattening out of the cells, and, at eruption, these lie between the enamel and the cells of the gingival margin.

Wilson and Hill (17, p. 518) state that "the process of eruption is very clearly seen to be attended by the flattening out of the enamel epithelium covering the tooth crown and its conversion into squamous epithelium with concomitant formation in it of epithelial 'nests' or 'pearls' close to the tooth cusps."

My own preparations include very few where the tooth is at the point of eruption, and in these there is no sign of any nuclear changes in the flattened ameloblasts such as are associated with mitosis. It is an interesting point, and one which will receive special attention in further researches, at what stage the ameloblasts, having passed through their period of functional activity, once more resume the power of cell division to form "nests."

The Ameloblastic Membranes.

Though the lateral outlines of the individual ameloblast cells appear to be lost in the more active stages of ameloblastic life, yet at all times the ends of the cell remain quite distinct and well-defined. The base of the ameloblast is characterised by a finely granular structure, which gives to oblique sections through this area the appearance of a distinct structure lying between the ameloblasts and the cells beyond, but that this cannot be the case is obvious, for at all stages the bases of the ameloblasts are connected with the cells of the stratum intermedium by fine cell-bridges (figs. 3, 6, 11, 12, *c.b.*), as are the cells of the stratum intermedium with one another (fig. 15, *c.b.*). At the secreting surface the free passage of the contents of the cell is prevented by the development of a differentiated area of the cytoplasm to form a cell-membrane (figs. 16 and 17). This is not a metaplasmic cuticular product, but is formed, as is usual for these structures in animal cells (8, p. 129), by a local concentration of certain elements of the cytoplasm which have the property of lowering surface energy. In addition, the free passage outwards of any fluid material occupying the intercellular spaces between the ameloblasts is prevented by the presence of a continuous thin intercellular material (*t.b.*), which, so to speak, cements together the outer ends of the cells where they come into contact. This cementing material constitutes the so-called terminal bars of authors, it is co-extensive with the margin of the cell-membrane above described, and gives a clear polygonal form to the secreting surface of the cell.

Thus we have the secreting surface of the ameloblasts showing a modification of the cytoplasm which takes the form of a thin layer finely granular in structure. Since the interspaces between the cells are closed, it follows that all products of cell activity within the enamel organ can only pass out through this surface of the ameloblasts.

Using iron-hæmatoxylin as a stain, it has been my experience that this portion of the cell usually destains rather suddenly, but if the hæmatoxylin bath is rendered alkaline by the addition of lithium carbonate, as suggested to me by the late Prof. Minchin, and the differentiation carried out in a weak solution of iron-alum under a water-immersion lens, there is no difficulty in arresting the process to show these structures quite clearly.

As in all other cytological studies, it is of the utmost importance that observations on the nature and structure of cell-membranes should be based on material of which

the fixation has been effected with special reference to such work. In badly fixed material, a layer may frequently be found lying between the secreting surfaces of the ameloblasts and the forming enamel, which appears to have been formed by an exudation from cells after death, for in properly fixed material there is never any trace of such a structure.

In my preparations there is no evidence to support MUMMERY'S view of an outer ameloblastic membrane *separating* the cells of the stratum intermedium from the ameloblasts, nor of an inner ameloblastic membrane *lying between* the ameloblasts and the forming enamel.

Concerning these structures he writes (5, p. 108): "In developing enamel a membrane has been described between the ameloblast cells and the forming enamel, and also between the enamel cells and the stratum intermedium of the enamel organ.

"Mr. CHARLES TOMES is inclined to look upon these membranes as artificial productions, but in sections which have not been treated with either alcohol or acid they are easily to be seen."

A little later (5, p. 110), he writes: "The presence of large globular calcospherites in the enamel also suggests the slow diffusion of lime salts, which would appear to be dialysed through the inner ameloblastic membrane, or what is functionally equivalent to such a membrane—the outer surface of the albuminoid material in which the deposit takes place . . . ."

With regard to the inner ameloblastic membrane, MUMMERY'S position is somewhat anomalous, for whilst he asserts the existence of a membrane lying between the ameloblasts and the forming enamel, through which dialysis of the lime-bearing secretion takes place, yet he accepts the Tomes' processes as prolongations of the ameloblastic cytoplasm, which therefore must either pass through the membrane, perforating it, and so rendering it useless as an osmotic membrane, or continuity of the process with the remainder of the cell must be severed at that point where the membrane intersects it.

C. S. TOMES (2, p. 117) not only denies the existence of a membrane separating the ameloblasts from the forming enamel, but, influenced by a belief in the unassailable accuracy of his observations, states that "there cannot be a membrane in this position," and (3, p. 179) "it is perfectly certain that there is no membrane over the free end of the ameloblasts after the formation of its fibrillar prolongation."

In view of his conception of the origin and nature of the Tomes' processes, TOMES at least shows a logical attitude in the above statements, for it is difficult to conceive the existence of a membrane in this position if these processes are cytoplasmic.

Briefly put, his conception of the ameloblast is that it is a cell at first columnar in form, with a well-defined margin towards the surface of the dentinal papilla. With the assumption of a functional life, the cell-body retreats, but leaves behind a process continuous with and identical with the interior "plasm" of the cell, and until calcification has progressed to some extent, "probably nearly or quite of the full diameter

of the cell." This process extends throughout the whole thickness of the forming enamel, and since the greater part of the enamel is laid down whilst but a small amount of dentine is forming, synthesis of the cytoplasm must be very rapid. Coincidently with this very great increase in the amount of cytoplasm, he holds that the cell also elaborates and secretes its lime-bearing fluid, so that active growth and active secretion—contrasted states of cell-activity—are taking place at one and the same time.

The conditions and changes in the ameloblasts described earlier in this paper lend no support to such a view.

On the Nature of the Tomes' Processes.

Tomes bases his belief as to the origin and nature of the Tomes' processes on three reasons, in his own words (2, p. 110) :—

(*a*) "They are obviously a continuation of the plasm of the cell."

(*b*) "As regards taking the stain, they always behave precisely as does the plasm of the ameloblasts."

(*c*) "There is no appearance of a cell-membrane across the end of the ameloblast."

Tomes has given us no particulars of the technique employed in his researches, but concerning the material from which his conclusions were drawn, he writes :—

"My material consisted of embryos of Macropods and of Hyrax, but unfortunately it is almost impossible to procure Marsupial embryos in first-rate histological preservation, as they are apt to have lain in the pouch for a time after death, and this has added to the difficulty of getting good sections."

Cytological conclusions based on material concerning the fixation of which there is any doubt are open to suspicion, and in the case of secreting cells, such as are the ameloblasts, I have shown that fixation must be rapid and complete since *post-mortem* changes are particularly active and give rise to a misleading behaviour in regard to stains.

On each and all of the points advanced by Tomes in proof of the correctness of his view concerning these processes, my observations are in direct contradiction.

His first contention, that the processes are obviously a continuation of the plasm of the cells, evidently refers to a physical resemblance between these structures and the cytoplasm. In my sections there is no such resemblance.

In all stages of their life-history, the ameloblasts are square-ended towards the dentine surface, and never of a tapering shape. During those stages (Stages I–III in this paper) of their life-history, during which the ameloblast preserves its well-defined cell-outlines laterally, the cytoplasm is densely granular towards the secreting surface, then a sharply defined line is seen, which is the cell-membrane in section, and beyond this lies the Tomes' process, appearing almost homogeneous.

In the stages represented in figs. 5 and 6 (Stages IV and V), where the cytoplasm of the cell has developed large vacuoles lying amongst a coarsely fibrillar cytoplasm,

the fibrils never extend beyond the cell-base. The contents of the vacuoles are obviously not identical with the material of which the processes are formed, for whilst the former present a coagulated milky appearance (when not dissolved or washed away by the reagents employed in preparing the sections), the processes appear homogeneous or faintly granular, and are not affected by the action of the alcohols, etc.

Even though the membrane closing the base of the cell had not been visibly demonstrated as it has been (*cf. ante*, p. 281), such a condition as just described would afford strong evidence for the existence of such a structure, which, whilst permeable to the passage of certain cell-constituents, retained others within the cell.

In the succeeding stages (VI, VII, VIII, and IX) no processes are visible, the relation of the cell to the forming enamel being well shown in fig. 10.

In those stages where the lateral outlines of the cell are well defined, such as are illustrated in fig. 4, each cell is seen to be furnished with Tomes' processes. Since the base of each cell is separated from its neighbours by terminal bars, as shown in figs. 16, 17, *t.b.*, this is what one would expect to find, since the contents of each cell could only emerge by its own cell-base, and a slight shrinkage would lead to the extension of individual droplets of the cell-secretion. But in those stages where the lateral outlines of the cell are lost (figs. 6 and 8), the processes are by no means regular in their occurrence. As the well defined membrane, with its terminal bars, can be seen at all stages, this points to the contents of the vacuoles in some cells being dialysed through the membrane, whilst in others such dialysis is not taking place at the moment. I have never been able to satisfy myself, in such stages, whether the walls of the vacuoles are complete, or whether the structure of the cytoplasm is an open network, though in the later stages (Stages VII and VIII) there is little doubt that the contents of the vacuoles are enclosed in complete cavities, giving to the cell a pseudo-alveolar structure, as figured in fig. 13.

The irregular occurrence of the processes would favour the former view.

The above description of Tomes' processes is based on material which had been decalcified, or had been softened by the employment of an acid fixative.

In the case of the sections from which figs. 18 and 19 were drawn, no decalcification was effected. These sections were prepared from a pouch-fœtus of *Didelphys aurita* obtained by Prof. Hill whilst in Brazil, and are in a very fine state of fixation, Zenker's fluid having been employed as the fixative. The head was imbedded in photoxylin before paraffin, and the sections—8 μ in thickness—stained on the slide by means of iron-hæmatoxylin followed by benzopurpurin.

The amount of shrinkage is extremely slight, and the Tomes' processes are conspicuous by their absence, for there is no sign of any definite structures continuous with the ends of the ameloblasts, but lying all along between the cells and the developing enamel there is a clear continuous homogeneous layer.

It was, of course, possible that the photoxylin had rendered the processes invisible, though, if these structures are actual prolongations of the cytoplasm,

identical in their nature with the bodies of the ameloblasts, it would be reasonable to expect them to show a similar reaction to stains, and to show the same physical structure.

To make certain on this point, certain of the slides, after having been examined, were unmounted, the photoxylin dissolved away, and the sections, having been restained, were again mounted. Beyond a certain amount of shrinkage, these sections showed no difference in appearance from those in which the photoxylin was present. In all the sections obtained from this embryo, the differentiation of the secreting surface to form a cell-membrane is beautifully shown.

With Tomes' statement that the processes stain identically with the cytoplasm, my observations are in contradiction.

In tissues where the fixation is bad, either from the material having been allowed to remain some time after death before immersion in the fluid, or because of the employment of a fixative whose action was not sufficiently rapid and penetrating, karyoplasm, cytoplasm, and Tomes' processes stain identically, but with material of such beautiful fixation as I have been so fortunate to have at my disposal, and with the employment of the most delicate technique, the Tomes' processes and the cytoplasm invariably show a different reaction to the stain.

At Stage IV, when the cell consists of large vacuoles and a coarse fibrillar cyto-mitoplasm, the contents of the vacuoles stain with difficulty and show a milky appearance, the fibrils of the cytomitoplasm are strongly basophile, whilst the transparent Tomes' processes are as markedly acidophile, a sharp line of demarcation invariably existing between the cell and the process.

In the earlier stages, employing such a delicate cytoplasmic stain as Lichtgrün-picric after iron-hæmatoxylin, the fibrils of the cytoplasm stain black, the remaining cytoplasm shows a special affinity for the green, whilst the Tomes' processes attract the yellow of the picric acid.

I have already brought forward evidence of the existence of the cell-membrane over the secreting surface. Since the passage of any substance between the ameloblasts is prevented by the terminal bars developed in these positions, it necessarily follows that the enamel is entirely the product of a secretion shed by the ameloblasts. The observations here recorded justify, in my opinion, the conclusion that Tomes' processes are not prolongations of the cytoplasm, but are merely forms assumed by the secretory product of the enamel cells.

The ameloblastic secretion is fluid in consistency and therefore is extensile. When there is shrinkage in the tissues prior to complete fixation this viscid secretion would necessarily be drawn out into structures of a form such as the Tomes' processes assume. That such shrinkage does take place even in tissues whose fixation is otherwise perfect is clear when such preparations as shown in fig. 2 are examined; here the enamel organ and the dentinal papilla have become widely separated through shrinkage, which has proceeded to a greater degree in the dentinal papilla, so that if

the surfaces of the inner enamel epithelium and the outer surface of the dentinal papilla were brought together they would no longer coincide. Yet in this tooth-germ cell structure is beautifully preserved and mitoses are very frequent throughout both the enamel and dentine organs.

Those readers familiar with the illustrations of literature concerning tooth-change in Marsupials will recall that in the greater number of cases where tooth-germs are figured this disproportionate shrinkage of enamel organ and dentinal papilla obtains.

In the embryo of *Didelphys aurita* described above no such shrinkage had taken place. It is, therefore, most probable that the Tomes' processes are formed by shrinkage in the surrounding tissues before the fixative has penetrated to and coagulated the ameloblastic secretions. To obviate such changes it is necessary to allow as little time as possible to elapse after death before immersion in the fixative and to employ for this purpose a fluid which penetrates equally, coagulates rapidly, does not dissolve the albuminous fluids and whose shrinking action is counteracted by the presence of acetic acid.

Further, in the forming enamel there is not the slightest evidence of the presence of any cytoplasmic structures such as Tomes and Mummery believe to be present, and the appearances on which they base their belief are open, as will be shown later, to quite a different interpretation.

The appearances described above constitute the more obvious morphological changes through which Marsupial enamel cells pass during functional activity, a much more exhaustive account, together with a discussion on their physiological significance, being embodied in a paper dealing with the cytology of the enamel organ in Vertebrates which is in course of preparation.

The essential difference in the life-cycle of Marsupial enamel cells from those of most other Mammals, lies in the fact that in the latter no stage is characterised by any marked difference in the cytoplasm or nuclei of the ameloblasts.

In the case of Marsupials, however, we find that there is a stage where the greater part of the cytoplasm has become transformed into metaplasmic material, the cyto-mitoplasm alone remaining.

The marked changes in the nuclei of the ameloblasts form another distinguishing feature, for the size of the nucleus is undoubtedly related to the functional activity of the cell, and therefore to the amount of undifferentiated cytoplasm, so that without going so far as to accept in their entirety Hertwig's views as to "Kernplasma-relation," we may safely infer that the great decrease in size of the nucleus is due to the fact that almost all the cytoplasm has been changed into metaplasm.

This great decrease in the size of the ameloblastic nuclei during the earlier stages of functional activity shows that substances from the karyoplasm must diffuse into the cytoplasm, whilst their subsequent increase during the stages of rejuvenation can only be explained by assuming that the nuclear chromatin has the power of taking

from the cytoplasm a material which restores to the nucleus its spherical form and typical structure.

My observations on Marsupial enamel cells lend no support to the views advanced by Leon Williams that the enamel rods are built up of globules formed in the ameloblasts, probably as a direct nuclear formation, that these pass out from the cell connected by "plasm strings," and that a separate product is taken by the ameloblasts from the cells of the stratum intermedium and poured about the rods to form the interprismatic material. As to the origin of the globules, he writes: "We observe in the nuclei of the ameloblasts, and also apparently emerging from and just outside of the nuclei, bodies which are evidently identical with those seen throughout the entire length of the ameloblasts in fig. 58," and suggests that "it is not improbable that these bodies are of the same nature as the Nebenkern often seen in other cells." This latter suggestion is of no value, for though many different theories have been advanced as to the nature of these structures, the view advanced by Mathews (10), that they are entirely of cytoplasmic origin, is generally accepted by cytologists, and, as regards the evidence of bodies in the nucleus, I have never observed in the course of prolonged investigations of the cytology of the enamel cells, not only in Marsupials, but in other Vertebrates, any appearance within the nucleus similar to the globules of metaplasmic material to be seen in the cytoplasm.

I have carefully carried out the microchemical tests for calcium given by Prof. McCallum (11), but neither in fresh cells nor in fixed material have I ever succeeded in obtaining the faintest reaction of calcium in the nuclei of any cells of the enamel organ.

Neither is there any evidence to suggest for the globules a nucleolar origin, such as gives rise to the yolk-nucleus in many cells, for, though often a stream of granules may be seen lying between the nucleolus and the nuclear membrane, and apparently passing in an altered condition into the cytoplasm, they disappear entirely, without leaving any evidence of their presence.

From Leon Williams' description, one would infer that the globules pass from the nucleus into the cytoplasm, and from thence emerge in a more or less unaltered condition, retaining their identity.

This is certainly not the case, for, though the accumulations of metaplasm within the cell form vacuolations, the contents of these are not definite bodies, they are not of uniform size, and, when not washed away, present a coagulated appearance, whilst the secretion, having passed beyond the cell, appears transparent or finely granular, and readily takes an acid stain.

Further, the secreting surface of an ameloblast at all times shows the presence of a specialised layer of cytoplasm of a finely granular structure, which undoubtedly performs the function of a dialysing membrane, and which would prevent the passage of a discrete body.

By the use of the word "body," Leon Williams implies a structural differentiation

and stability which do not exist, and which would lead one to infer that secretion—the expulsion of the metaplasmic product—was an active process of the ameloblast, also that these cells exert a constructional influence on the elaborated secretion.

The same criticism applies to MUMMERY's description of the enamel rod as "formed of regular bodies built up like a row of bricks."

In the changes which characterise the life-cycle of enamel cells in Marsupials, there is no support for any theory which endeavours to explain the appearances found in formed or forming enamel, as being due to intermittent rhythmical secretion from these cells, such as LEON WILLIAMS adopts when he speaks of the enamel rod as "built up by the successive rhythmical orderly deposit of bodies of uniform size formed in the ameloblast."

CHANGES IN THE AMELOBLASTIC SECRETION.

The problem to be solved in the calcification of enamel is the transformation of a colloidal fluid poured out by the ameloblasts, and comparatively rich in organic material, into a solid substance of a definite structure, composed almost entirely of inorganic material. No theory is sufficient which does not give an explanation of the changes by which this transformation can be continuously and completely effected. In the physico-chemical changes involved in the crystallisation of colloidal media, we possess data which at least enable us to interpret the progressive changes which the forming enamel is observed to undergo, and to explain the structure of the completed tissue.

The Development of the Fibril System.

In sections, the secretion which has passed out from the ameloblasts consists at first of a continuous homogeneous layer, in which no trace of structures can be seen (fig. 3). The first appearance of any structural differentiation is recognisable as a characteristic layer lying just beneath the ameloblasts. The interpretation of this layer is arrived at most easily by studying sections in which a fair thickness of enamel is in process of formation.

In fig. 22, which is a tangential section through the enamel organ of a developing tooth of *Didelphys aurita* cut without previous decalcification, it is seen that the layer in question (*f.f.l.*) is not merely a perforated lamina, but is an irregular sponge-work enclosing areas the boundaries of which are not always complete. These areas are occupied by a transparent fluid coagulum, which takes the acid stain; they are not uniform in size, as TOMES erroneously states, though his illustration (11, Plate XVI, fig. 5) does not support his description, and even the smallest are considerably larger than are the bases of the ameloblasts, or of the prisms of the formed enamel.

In the substance of the septa which constitute the sponge-work skeleton, fibril-

like structures (*f.*) are to be seen winding a sinuous course as they follow the outlines of the alveoli (*alv.*) (fig. 23). These structures are evidently distinct from the lamellæ which form the walls of the alveoli, for on transverse section they show as well-defined rounded dots.

At a little later stage (fig. 24) the lamellæ disappear, but the fibrils persist, forming a true open-network or reticular system, the fibrils (*f.*), of which can be clearly traced into the forming enamel (fig. 28), where their staining capacity increases, and, retaining their sinuous course, they constitute the fibril system of the enamel.

In fig. 25, which is a tangential section of a developing tooth of *Trichosurus vulpecula*, the outlines of the spongework are to be seen at the surface of junction with the dentine; the partitions are seen to consist of granules arranged hexagonally, and from the nodal points of these irregular hexagons, the fibrils (*f.*) pass upwards, showing no trace of connecting lamellæ.

The appearance of this fibril-forming layer in vertical section is shown in figs. 26, 18, 19, where its alveolar nature is not evident, but the origin and direction of the fibrils is clearly seen.

In other sections from the same series the fibrils are not visible throughout the whole area of the forming enamel, but these sections were prepared without decalcification, and subsequent to fibril formation the transparent coagulum which occupied the cavities of the alveoli undergoes precipitation and stains deeply with the hæmatoxylin. If such a section is treated with weak acid, this precipitate dissolves, and the "fibrils" are seen passing from the fibril-forming layer to the surface of the dentine, as shown in fig. 18.

Earlier stages of enamel formation, which alone would be difficult to interpret correctly, are readily solved by comparison with such sections as shown in fig. 22.

In fig. 26 the first appearance of structures in the ameloblastic secretion (which is at first homogeneous, as shown in fig. 3) is seen in the form of a number of pyriform bodies whose apices lie towards the dentine. These are obviously early stages of the structures seen in the developing enamel in figs. 18 and 19, and they are, therefore, the first appearance of the fibril-forming layer (*f.f.l.*) in optical section. The fibrils appear to be formed by the coalescence of minute droplets or globules, which arise within the interalveolar septa.

The fibril-forming layer just described is evidently identical with the structure seen by Tomes lying on the surface of forming enamel, and which he has described as a "sort of honeycomb, the septa of which take the stain strongly. The Tomes' processes and their continuations, the fibrils, enter the spaces of the honeycomb, and in favourable sections which happen to be transverse to the honeycomb are seen to fill the holes. This conspicuous honeycomb is exactly the same thing as the fenestrated membrane already alluded to in human and other Mammalian enamels. It is . . . . a transitional structure belonging to one—the earliest—stage of enamel calcification, and when a little further advanced towards completion, it loses its

resistant power and becomes dissolved by the decalcifying agent." Later (2, p. 117) he writes that it "is not a continuous sheet; at least, it is a much perforated sheet, the perforations corresponding in size and position with the ends of the forming enamel prisms."

Mummery (4), though he misses the correct interpretation, gives a description which is more accurate when he states: "The Tomes' fibres extending from the ameloblasts as broad prolongations of the fibrillar cell contents, as far as the honeycomb, have 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, turn at an angle to their former course, and extend all across the wide area of the forming enamel, to the dentine." He regards the honeycomb as developed to act as a "directing membrane or guiding frame to control the orderly arrangement of the folding prisms and spaces between them.

That the conceptions of both Tomes and Mummery are incomplete and erroneous is apparent on examining such sections as are shown in the drawings which appear in the accompanying plates.

Changes in the Ameloblastic Secretion subsequent to Fibril Formation.

I have endeavoured to show that the first layer of the ameloblastic secretion to be deposited on the dentine is homogeneous, but that, very early, structural differentiation takes place, with the result that it takes the form of a layer having an open-network pattern, and that this layer gives origin to the fibrils characteristic of Marsupial enamel.

The physical changes which take place in the residue of the ameloblastic secretion, *i.e.* the transparent fluid coagulum occupying the spaces between the fibrils, after formation of this layer, are responsible for the typical structure of the enamel, for there is no evidence that the ameloblasts have any direct influence in affecting the pattern assumed in the completely calcified tissue.

In dealing with the calcification of enamel, we are handicapped by the lack of definite, well-authenticated data as to how the lime salts are attracted from the blood and conveyed to the cells of the enamel-organ; what changes they undergo therein; the chemical composition of the secretion which is shed from the ameloblasts; and the agency or agencies by which the colloidal secretion is precipitated.

We have no indisputable chemical analysis of the completed enamel, since the results cited in text-books have been arrived at by the employment of the ashing method which, as Gideon Wells (12) has pointed out, gives most misleading results where more than one salt is concerned and where organic material may be present.

Further, we possess no exhaustive examination of its optical properties, such as Römer (13) has given us for shell-structures in Mollusca, a research carried out with the advantage of accessibility to the advice and direction of Prof. Bütschli, whose

great authority on all questions relating to organic calcification is embodied in his memoir, 'Untersuchungen über Organische Kalkgebilde' (14).

Even the question of the structure of the completed enamel is disputed, for though the majority of those who have studied this tissue believe in the existence of an interprismatic material, yet Otto von Walkhoff (15), a cautious and careful observer, whose opinions command the utmost respect, reverts to the view of Waldeyer, and denies the existence of such a substance.

Also opinions are divided concerning the origin of the structural basis of enamel, one school holding that it is formed by a deposition of lime in the distal area of the cytoplasm of the ameloblasts, which is replaced by a further growth around the nucleus—a view usually known as the conversion theory—and another school holding that enamel is formed from a substance shed by the enamel cells—the secretion theory. The views of Tomes and Mummery on enamel calcification, quoted earlier in this paper, seem to be a blending of the views held by both schools.

The progress of knowledge is retarded rather than advanced by speculative theories based on inconclusive experiments and incomplete data, and, with such meagre knowledge as we possess concerning enamel, one writes with the greatest reserve when endeavouring to interpret the appearances seen in the progressive solidification of the tissue. Whilst not attempting to advance a complete theory of enamel calcification, the more obvious changes which the secretion undergoes may be safely described, corroborated as they are not only by the illustrations attached to this paper, but also by a considerable amount of material of enamel development in other Vertebrates.

The effect of precipitation from the ameloblastic secretion of a considerable portion of its organic material to form the fibrils must be to increase the concentration of calcium salts in the remainder of the secretion. This concentration is accompanied by a further organic precipitation or gel formation, which assumes a definite honeycomb pattern, and is obviously of a different chemical nature to the fibrils, for it is easily dissolved and, whilst taking the basic stain with difficulty, destains very readily.

These changes are well shown in fig. 27, drawn from a section of developing enamel of *Macropus ruficollis*, cut without previous decalcification, and stained with hæmatoxylin, a stain which is, as Adami has pointed out, an excellent general microchemical test for calcium.

The ameloblasts are seen in longitudinal section, but the forming enamel is cut obliquely, since, as may be seen by reference to fig. 8, the axis of the cell does not correspond to the direction of the forming rods.

Lying immediately beneath the ameloblasts is the fibril-forming sponge work layer (*f.f.l.*), much more uniform in the size of its alveoli (*alv.*) in this section than is usually the case. These alveoli are not empty, as the drawing would seem to show, but are occupied by a transparent substance faintly tinged by the acid stain.

A little deeper the alveolar appearance has disappeared, giving way to a number of more or less irregularly shaped areas (*e.r.*), deeply stained by the hæmatoxylin, and lying in a transparent medium.

Yet deeper these areas have become more disperse, are more or less polygonal, and do not stain so deeply.

The areas (*e.r.*) in question appear as the result of a change in the substance occupying these alveoli, and the clear region about them is misleading, since it suggests either that they lie in a fluid medium which has been dissolved, or that they were originally in contact, but have undergone shrinkage, and drawn apart from one another, to again swell a little farther within the forming enamel.

Actually this clear area is a definite structure, hyaline in nature, and its invisibility is due to that fact that it does not stain readily, or rather destains very easily. This may be demonstrated by using iron hæmatoxylin as a stain, when the whole area of devoloping enamel stains very deeply, but in differentiation the matrix destains very rapidly and completely, causing the areas (fig. 27, *e.r.*) to appear as though spaces existed between them. By checking differentiation at the right moment, the matrix (*i.p.m.*) may be demonstrated to be a continuous layer forming a honeycomb the walls of which are more or less hexagonal (fig. 19), and in its cavities lie the more deeply staining structures (*e.r.*), which are identical with those seen in fig. 27.

The real nature of these areas is shown by comparing such sections shown in fig. 27 with fig. 28, which is a drawing of a section of developing enamel of *Macropus ruficollis* showing the first organic coagulation constituting the fibril-forming layer (*f.f.l.*), the meshes of which are comparatively large when compared to the long straight dark bands, alternating with light ones (*e.*), which constitute the remainder of the figure. In fig. 29 we have a drawing of a section of partially decalcified enamel from the incisor of the same specimen of *Macropus ruficollis* from which fig. 28 was prepared, and here it is evident that the dark areas (*e.r.*) seen in this figure are identical with those seen in fig. 27 (*e.r.*). In both cases these areas are the developing enamel rods seen in transverse or oblique sections, and lying between them is the honeycomb matrix.

The enamel rods would appear to arise by changes in the homogeneous contents of the alveoli of the fibril-forming layer (fig. 27, *f.f.l.*) lying just beneath the ameloblasts. They are obviously not continuous with the cytoplasm of the ameloblasts, for in none of my sections is there any evidence of well-defined structures lying in the spaces of the alveoli; on the contrary, as stated above, these are entirely filled by a transparent faintly-stained coagulum. In forming Marsupial enamel there is no trace of the existence of such prolongations of the cytoplasm of the ameloblasts as those on which Tomes has based his theory of enamel development.

Fig. 27 of this paper evidently corresponds to such sections as those of which Tomes (11, p. 113) has given the following description:—

"It will be noticed that in these oblique sections, the partitions of the honeycomb

are well stained, as they usually are; but as we progress further into the more formed enamel, the corresponding areas become light, and it is the contained fibre alone that is stained. In fact the septa have almost disappeared under the action of the decalcifying acid, and still further, in yet more developed enamel, they quite disappear, leaving the fibres free."

Also, "In the openings of the honeycomb dark bodies, the fibres cut across occur in many places, whilst in others they have fallen out; but enough remains to show their identity with the row of dark oval bodies which, with light interspaces, constitute the older more solid portion."

Evidently the areas seen in fig. 27 (*e.r.*) are identical with those which TOMES describes as fibres, and which, in his opinion, are prolongations of the ameloblastic cytoplasm usually termed Tomes' processes.

As stated earlier in this paper, I believe we have the strongest evidence that Tomes' processes are not cytoplasmic, but are purely metaplasmic. This view is corroborated by the study of sections such as the one we have under consideration.

Moreover, there is no evidence of the enamel cells secreting any product except the ameloblastic secretion, nor is there any evidence of the pouring out of a substance about the prisms, a view which both TOMES and MUMMERY hold, though neither of these authors gives any indication of its origin. If this latter view were correct, spaces must necessarily be present between the prisms, but I have never seen the slightest evidence of the existence of any such spaces, not even at the amelo-dentinal surface, an area which MUMMERY regards as possessing them, and on the existence of which he has founded his theory of dentinal penetration. With the first precipitation or coagulation (which I have endeavoured to show gives origin to the "fibrils") the interstices are seen, in carefully fixed tissues, to be occupied by a clear, well-stained substance, which ultimately undergoes calcification, to form the prismatic enamel. Since the process of fixation is a process of coagulation, it is probable that, with imperfect fixation, this substance may be washed away or dissolved, leaving what appears to be a space. In the great number of sections which I have examined, I have never seen a single case where there was any appearance of such a condition, and have always found Marsupial enamel to be as complete in its structure as that of other Mammals.

The forming rods appear as a very fine granular precipitate, which gives a marked reaction to all the microchemical tests for calcium. The honeycomb matrix does not consist, in Marsupials, of thin solid laminæ forming the walls of the cavities of the honeycomb (as there is some evidence in favour of its so doing in most Mammals), but appears to exhibit a microstructure in some parts identical with its gross structure, whilst in others its structure would seem to be open spongework, the cavities of which communicate with one another. On this point I would wish to write with reserve, for, in the examination of partially calcified material, the difficulties of technique are very great, and the possibilities of causing artificial

appearances are many. Consequently, it is unsafe to lay down definite conclusions until ample confirmation has been accumulated.

The enamel of Higher Mammals is usually laid down slowly, and coincidently undergoes almost complete calcification. In Marsupials, however, this is not the case, for the enamel matrix is laid down very rapidly, practically the whole thickness of the tissue being deposited, whilst but a slight amount of dentine has been formed. The outline of the enamel cells and the developing enamel is at all times regular.

If, however, an unerupted tooth is removed from its bony crypt in a dried skull, the enamel is seen to be disposed in a series of prominent ridges, running parallel to the long axis of the tooth. This appearance can only be interpreted by assuming that the forming enamel has undergone incomplete calcification, and so has become distorted in drying. Conclusions based on the microscopical examination of enamel from such a source are thus open to suspicion.

At this point the record of direct observations ends.

To recapitulate briefly, we have at first, lying between the ameloblasts and the dentine, a homogenous layer, the ameloblastic secretion, which undergoes coagulation with the result that a spongework skeleton is formed, the meshes of which are usually many times larger than the area of the base of a formed enamel prism, and it is in the walls of this spongework that the fibrils arise. In its interstices there lies a transparent substance continuous with the contents of the neighbouring cavities of the spongework.

Later, this transparent substance also undergoes a further coagulation, which assumes the form of a honeycomb, the cavities of which are not continuous with one another, and in transverse section are much smaller than the cavities of the spongework, but are of the size of the completed enamel prisms, of which they form the skeleton.

Thus two distinct coagulations occur, the first having a comparatively coarse and irregular structure, whilst the second is much finer and more regular in pattern, the latter lying within the former.

The secretion in which these changes take place is a colloidal fluid comparatively rich in organic material, and though at present we have no definite knowledge of the precise agency or agencies by means of which the transformations described above have been brought about, yet they are in conformity with similar changes observed in the solidification of other colloidal media, where these agencies are known.

It is a well-established fact that the proteins in blood serum are separated out by the addition of certain salts, and that the proteins precipitated vary with the degree of salt concentration. When ammonium sulphate is employed, precipitation of the globulins commences with a salt concentration of 24 per cent., and is completed at 36 per cent., and on increasing the concentration to 70–80 per cent. the albumins are precipitated (Fenton).

Further, certain protein gels take the form of an open network, which is the

structure of the layer forming the fibrils described in this paper, whilst other proteins give rise to gels having the form of a honeycomb, which is the structure observed in that coagulation which affords the skeleton of the developing enamel prisms.

To arrive at a precise knowledge of the agencies effecting the changes seen in the progressive solidification of the enamel, it is necessary to grow tooth germs artificially, when, with a medium of which the chemical constitution is known definitely and which can be controlled and varied, it is reasonable to hope that the factors determining such changes may be solved.

This investigation is in hand, and I have been able to grow the isolated germs *in vitro* successfully; research on these lines is necessarily slow, but until definite results have been obtained by such methods, it is unlikely that a complete theory of enamel calcification can be evolved.

The honeycomb matrix must possess a strong adsorption affinity for calcium salts, and this results in its ultimate conversion into a tissue in which the percentage of organic material is extremely slight, for when sections of adult Marsupial enamel are subject to a decalcifying agent, the whole of the tissue, excepting the fibrillar system, is dissolved. The probability that the honeycomb is not solid, but has a micro-structure similar to its gross structure, shows that by this means a relatively great surface is afforded for the action of adsorption.

Thus it seems most probable that calcification of the enamel begins as an adsorption process due to surface phenomena, where the salts are deposited on or in an organic matrix, which has assumed a honeycomb form in conformity with the changes which take place in the coagulation of colloids. It thus differs from the method of formation of true crystals, since at present we have no evidence that the rods grow by accretion on the surface of a nucleus of the same nature as the crystallising substance.*

As previously stated, Tomes considers the fibrils to lie within the prisms, whilst Mummery considers them to lie in the interprismatic material.

Tomes, however, alluding to microphotographs of Marsupial enamel prepared by Leon Williams, admits that they show the tubules "undoubtedly lying within the prisms and also in the interprismatic material." In my own sections, prepared by grinding adult teeth, this is undoubtedly the case.

The view of the origin of the fibril system and the subsequent changes in the enamel-forming secretion outlined in this paper would account for the presence of the "fibrils" either within the prisms, in the interprismatic material, or in both.

The presence of a system of fibrils in the enamel is associated with certain characteristic changes in the ameloblasts during their functional life, which are in turn associated with an extremely rapid development of the enamel. As a rule, where the fibrils are present, the milk-dentition is in a more or less completely suppressed condition, not only in Marsupials, but also in other Mammals possessed of fibrillar enamel.

* It is my pleasure to acknowledge my deep indebtedness to Prof. Bayliss, F.R.S., and Dr. H. J. H. Fenton, F.R.S., for their advice and direction on the physico-chemical side of this paper.

Such, briefly, are the more important changes passed through by the ameloblasts during their functional life, and of their secretion in its transformation into the completely formed enamel, but there are certain features in the changes which take place in the cells constituting the stellate reticulum which throw light on the origin and nature of the ameloblastic secretion.

The Stellate Reticulum and its Blood Supply.

The cells constituting the enamel organ are not morphologically distinct from one another; those of the external epithelium merge into the cells of the stellate reticulum, and these again into the cells of the stratum intermedium. It is true that the cells constituting the stellate reticulum have a very distinctive appearance, but this is due to the gradual accumulation within their cytoplasm of globules of metaplasmic material, which coalesce and force the cytoplasm of neighbouring cells into contact with one another until they appear to merge.

In very thin sections the stellate reticulum appears as a net-like structure with the nuclei at the nodal points: in thicker sections it appears as an alveolar structure, the walls being composed of the cytoplasm of the cells (fig. 20), and the alveoli occupied by the metaplasmic material destined to be eventually elaborated by the ameloblasts, and to furnish the material of which the enamel is formed. It thus acts as a storehouse, attaining large dimensions before the ameloblasts have undergone much differentiation, and before there is any appearance of calcification in either enamel or dentine.

Leon Williams (7, p. 287) states that the "reticulum of the enamel organ is not composed of cells which have been changed from a polygonal to a stellate form. The stellate structure is simply the intercellular substance which is left after the removal of the cell contents."

This explanation is incorrect, for it is a simple matter to trace the assumption of the stellate form from the simple cell, and to see that the change of its shape is due to the gradual accumulation within its cytoplasm of metaplasmic contents, and further, to observe the resumption of the original form after the metaplasm has been given up to the enamel cells. Also Leon Williams' own illustrations (7, pp. 289 and 309) show the nuclei surrounded by cytoplasm connected by processes with that investing neighbouring nuclei.

When functional activity has begun with the deposition of their secretion, the ameloblasts rapidly recede from the dentinal surface into the stellate reticulum, taking up and elaborating the material accumulated within these cells. This transference is usually through the medium of the cells of the stratum intermedium, though this is not invariably the case, for, when growth is very rapid, the stellate reticulum (fig. 7, *s.r.*) may be seen in contact with the ameloblasts (a) without the intervention of the stratum intermedium. In properly fixed material, there is never any sign of the nuclei undergoing disintegration whilst still distributed throughout

the stellate reticulum, but, with the rapid utilisation of these cells and the disappearance of their metaplasmic contents, they lose their typical form, and lie about the bases of the ameloblasts, interspersed with and indistinguishable from the cells of the stratum intermedium.

Lying just outside the external epithelium blood-vessels are to be seen, frequently so richly developed as to form a capillary system closely investing the whole enamel organ. With the progress of enamel deposition, these capillaries penetrate into the stellate reticulum, where they branch (fig. 21), their ramifications extending often as far as the cells of the stratum intermedium.

Hopewell-Smith and Marett-Tims (9), referring to the vascularity of the enamel organ in *Macropus billardieri*, state that they were unable to trace the capillaries beyond a point midway between the outer and inner enamel epithelium, but, in my preparations, vessels of a lumen from 20 μ to 30 μ in diameter are frequently seen lying in contact with the cells of the stratum intermedium.

The capillary walls are seen to be formed of a single layer of endothelial cells, and lying immediately in contact with them are the cells of the stellate reticulum, so that these can readily assimilate the lime-bearing fluid derived from the blood.

Summary.

The organic "fibrils" present in Marsupial enamel, and extending almost but not quite to its free surface, are not fortuitous in origin, but constitute a well defined system, and arise from the coagulation or gel-formation of an organic substance not usually present in the colloidal secretion shed by the enamel cells of most other Mammals.

This coagulation takes the form of a spongework, and the "fibrils" arise within its walls; they follow the outline of the alveoli of the spongework, and so pursue a sinuous spiral course, which persists in the completely calcified enamel. Their mode of origin also accounts for the dilated "joint" and abrupt bend so often seen at the point of junction with the dentine.

The fibrils are resistant to acids and alkalis, usually attract an acid stain, are precipitated at a relatively small concentration of calcium salts, for which they exhibit a feeble adsorption affinity, as is shown by the fact that they do not become impregnated by the calcium, and in ground sections of fully formed enamel they frequently become detached, as though they lay imbedded in the calcified matrix, but were not attached intimately to it.

The development of this system of "fibrils" is associated with certain well-defined changes in the enamel-cells not seen in the development of non-fibrillar enamel for these cells—the ameloblasts—pass through a stage where they undergo a great increase in length, the greater part of the cytoplasm becomes transformed into metaplasm, a fine cytomitoplasm remaining, whilst the cells lose their marked individual columnar form. Coincidently the nuclei become much reduced in size, and in the greater number of cases assume an irregular form.

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Following this stage of cytoplasmic differentiation the ameloblasts pass through a stage of rejuvenation, in which the cytomitoplasm is probably the active agent, since it is seen to break down into granules from which, or about which, the new cytoplasm is built up, and the cells once more resume their columnar individual forms separated from their neighbours by a well-marked interval occupied by an intercellular fluid.

In the earlier stages of this rejuvenation the nucleus lies in a neutrophile area, which is, however, traversed by faintly staining fibrils terminating in the nuclear membrane, and it is at the expense of this area that the nucleus recovers its original shape and structure.

This rejuvenation of the ameloblasts is coincident with the dissolution and disappearance of certain cells of the stratum intermedium, and since these cells are connected with the ameloblasts by means of cell-bridges, it is probable that the products of the disintegration of the cells of the stratum intermedium are fundamental in the rejuvenation of the ameloblasts.

With the commencement of functional activity the cytoplasm of the end of the ameloblasts lying towards the forming enamel becomes modified to act as a cell-membrane, and is characterised by a densely granular structure; where these specialised areas of cytoplasm come into relation with those of contiguous cells, structures are developed which close these intercellular spaces. Consequently any product of ameloblastic activity can only emerge from the cell by dialysis through this specialised layer of cytoplasm.

Employing the technique ordinarily adopted for investigating enamel development, it is usual to find structures passing from the cell-membrane described above towards the surface of the developing enamel. These structures, usually designated Tomes' processes, are not prolongations of the cytoplasm, but are forms assumed by the ameloblastic secretion, and are a purely metaplasmic product. They are not seen in the later stages of the functional life of the ameloblasts, and when special precautions are adopted to prevent unequal shrinkage, the secretion does not assume such forms, but appears as a continuous layer.

The enamel is regarded as the product of changes similar to those passed through by other colloidal media in solidification, *i.e.* a continuous phase, organic in nature and of a honeycomb structure, being produced by gel formation, in the cavities of which lies a dispersed phase rich in calcium but poor in organic material.

The enamel appears to calcify from adsorption of the calcium into the organic matrix, and this proceeds until eventually the cavities of the honeycomb are completely filled and obliterated, when the pattern of the fully-formed tissue can be made evident only by the use of a decalcifying agent, which acts unequally on the rods and on the interprismatic material.

The fibrils appear to lie both within the substance of the rods and in the meshes comprising the interprismatic material, and they are not present in the outer zone of the enamel which is laid down by the ameloblasts after their rejuvenation.

In conclusion, it is my most pleasant duty to record my deep obligation and grateful thanks to Prof. HILL, F.R.S., for an unlimited supply of material and for much invaluable advice; to Miss MABEL RHODES, of the Lister Institute, for the beautiful drawings used to illustrate this paper; and to Mr. F. J. PITTOCK, of the Zoological Department of University College, who has placed his great skill and experience in all branches of micrology at my disposal throughout an extended research, of which the observations embodied in this communication form but a small part.

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DESCRIPTION OF PLATES.

All the drawings were made direct by means of the Abbé camera lucida.

The following is a list of the reference letters common to the various figures :—

a. Ameloblasts.
alv. Alveolus.
a.s. Ameloblastic secretion.
cap. Capillary vessel.
c.b. Cell-bridge.
c.m. Cell-membrane.
cmt. Cytomitoplasm.
cyt. Cytoplasm.
d. Dentine.
d.p. Dentinal papilla.
e. Enamel.
e.e. External epithelium of the enamel organ.
e.r. Enamel rod.
f. Fibril.
f.f.l. Fibril-forming layer.
i.a.m. Inner ameloblastic membrane.
i.p.m. Interprismatic material.
lam. Lamellæ.
n. Nucleus.
n.a. Neutrophile area.
nlo. Nucleolus.
o.a.m. Outer ameloblastic membrane.
s.i. Stratum intermedium.
s.r. Stellate reticulum.
t.b. Terminal bar.
t.p. Tomes' process.
vac. Vacuole.

Plate 7.

Fig. 1.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the relatively large size of the nucleus (*n*), and the chromatin dispersed throughout the karyoplasm in the form of minute fragments. ×1000.

Fig. 2.—*Dasyurus viverrinus.* Head-length 12·5 mm. Longitudinal section, showing the margin of the bell-shaped enamel organ, slightly separated from the dentinal papilla (*d.p.*).

The nuclei of the ameloblasts (*a*) are relatively large and elongated in shape, with the chromatin dispersed as in fig. 1.

At the junction of ameloblasts with the external epithelium (*e.e.*) the nuclei are seen cut across transversely. The central area of the enamel organ is occupied by cells which become differentiated into the stellate reticulum (*s.r.*) and stratum intermedium (*s.i.*). Many of the nuclei have assumed a markedly elongated shape. × 600.

Fig. 3.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section, showing the columnar ameloblasts (*a*) with nuclei (*n*) of relatively large size, and the cytoplasm undergoing differentiation and becoming faintly fibrillar.

At their bases lie the cells of the stratum intermedium (*s.i.*), connected with one another by means of cell-bridges (*c.b.*), and on the right of the section similar bridges are seen connecting these cells to the bases of the ameloblasts.

The cells are seen to terminate in a well-defined margin, beyond which lies a continuous faintly granular layer (*a.s.*), which is the first deposition of the ameloblastic secretion. × 1500.

Fig. 4.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section through the ameloblasts (*a*), drawn from another part of the same enamel organ as fig. 3.

The cell has increased greatly in length, and the fibrils of the cytoplasm are more distinct.

The nuclei are relatively and actually much smaller than in the preceding stage. The cells terminate in a well-defined margin, and in place of the continuous layer (*a.s.*) seen in fig. 3, well-defined processes (*t.p.*)—the Tomes' processes—are seen stretching across to the forming enamel. × 600.

Fig. 5.—*Macropus ruficollis.* Head-length 6 cm. Section through the enamel organ at a fold in the surface of the enamel, showing the ameloblasts in longitudinal and transverse section. The well-defined cell outlines of the previous stage have disappeared, and their place is taken by a coarsely fibrillar cytomitoplasm (*cmt.*) and vacuoles (*vac.*) in the cytoplasm.

The nuclei have further decreased in size. Where the ameloblasts are seen in longitudinal section they are seen to be separated from the cells of the stratum intermedium by a sharply defined border (*o.a.m.*), but where the section is transverse there is no sign of any separation of the two layers. × 450.

Fig. 6.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts have decreased greatly in length. The cytoplasm at the base of the cell is alveolar, whilst towards the secreting surface it is coarsely fibrillar, and the cell-outlines are indistinguishable. The nuclei (*n*) have lost their typically oval form, and fibrils of the cytomitoplasm

(*cmt.*) terminate in the nuclear membrane. There is no sign of branching of the nucleus. × 600.

Fig. 7.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts (*a*) have further decreased in length from the stage shown in fig. 7, and the nuclei (*n*) are more deeply chromatic and irregular in shape. The fibrils of the cytomitoplasm (*cmt.*) are seen to be assuming an arrangement which foreshadows the resumption by the cell of a columnar form. The cells of the stratum intermedium are absent, and the processes of cells of the stellate reticulum (*s.r.*) are in continuity with the bases of the ameloblasts (*a*).

Fig. 8.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts are seen to have increased in length, and though the outlines of individual cells are not defined, the fibrils of the cytomitoplasm (*cmt.*) pass from the nucleus (*n*) in a more or less direct line to the secreting end (*i.a.m.*) of the cell, where they terminate in small deeply basophile granules. The nuclei (*n*) are small and frequently irregular in shape. × 600.

Plate 8.

Fig. 9.—*Macropus ruficollis.* Head-length 2 cm. Various stages in the process of disintegration of the nuclei of cells of the stratum intermedium. In 1 and 2 the nucleus is shown as in active cells. In 3 the nucleolus (*nlo.*) is seen surrounded by a clear annular zone, whilst the chromatin has aggregated into large spherules. In 4 the chromatin has collected on the inner surface of the nuclear membrane, and the achromatic contents of the nucleus are visible. At 5 the karyoplasm has undergone coagulation and the chromatin has assumed a crescentic arrangement, whilst at 6 the nucleus is seen to have drawn away from the cytoplasm (*cyt.*), which is coarsely alveolar. × 1,500.

Fig. 10.—*Macropus ruficollis.* Head-length 2 cm. Longitudinal section. The ameloblasts (*a*) have increased in length from the stage shown in fig. 8, and have resumed their individuality. The cytoplasm is faintly fibrillar, and the area about the nucleus stains with difficulty. The nuclei (*n*) have increased in size and have again assumed their spherical or oval form, with the chromatin distributed in small spherules.

The cells of the stratum intermedium (*s.i.*), lying in contact with the bases of the ameloblasts (*a*), are seen undergoing degenerative change. The forming enamel (*e*), which stains intensely black, is seen lying in close contact with the cell-membrane (*i.a.m.*). × 600.

Fig. 11.—*Macropus ruficollis.* Head-length 6 cm. Longitudinal section. The ameloblasts (*a*) have once more become well-defined cells, separated from their neighbours by an intercellular area, which is traversed by

cell-bridges (*c.b.*) These pass not only from ameloblast to ameloblast, but also from these cells to the cells of the stratum intermedium (*s.i.*). The ameloblastic cytoplasm has become coarsely alveolar; it does not extend to contact with the nuclear membrane, being separated by a clear area (*n.a.*), which is traversed by fine fibrils. The ameloblasts are seen to vary in length. × 1000.

Fig. 12.—*Macropus ruficollis.* Head-length 6 cm. Longitudinal section. The ameloblasts (*a*) show the same features as in fig. 11, but cells from the stratum intermedium (*s.i.*) are seen to be inserting themselves between the ameloblasts. × 1000.

Fig. 13.—*Macropus ruficollis.* Head-length 6 cm. Transverse section through the ameloblasts (Stage VII). The cytoplasm is seen to be coarsely alveolar, the cavities (*vac.*) being occupied by a fluid which disappears in the later stages. The cell-bridges (*c.b.*) connecting the ameloblasts are very clearly shown. × 1500.

Fig. 14.—*Macropus ruficollis.* Longitudinal section through the apex of the enamel organ just prior to eruption. The ameloblasts (*a*) are seen gradually to lose their columnar form, first becoming cuboidal, then flattened.

The cytoplasm, which in the preceding stage was alveolar, has become faintly fibrillar, but as the cell loses its typical ameloblastic form, the fibrillar structure gives place to a finely granular cytoplasm. × 450.

Fig. 15.—*Macropus ruficollis.* Head-length 2 cm. Transverse section, showing the cells of the stratum intermedium varying in size and connected to their neighbours by intercellular bridges (*c.b.*) The interspace between the cells is occupied by a feebly staining acidophile fluid. × 600.

Fig. 16.—*Didelphys aurita.* Head-length 20 mm. Transverse section through the bases of the ameloblasts (*a*), showing this area of the cell modified to act as a cell-membrane (*c.m.*), the bases of the cells being separated one from another by the development of terminal bars (*t.b.*). × 600.

Plate 9.

Fig. 17.—*Didelphys aurita.* Head-length 20 mm. Oblique section through the ameloblasts, showing the nuclei (*n*), then the cell-bodies (*cyt.*), granular in appearance (the dots being optical sections of the fibrils of the cytoplasm), and in the lower portion of the drawing the finely granular cell-ends (*c.m.*) are seen with the terminal bars (*t.b.*) closing the intercellular areas. × 600.

Fig. 18.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the area of developing enamel lying between the ameloblasts (*a*) and

the dentine (*d*). The structures (*f.f.l.*), which taper in their course from the ameloblasts to the dentine, are the lamellæ of the layer which gives origin to the fibrils, seen in section. × 600.

Fig. 19.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the ameloblasts (*a*), the fibril-forming layer (*f.f.l.*) with its incomplete alveoli (*alv.*), the fibrils (*f*) pursuing a sinuous course, the forming enamel rods (*e.r.*) lying in the cavities of a honeycomb matrix—the interprismatic material (*i.p.m.*).

Fig. 20.—*Macropus ruficollis.* Head-length 20 mm. Longitudinal section, showing the spongiform structure of the stellate reticulum (*s.r.*).

Fig. 21.—*Macropus ruficollis.* Head-length 5·2 cm. Transverse section through the stellate reticulum (*e.r.*), showing branching of a capillary vessel (*cap.*) and the cells of the stellate reticulum lying immediately in contact with the endothelial capillary walls. × 450.

Fig. 22.—*Didelphys aurita.* Head-length 20 mm. Tangential section cut without decalcification. Lying immediately beneath the ameloblasts (*a*) is the fibril-forming layer (*f.f.l.*), showing continuity of the fibrils (*f*) past different alveoli. Beneath this layer the material, clear in the alveoli of the fibril-forming layer, is seen to have undergone precipitation. × 450.

Plate 10.

Fig. 23.—*Didelphys aurita.* Head-length 20 mm. Portion of the fibril-forming layer (*f.f.l.*) of fig. 22. The fibrils (*f*) may be seen following the outlines of the incomplete alveoli (*alv.*) × 1500.

Fig. 24.—*Trichosurus vulpecula.* Head-length 5·2 cm. Longitudinal section through a decalcified tooth-germ, showing the fibril system (*f*) passing from just beneath the ameloblasts (*a*) to the surface of the dentine (*d*). In some places the fibrils pursue an almost straight course, but the majority are seen to be sinuous. × 300.

Fig. 25.—*Trichosurus vulpecula.* Head-length 5·2 cm. Section slightly oblique to the long axis of the tooth, showing the alveoli (*alv.*) of the fibril-forming layer at the point of junction with the dentine (*d*). They are irregular in form and vary in size, and appear as fine granules arranged more or less hexagonally. × 600.

Fig. 26.—*Didelphys aurita.* Head-length 20 mm. Drawing of a vertical section through a developing incisor. A considerable area of dentine (*d*) has been laid down. Between the ameloblasts (*a*) and the dentine (*d*), and lying on the surface of the latter, are seen a number of pyriform structures (*f.f.l.*), between which lies a homogeneous transparent substance. These pyriform structures are the first appearance of the fibril-forming layer. (Compare figs. 18, 19 and 22). × 350.

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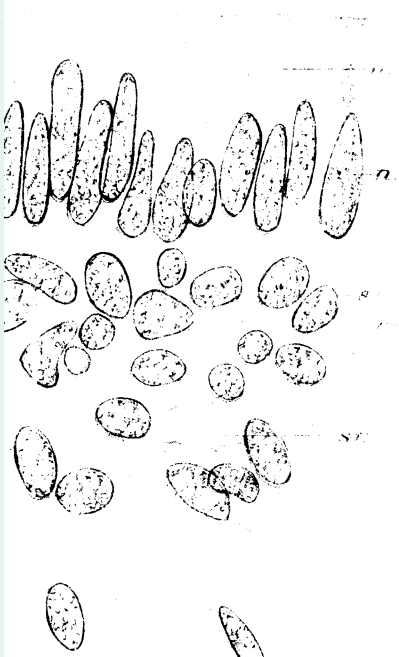

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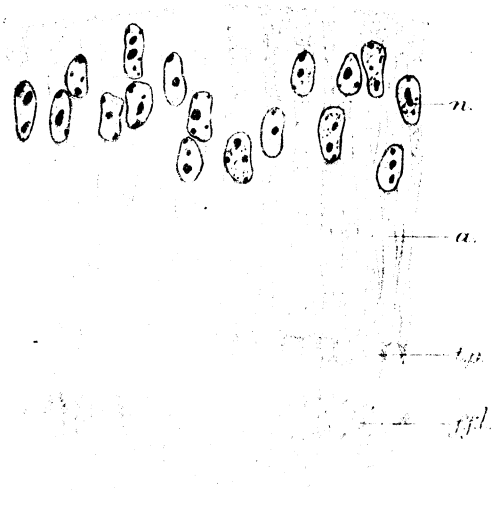

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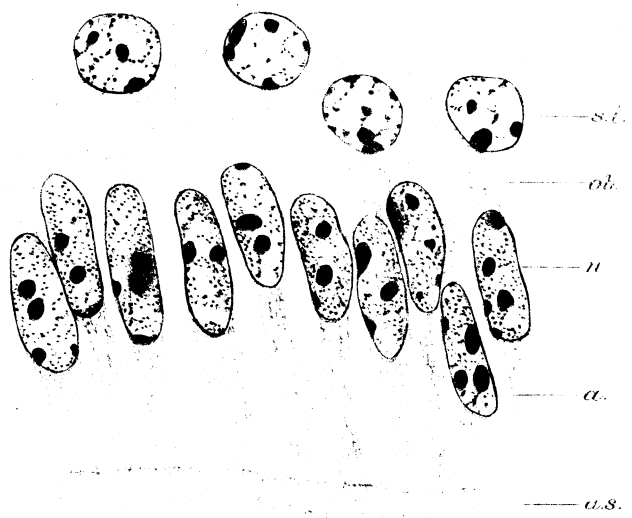

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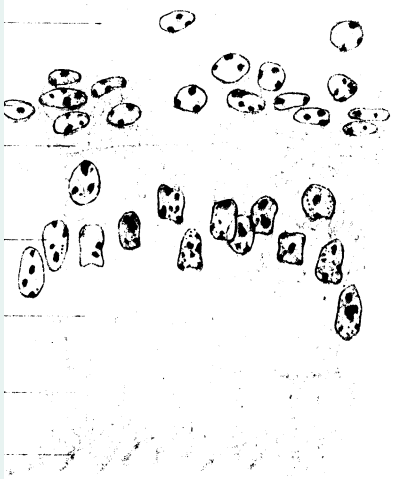

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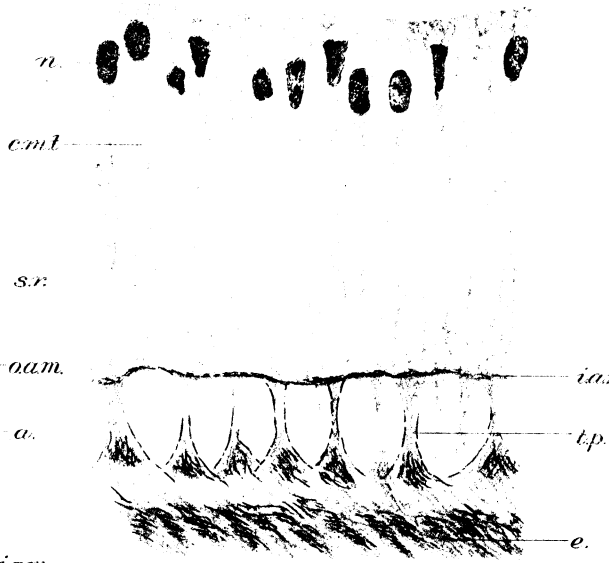

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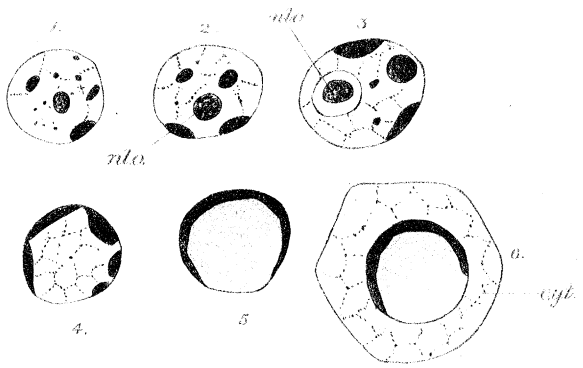

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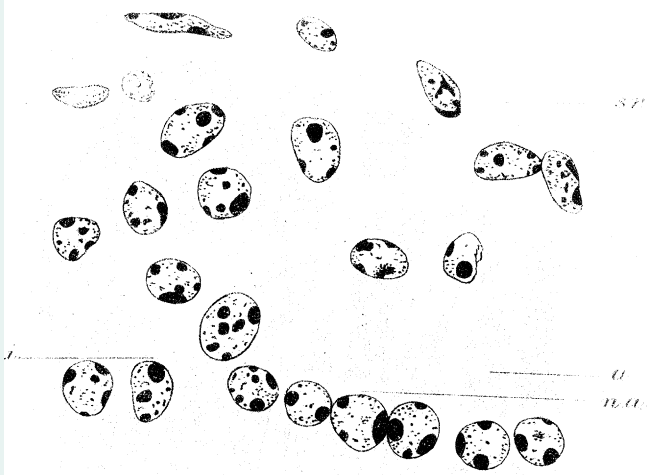

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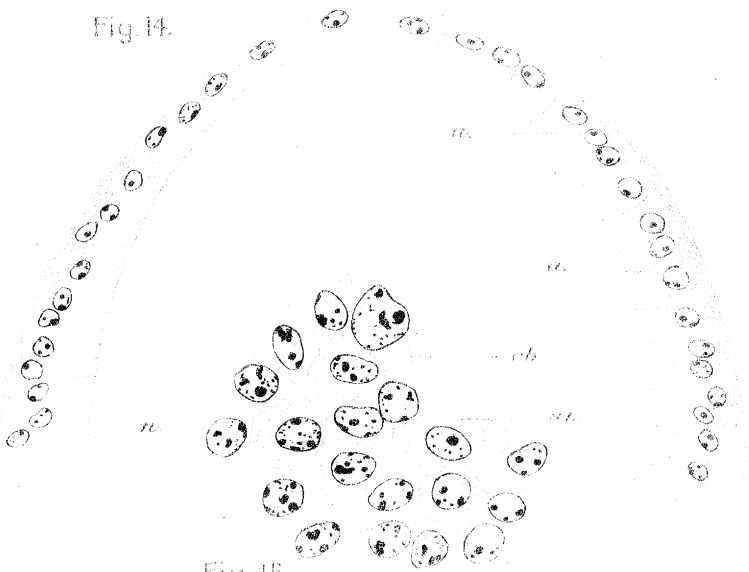

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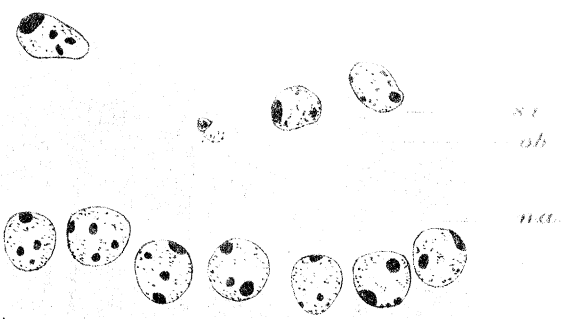

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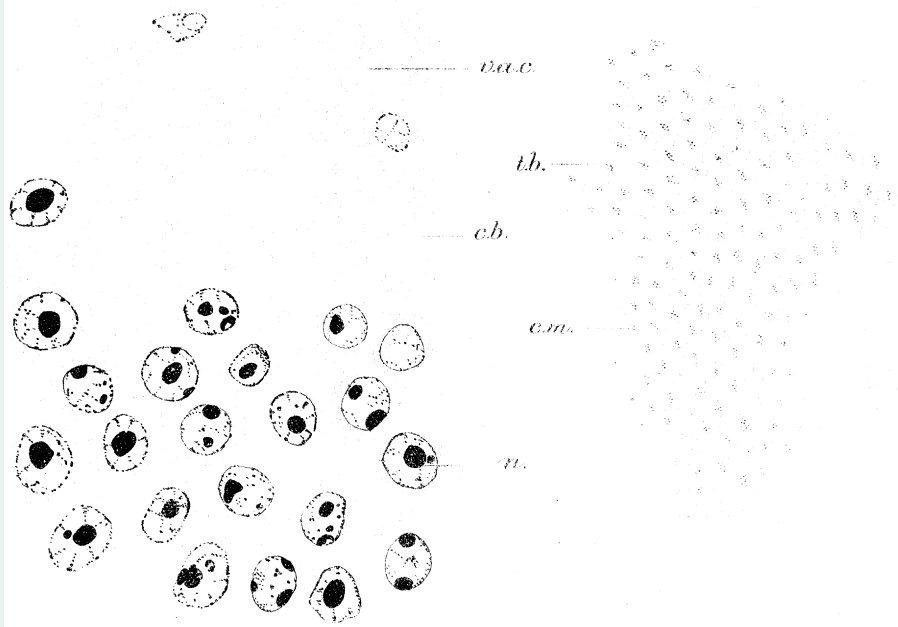

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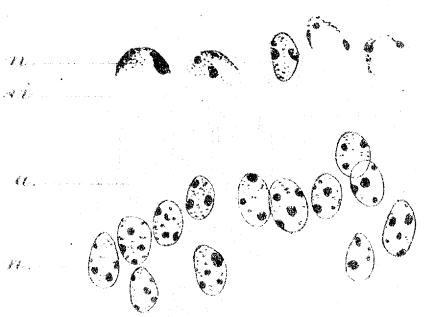

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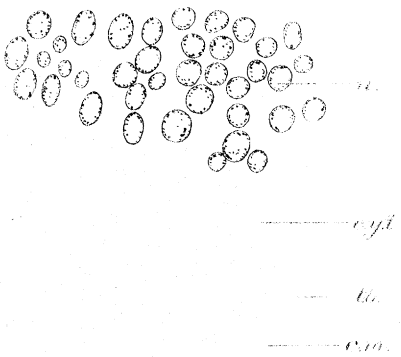

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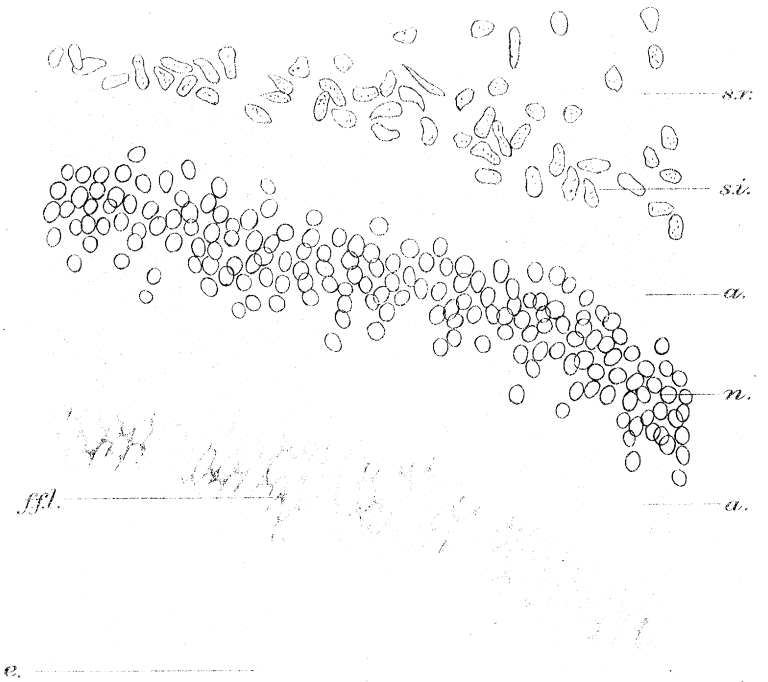

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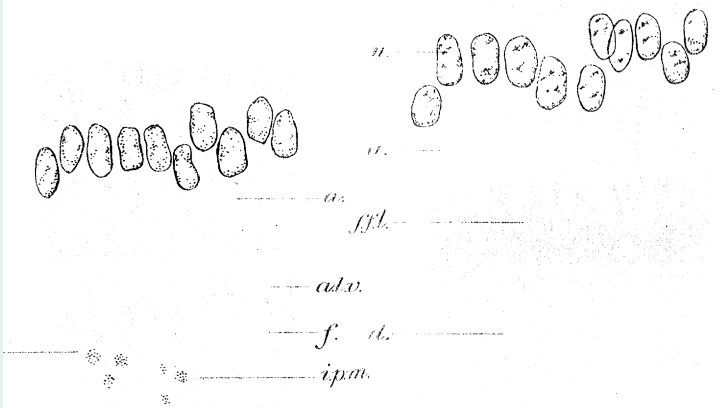

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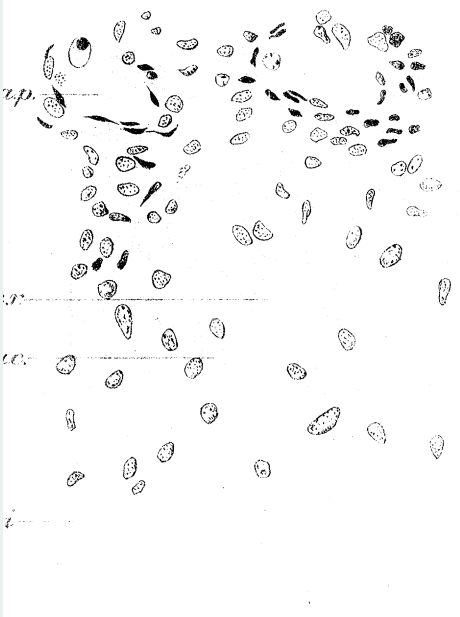
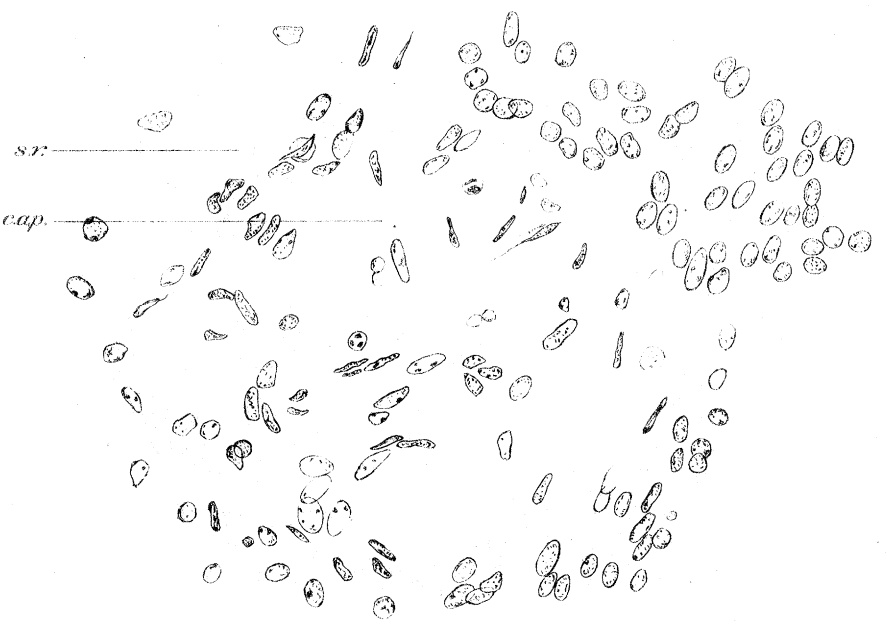


Fig. 20.



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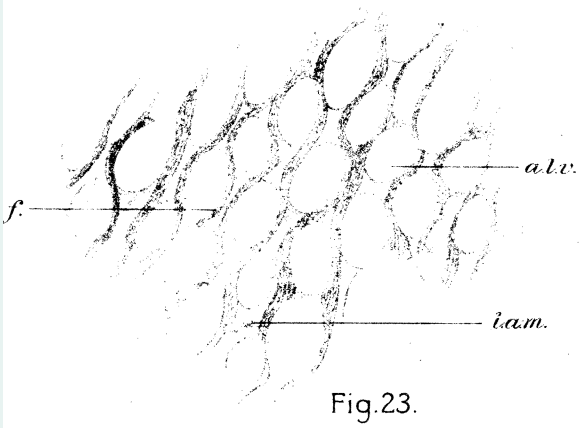
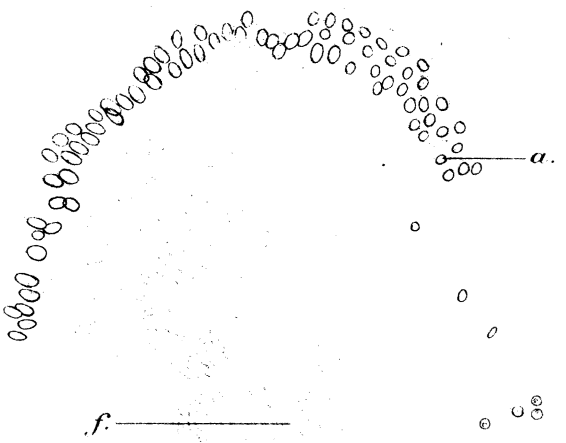


Fig. 23.



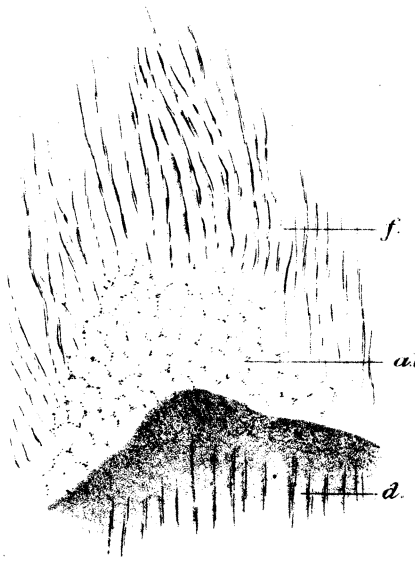


Fig. 25.

Fig. 24.

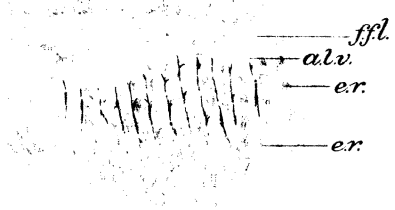


Fig. 27.

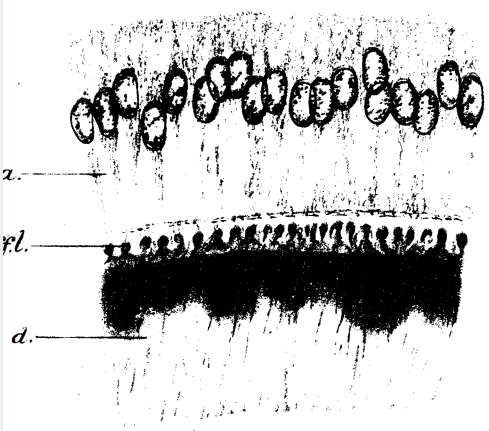
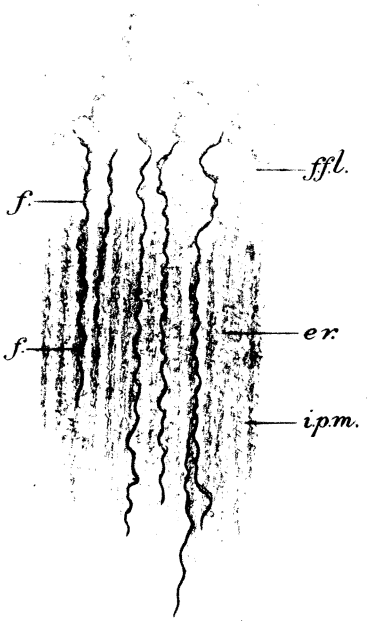


Fig. 26.



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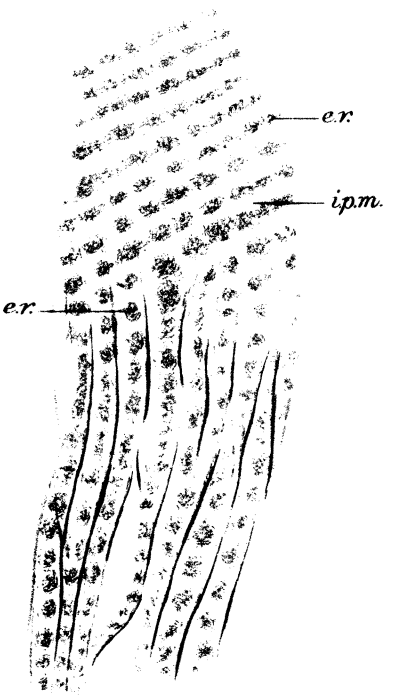

Fig. 29.

Fig. 27.—*Macropus ruficollis.* Head-length, 2 cm. Longitudinal section of ameloblasts and developing enamel, cut without previous decalcification. The ameloblasts (*a*) are seen closely packed together; beneath them is the fibril-forming layer (*f.f.l.*) which gives place to a number of dark areas (*e.r.*) lying between structures continuous with the fibril-forming layer. These apparently disappear a little deeper in the section, and the dark areas (*e.r.*) have become more diffuse and less deeply stained. × 600.

Fig. 28.—*Macropus ruficollis.* Vertical section through the margin of the developing enamel, showing the fibril-forming layer (*f.f.l.*) and the fibrils (*f*) passing into the enamel (*e*). The rods (*e.r.*) stain more deeply than the interprismatic material (*i.p.m.*). × 600.

Fig. 29.—*Macropus ruficollis.* Section of developing enamel. The rods (*e.r.*) are shown in longitudinal section in the upper portion of the figure, whilst in the lower portion their ends are seen in section. The interprismatic material (*i.p.m.*) is seen less deeply stained. × 1000.

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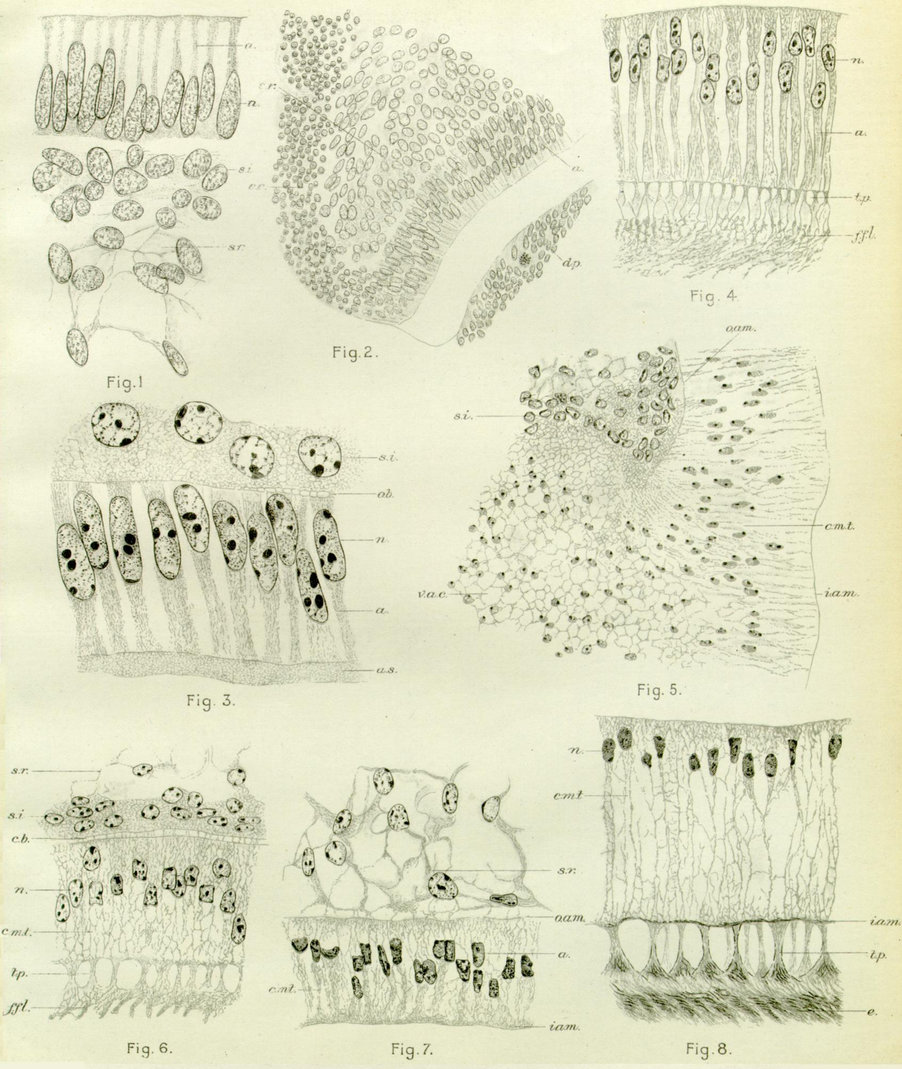


PLATE 7.

Fig. 1.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the relatively large size of the nucleus (*n*), and the chromatin dispersed throughout the karyoplasm in the form of minute fragments. × 1000.

Fig. 2.—*Dasypus viverrinus.* Head-length 12·5 mm. Longitudinal section, showing the margin of the bell-shaped enamel organ, slightly separated from the dentinal papilla (*d.p.*).

The nuclei of the ameloblasts (*a*) are relatively large and elongated in shape, with the chromatin dispersed as in fig. 1.

At the junction of ameloblasts with the external epithelium (*e.e.*) the nuclei are seen cut across transversely. The central area of the enamel organ is occupied by cells which become differentiated into the stellate reticulum (*s.r.*) and stratum intermedium (*s.i.*). Many of the nuclei have assumed a markedly elongated shape. × 600.

Fig. 3.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section, showing the columnar ameloblasts (*a*) with nuclei (*n*) of relatively large size, and the cytoplasm undergoing differentiation and becoming faintly fibrillar.

At their bases lie the cells of the stratum intermedium (*s.i.*), connected with one another by means of cell-bridges (*c.b.*), and on the right of the section similar bridges are seen connecting these cells to the bases of the ameloblasts.

The cells are seen to terminate in a well-defined margin, beyond which lies a continuous faintly granular layer (*a.s.*), which is the first deposition of the ameloblastic secretion. × 1500.

Fig. 4.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section through the ameloblasts (*a*), drawn from another part of the same enamel organ as fig. 3.

The cell has increased greatly in length, and the fibrils of the cytoplasm are more distinct.

The nuclei are relatively and actually much smaller than in the preceding stage. The cells terminate in a well-defined margin, and in place of the continuous layer (*a.s.*) seen in fig. 3, well-defined processes (*t.p.*)—the Tomes' processes—are seen stretching across to the forming enamel. × 600.

Fig. 5.—*Macropus ruficollis.* Head-length 6 cm. Section through the enamel organ at a fold in the surface of the enamel, showing the ameloblasts in longitudinal and transverse section. The well-defined cell outlines of the previous stage have disappeared, and their place is taken by a coarsely fibrillar cytomitoplasm (*cmt.*) and vacuoles (*vac.*) in the cytoplasm.

The nuclei have further decreased in size. Where the ameloblasts are seen in longitudinal section they are seen to be separated from the cells of the stratum intermedium by a sharply defined border (*o.a.m.*), but where the section is transverse there is no sign of any separation of the two layers. × 450.

Fig. 6.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts have decreased greatly in length. The cytoplasm at the base of the cell is alveolar, whilst towards the secreting surface it is coarsely fibrillar, and the cell-outlines are indistinguishable. The nuclei (*n*) have lost their typically oval form, and fibrils of the cytomitoplasm (*cmt.*) terminate in the nuclear membrane. There is no sign of branching of the nucleus. × 600.

Fig. 7.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts (*a*) have further decreased in length from the stage shown in fig. 7, and the nuclei (*n*) are more deeply chromatic and irregular in shape. The fibrils of the cytomitoplasm (*cmt.*) are seen to be assuming an arrangement which foreshadows the resumption by the cell of a columnar form. The cells of the stratum intermedium are absent, and the processes of cells of the stellate reticulum (*s.r.*) are in continuity with the bases of the ameloblasts (*a*).

Fig. 8.—*Macropus ruficollis.* Head-length 2·5 cm. Longitudinal section. The ameloblasts are seen to have increased in length, and though the outlines of individual cells are not defined, the fibrils of the cytomitoplasm (*cmt.*) pass from the nucleus (*n*) in a more or less direct line to the secreting end (*i.a.m.*) of the cell, where they terminate in small deeply basophile granules. The nuclei (*n*) are small and frequently irregular in shape. × 600.

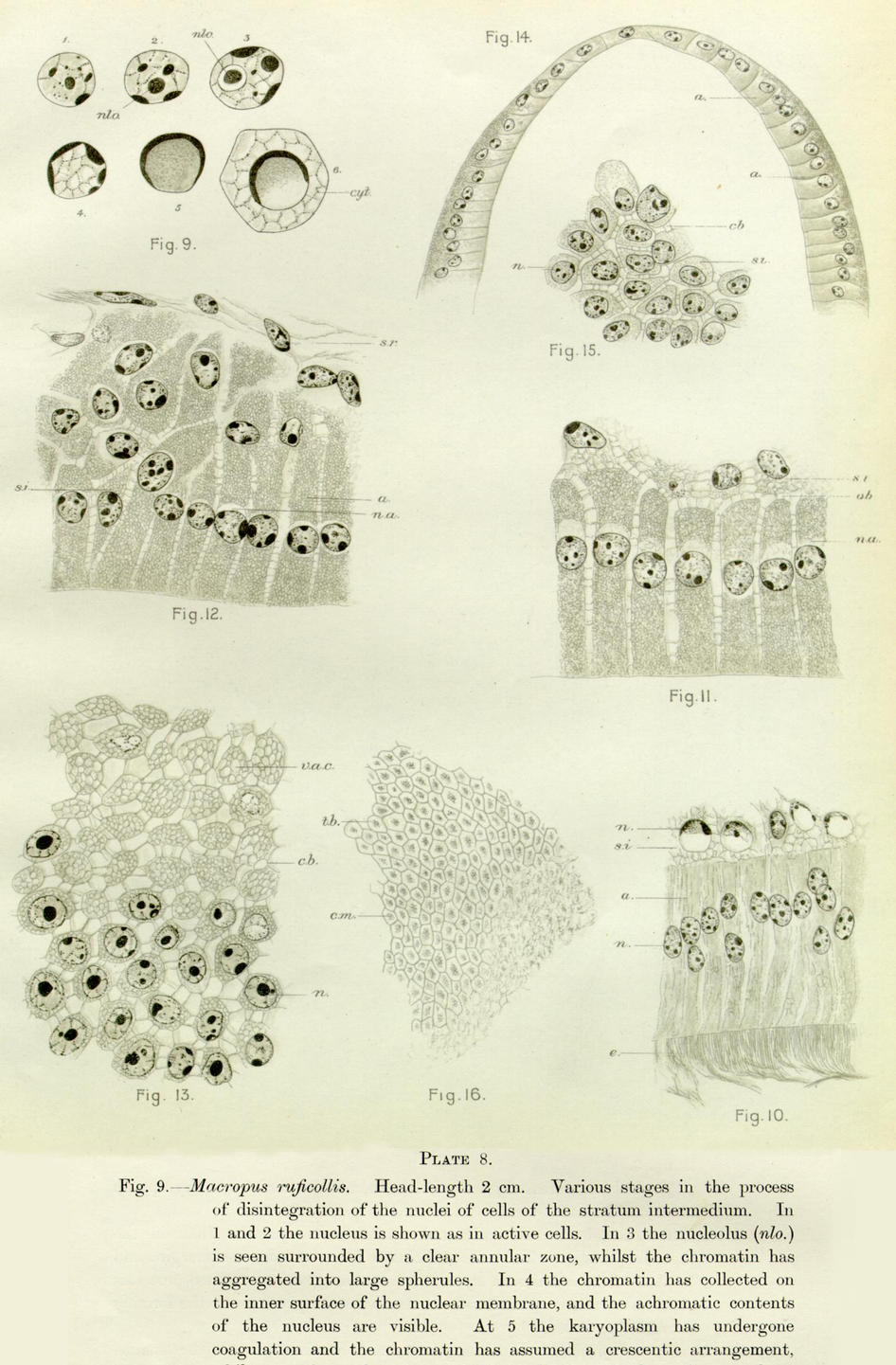


PLATE 8.

Fig. 9.—*Macropus ruficollis.* Head-length 2 cm. Various stages in the process of disintegration of the nuclei of cells of the stratum intermedium. In 1 and 2 the nucleus is shown as in active cells. In 3 the nucleolus (*nlo.*) is seen surrounded by a clear annular zone, whilst the chromatin has aggregated into large spherules. In 4 the chromatin has collected on the inner surface of the nuclear membrane, and the achromatic contents of the nucleus are visible. At 5 the karyoplasm has undergone coagulation and the chromatin has assumed a crescentic arrangement, whilst at 6 the nucleus is seen to have drawn away from the cytoplasm (*cyt.*), which is coarsely alveolar. × 1,500.

Fig. 10.—*Macropus ruficollis.* Head-length 2 cm. Longitudinal section. The ameloblasts (*a*) have increased in length from the stage shown in fig. 8, and have resumed their individuality. The cytoplasm is faintly fibrillar, and the area about the nucleus stains with difficulty. The nuclei (*n*) have increased in size and have again assumed their spherical or oval form, with the chromatin distributed in small spherules.

The cells of the stratum intermedium (*s.i.*), lying in contact with the bases of the ameloblasts (*a*), are seen undergoing degenerative change. The forming enamel (*e*), which stains intensely black, is seen lying in close contact with the cell-membrane (*i.a.m.*). × 600.

Fig. 11.—*Macropus ruficollis.* Head-length 6 cm. Longitudinal section. The ameloblasts (*a*) have once more become well-defined cells, separated from their neighbours by an intercellular area, which is traversed by cell-bridges (*c.b.*) These pass not only from ameloblast to ameloblast, but also from these cells to the cells of the stratum intermedium (*s.i.*). The ameloblastic cytoplasm has become coarsely alveolar; it does not extend to contact with the nuclear membrane, being separated by a clear area (*n.a.*), which is traversed by fine fibrils. The ameloblasts are seen to vary in length. × 1000.

Fig. 12.—*Macropus ruficollis.* Head-length 6 cm. Longitudinal section. The ameloblasts (*a*) show the same features as in fig. 11, but cells from the stratum intermedium (*s.i.*) are seen to be inserting themselves between the ameloblasts. × 1000.

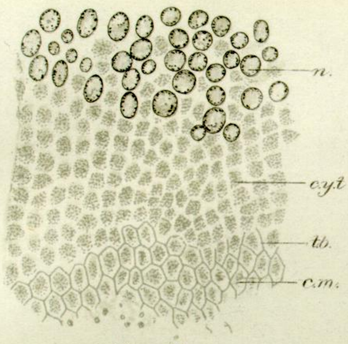
Fig. 13.—*Macropus ruficollis.* Head-length 6 cm. Transverse section through the ameloblasts (Stage VII). The cytoplasm is seen to be coarsely alveolar, the cavities (*vac.*) being occupied by a fluid which disappears in the later stages. The cell-bridges (*c.b.*) connecting the ameloblasts are very clearly shown. × 1500.

Fig. 14.—*Macropus ruficollis.* Longitudinal section through the apex of the enamel organ just prior to eruption. The ameloblasts (*a*) are seen gradually to lose their columnar form, first becoming cuboidal, then flattened.

The cytoplasm, which in the preceding stage was alveolar, has become faintly fibrillar, but as the cell loses its typical ameloblastic form, the fibrillar structure gives place to a finely granular cytoplasm. × 450.

Fig. 15.—*Macropus ruficollis.* Head-length 2 cm. Transverse section, showing the cells of the stratum intermedium varying in size and connected to their neighbours by intercellular bridges (*c.b.*) The interspace between the cells is occupied by a feebly staining acidophile fluid. × 600.

Fig. 16.—*Didelphys aurita.* Head-length 20 mm. Transverse section through the bases of the ameloblasts (*a*), showing this area of the cell modified to act as a cell-membrane (*c.m.*), the bases of the cells being separated one from another by the development of terminal bars (*t.b.*). × 600.



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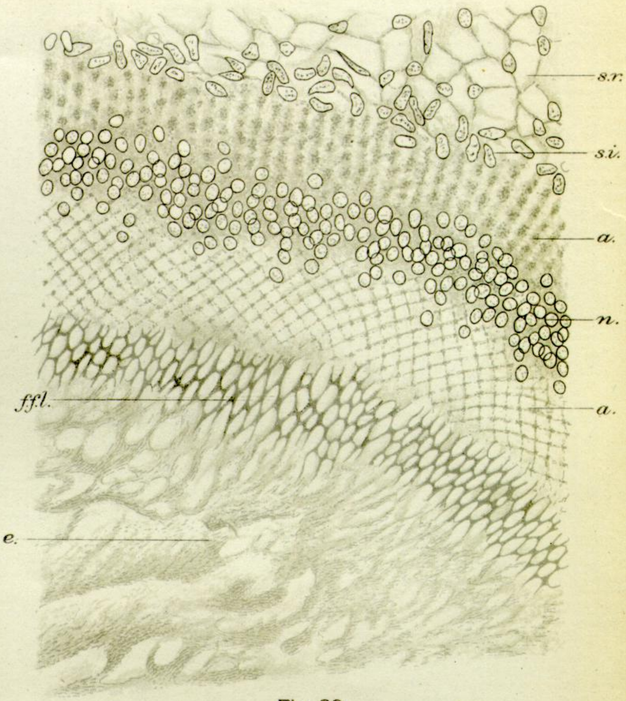


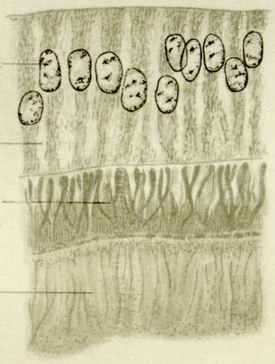
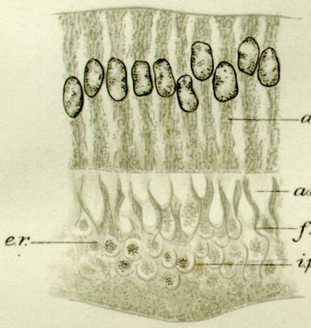


Fig. 18.

Fig. 22.



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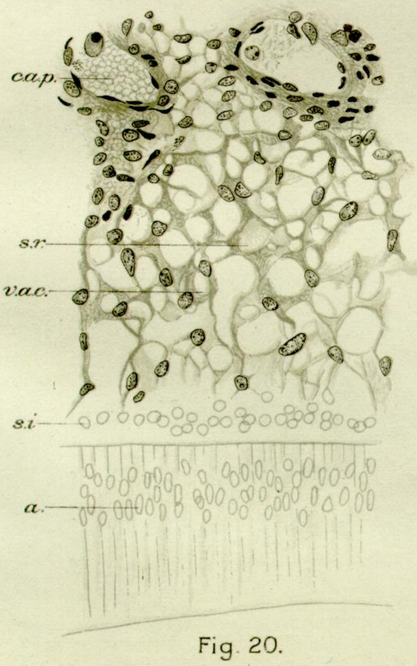

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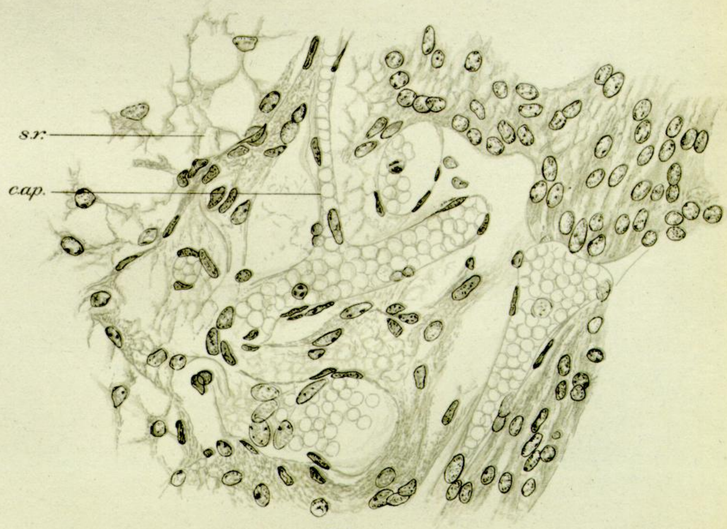

Fig. 21.

PLATE 9.

Fig. 17.—*Didelphys aurita.* Head-length 20 mm. Oblique section through the ameloblasts, showing the nuclei (*n*), then the cell-bodies (*cyt.*), granular in appearance (the dots being optical sections of the fibrils of the cytoplasm), and in the lower portion of the drawing the finely granular cell-ends (*c.m.*) are seen with the terminal bars (*t.b.*) closing the intercellular areas. × 600.

Fig. 18.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the area of developing enamel lying between the ameloblasts (*a*) and the dentine (*d*). The structures (*f.f.l.*), which taper in their course from the ameloblasts to the dentine, are the lamellæ of the layer which gives origin to the fibrils, seen in section. × 600.

Fig. 19.—*Didelphys aurita.* Head-length 20 mm. Longitudinal section, showing the ameloblasts (*a*), the fibril-forming layer (*f.f.l.*) with its incomplete alveoli (*alv.*), the fibrils (*f*) pursuing a sinuous course, the forming enamel rods (*e.r.*) lying in the cavities of a honeycomb matrix—the interprismatic material (*i.p.m.*).

Fig. 20.—*Macropus ruficollis.* Head-length 20 mm. Longitudinal section, showing the spongiform structure of the stellate reticulum (*s.r.*).

Fig. 21.—*Macropus ruficollis.* Head-length 5·2 cm. Transverse section through the stellate reticulum (*s.r.*), showing branching of a capillary vessel (*cap.*) and the cells of the stellate reticulum lying immediately in contact with the endothelial capillary walls. × 450.

Fig. 22.—*Didelphys aurita.* Head-length 20 mm. Tangential section cut without decalcification. Lying immediately beneath the ameloblasts (*a*) is the fibril-forming layer (*f.f.l.*), showing continuity of the fibrils (*f*) past different alveoli. Beneath this layer the material, clear in the alveoli of the fibril-forming layer, is seen to have undergone precipitation. × 450.

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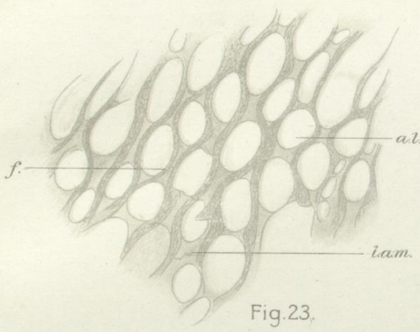

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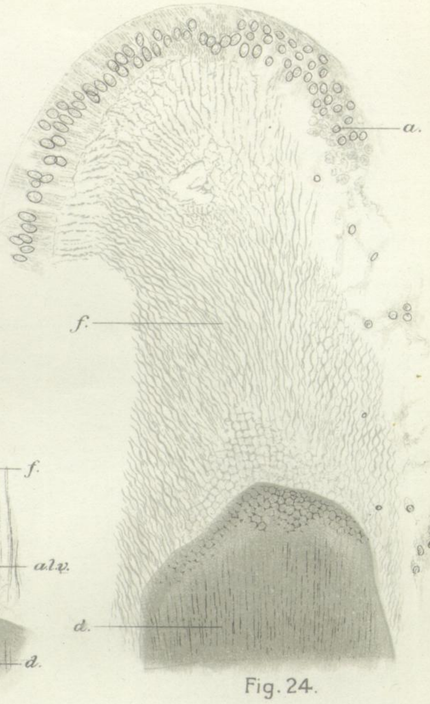

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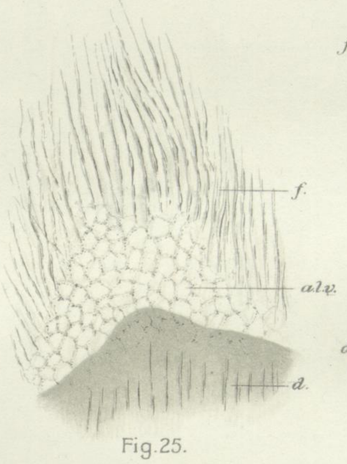

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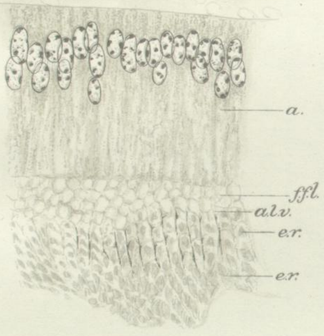

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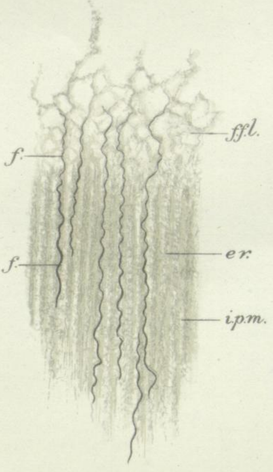

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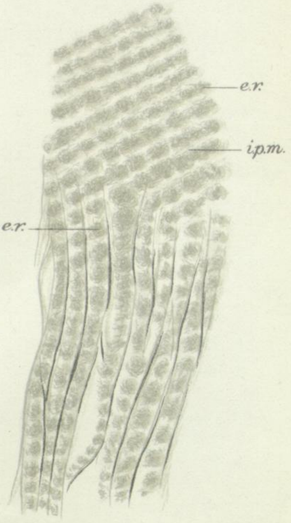

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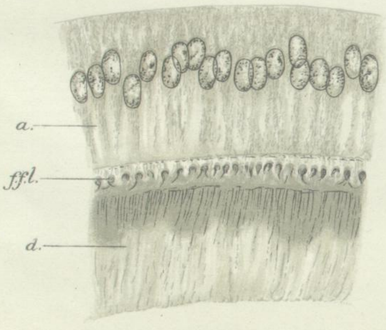

Fig. 26.

Plate 10.

Fig. 23.—*Didelphys aurita.* Head-length 20 mm. Portion of the fibril-forming layer (*f.f.l.*) of fig. 22. The fibrils (*f*) may be seen following the outlines of the incomplete alveoli (*alv.*) × 1500.

Fig. 24.—*Trichosurus vulpecula.* Head-length 5·2 cm. Longitudinal section through a decalcified tooth-germ, showing the fibril system (*f*) passing from just beneath the ameloblasts (*a*) to the surface of the dentine (*d*). In some places the fibrils pursue an almost straight course, but the majority are seen to be sinuous. × 300.

Fig. 25.—*Trichosurus vulpecula.* Head-length 5·2 cm. Section slightly oblique to the long axis of the tooth, showing the alveoli (*alv.*) of the fibril-forming layer at the point of junction with the dentine (*d*). They are irregular in form and vary in size, and appear as fine granules arranged more or less hexagonally. × 600.

Fig. 26.—*Didelphys aurita.* Head-length 20 mm. Drawing of a vertical section through a developing incisor. A considerable area of dentine (*d*) has been laid down. Between the ameloblasts (*a*) and the dentine (*d*), and lying on the surface of the latter, are seen a number of pyriform structures (*f.f.l.*), between which lies a homogeneous transparent substance. These pyriform structures are the first appearance of the fibril-forming layer. (Compare figs. 18, 19 and 22). × 350.

Fig. 27.—*Macropus ruficollis.* Head-length, 2 cm. Longitudinal section of ameloblasts and developing enamel, cut without previous decalcification. The ameloblasts (*a*) are seen closely packed together; beneath them is the fibril-forming layer (*f.f.l.*) which gives place to a number of dark areas (*e.r.*) lying between structures continuous with the fibril-forming layer. These apparently disappear a little deeper in the section, and the dark areas (*e.r.*) have become more diffuse and less deeply stained. × 600.

Fig. 28.—*Macropus ruficollis.* Vertical section through the margin of the developing enamel, showing the fibril-forming layer (*f.f.l.*) and the fibrils (*f*) passing into the enamel (*e*). The rods (*e.r.*) stain more deeply than the interprismatic material (*i.p.m.*). × 600.

Fig. 29.—*Macropus ruficollis.* Section of developing enamel. The rods (*e.r.*) are shown in longitudinal section in the upper portion of the figure, whilst in the lower portion their ends are seen in section. The interprismatic material (*i.p.m.*) is seen less deeply stained. × 1000.