39. Tooth-germs in the Wallaby *Macropus billardieri*.

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(Plate XLVII.* and Text-figures 181–189.)

The dentition of the Marsupials offers, in some respects, points of greater interest than does that of other mammals. Though much has been already written upon this subject, there are still problems awaiting solution.

The complete history of the tooth-genesis has been worked out in few members of this class, due no doubt to the difficulty of obtaining a sufficiently complete series of embryos of any one species. Any contribution, therefore, however small, may become of importance as forming a link in completing the chain of evidence.

The material upon which these observations are based consisted of three embryos of *Macropus billardieri*, obtained from the Seven Sisters Islands by Mr. Brooke Nicholls of Melbourne, to whom we are greatly indebted for his kindness in sending them for examination. These embryos give the following measurements :—

	Stage I.	Stage II.	Stage III.
	mm.	mm.	mm.
Tip of snout to occiput (circumferentially)	21	35	38
,, ,, ,, (in straight line)	15	27	27
Tip of snout to root of tail (circumferentially)	64	104	110
Vertex of head ,, ,, (in straight line)	35	66	52
Tail length	15	31	27
Total length from tip of snout to tip of tail (circumferentially)	79	135	137

Serial sections were cut in a vertical transverse direction though the skulls of Stages I and II and stained with borax carmine. The jaws of Stage III, which gave measurements approximately equal to those of Stage II and presumably therefore of an embryo of about the same age, were dissected and clarified in oil of cloves after the method recommended by Huxley, to show the relative positions of the calcified teeth.

The most detailed accounts of the development of the teeth in Diprotodont Marsupials are those given by M. F. Woodward (12)

* For explanation of the Plate see p. 942.

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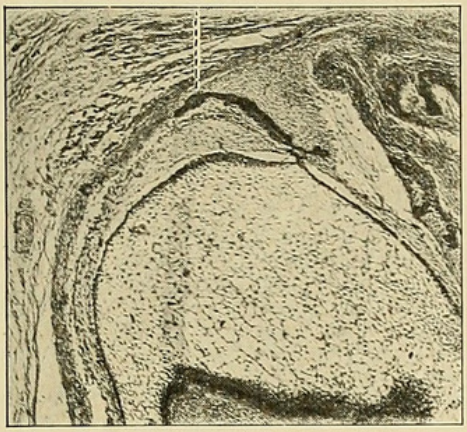

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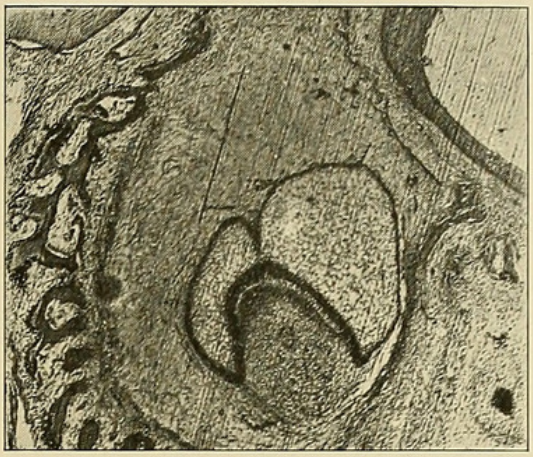

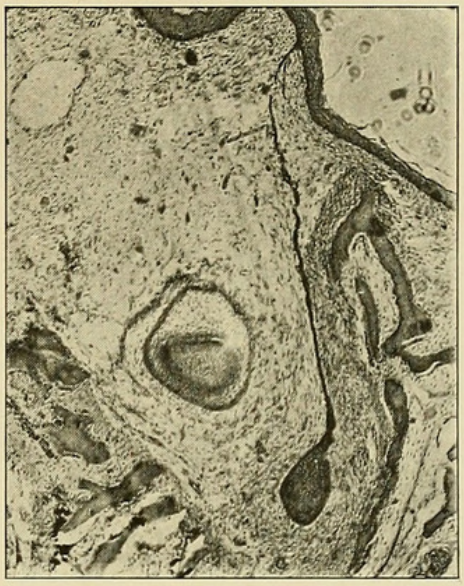

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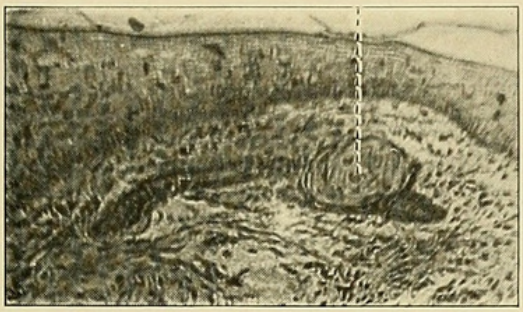

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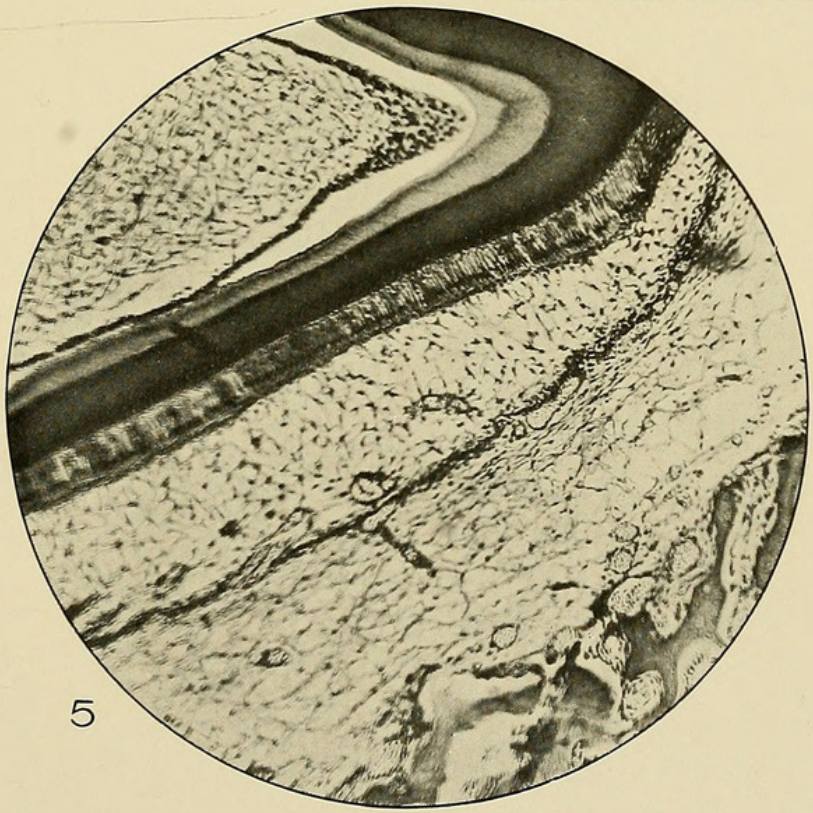

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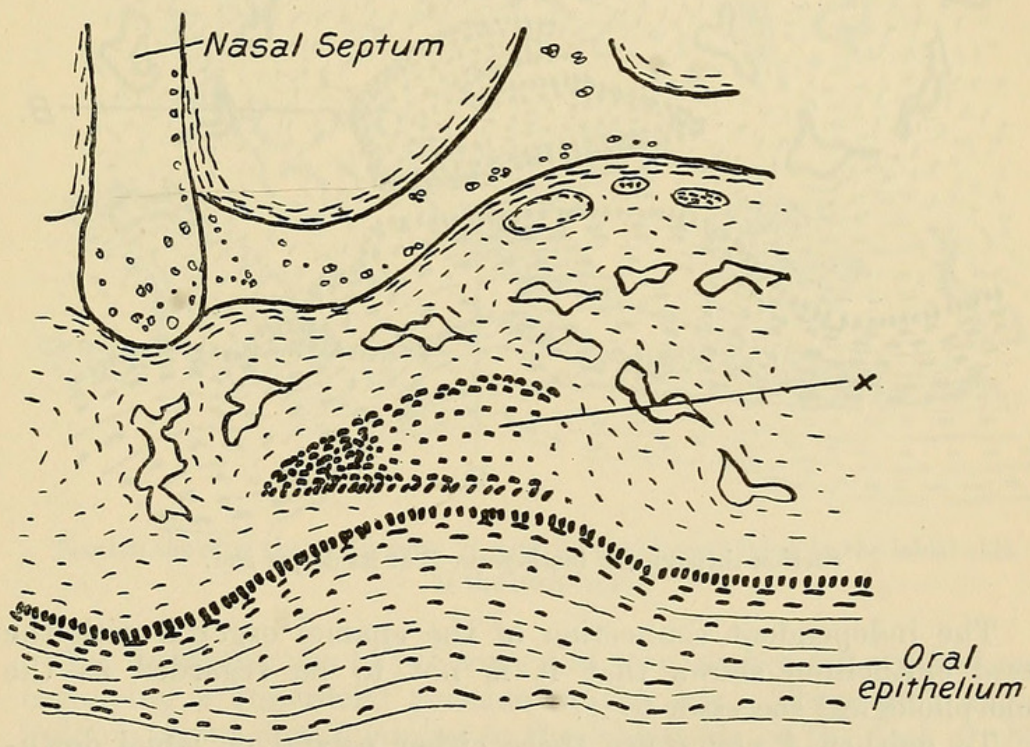
TOOTH-GERMS IN MACROPUS BILLARDIERI.

and by Deppendorf (1); the latter, however, does not appear to have examined any specimen of *Macropus*. Woodward has furnished the results of his examination of 14 embryos belonging to four species, viz. *M. giganteus*, *M. eugenii*, *M. bennetti*, and *M. brachyurus*. From the measurements given by him, allowing for the differences in size of the adult animals of the different species, our Stage I would appear to be considerably younger than any of the embryos which he examined. Before discussing the general considerations arising from our observations, we will proceed to a description of the conditions found in our material.

Stage I.

Upper Jaw.—At the anterior extremity of the upper jaw the oral epithelium sinks into the substance of the jaw, forming a broad triangular cellular mass the outline of which is very irregular. There are projections of some size extending laterally into the premaxillary region. None of these would, by themselves, suggest tooth vestiges, but the possibility of their being of such a nature is not entirely negatived because, as will be shown in the sequel, similar but more definite structures are to be seen in the lower jaw. The evidence here is, however, too problematical to permit of definite conclusions being drawn.

Text-fig. 181.



Section showing the tooth-band (×) passing horizontally inwards and forming the anterior part of the tooth-germ *B*.

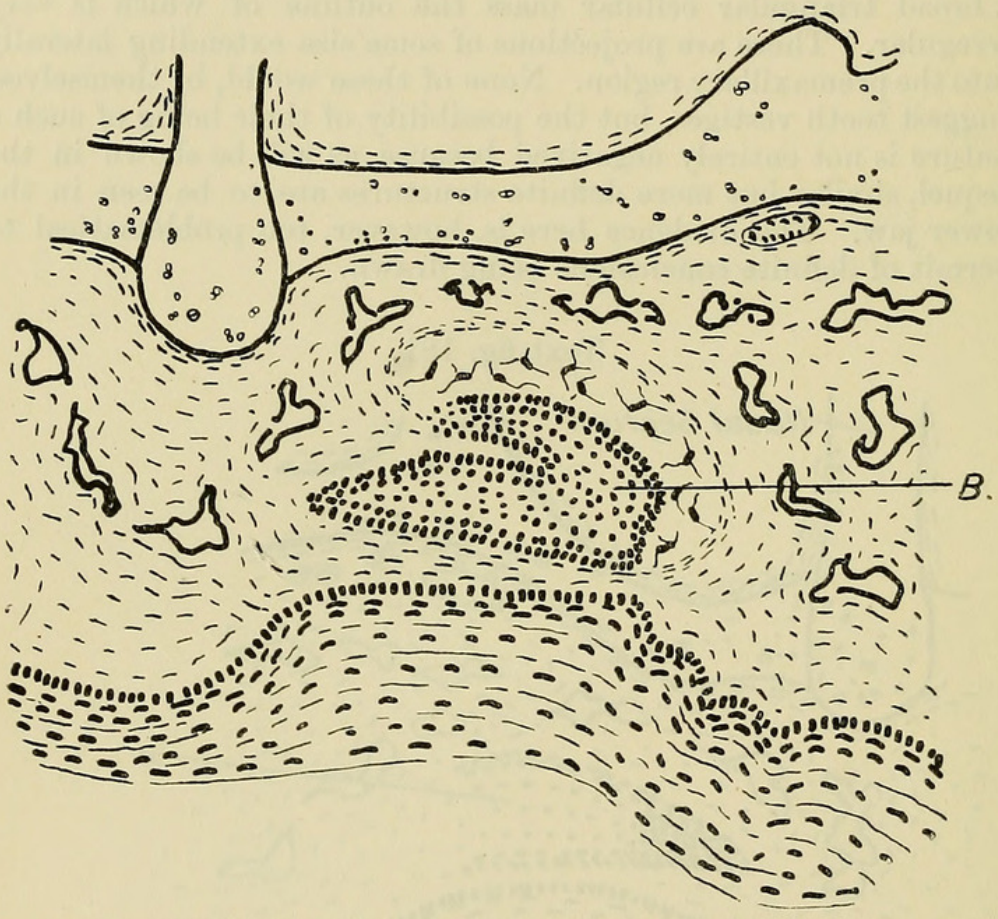
On either side of the middle line close to the nasal septum is a minute slightly calcified tooth-germ with a dental papilla of

definite shape. This germ will for the present be indicated by the letter *A*.

From a point just external to the point of connection of the neck of the enamel organ of *A* to the oral epithelium, the tooth-band passes horizontally inwards towards the middle line (text-fig. 181)*. This rapidly increases in size, forming on section a conical mass of cells which soon loses all connection with the oral epithelium. From the histological characters of the cells and from the fact that in the more posterior sections a portion of the stellate reticulum of the enamel organ is visible, this structure must be regarded as a second tooth-germ (*B*).

It is considerably larger than the preceding germ *A*, though there is not the same amount of differentiation into the more typical dental tissues.

Text-fig. 182.



Section through the tooth-germ *B* at its deeper end.

The independent connection of the enamel organ *B* with the oral epithelium shows that it is not to be regarded as the morphological successor to *A*.

To neither *A* nor *B* are there either palatal or labial down-

* Owing to the difficulty of accurate orientation, the anterior sections of a jaw cut in the vertical-transverse direction will be in a plane parallel with the outer surface of the jaw. This has been borne in mind in describing the relative positions of the various structures.

growths which could serve as an indication as to which dentition the germs should be referred.

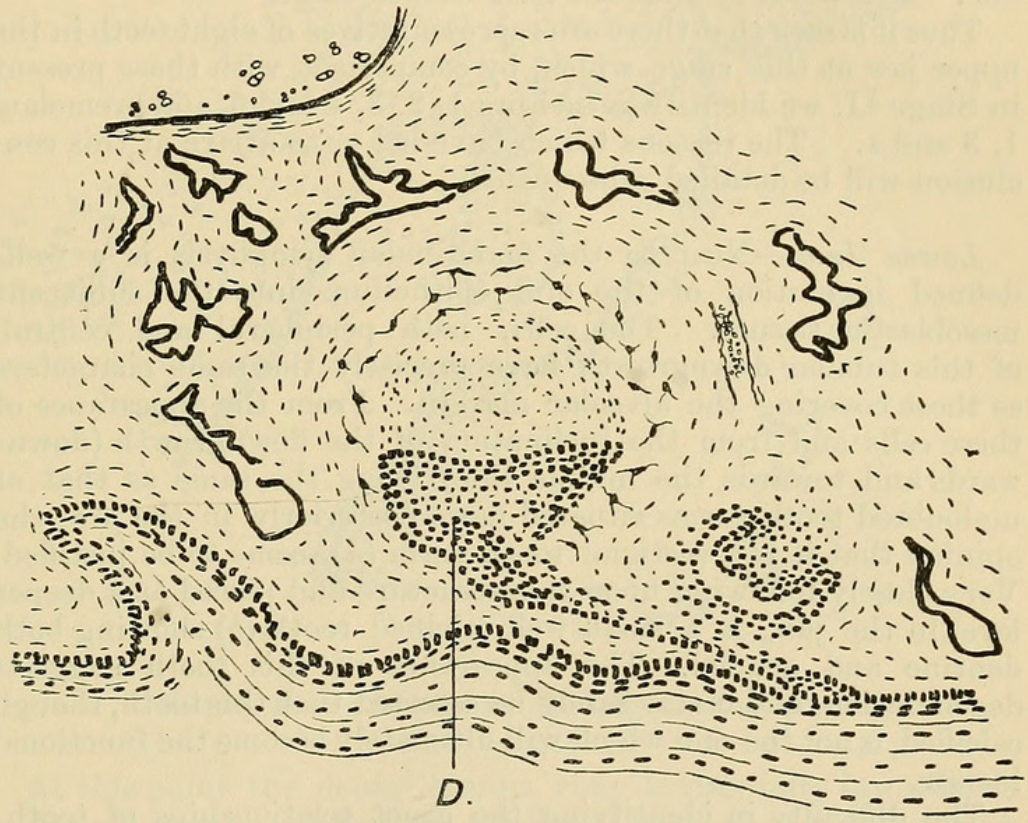
As this tooth is traced backwards it assumes a peculiar shape on section. Its inner margin is sharp, the outer one rounded, while on the dorsal surface is a relatively deep fissure (text-fig. 182).

The peculiarity of the adult tooth is thus early indicated.

As *B* disappears from the sections a new tooth-germ (*C*) makes its appearance. Though minute it has a fully formed enamel organ and dental papilla, but without any trace of calcification. This germ has a very superficial position in the jaw.

Closely following upon *C* is a large uncalcified tooth (*D*) lying to the palatal aspect of and slightly posterior to the preceding tooth-germ. Connected with the neck of the enamel organ is a minute predecessor slightly invaginated by a rudimentary dental papilla (text-fig. 183).

Text-fig. 183.



Section showing the tooth-germ *D* with its predecessor lying to the labial side of the neck-band.

Yet another germ (*F*) of some size soon makes its appearance, occupying a superficial position in the jaw. There is a slight bulging of the dental lamina to the labial side of the neck of this enamel organ, which may or may not indicate an abortive attempt at the formation of a predecessor.

The dental lamina from this point is continued backwards for some distance as a distinct band without showing any definite

trace of a tooth-germ. After an interval, another tooth (*H*) of considerable size appears, lasting through three slides. It has a dental papilla with blunted apex. As yet there is no definite evidence of calcification. There are neither palatal nor labial downgrowths of the dental lamina. This tooth we identify as the first of the cheek series.

The tooth-band continues backwards in a well-marked condition giving evidence of a distinct swelling which in Stage II has developed into an undoubted tooth-germ. Without a knowledge of the subsequent history of this swelling, one would have hesitated to attach importance to it.

Posteriorly to this is the large enamel organ of Stage I from the neck of which springs a definite palatal downgrowth of the dental lamina. The shape of the dental papilla is definitely molariform, the outer cusp already attaining to a higher level than the inner.

One more enamel organ (*J*), the most posterior in the jaw at this stage, is to be seen following closely upon the preceding one. It has but reached the flask-shaped stage.

Thus it is seen that there are representatives of eight teeth in the upper jaw at this stage which, by comparison with those present in Stage II, we identify as incisors 1, 2, 3, 4 and 6, and premolars 1, 3 and 4. The reasons which have led us to arrive at this conclusion will be detailed subsequently.

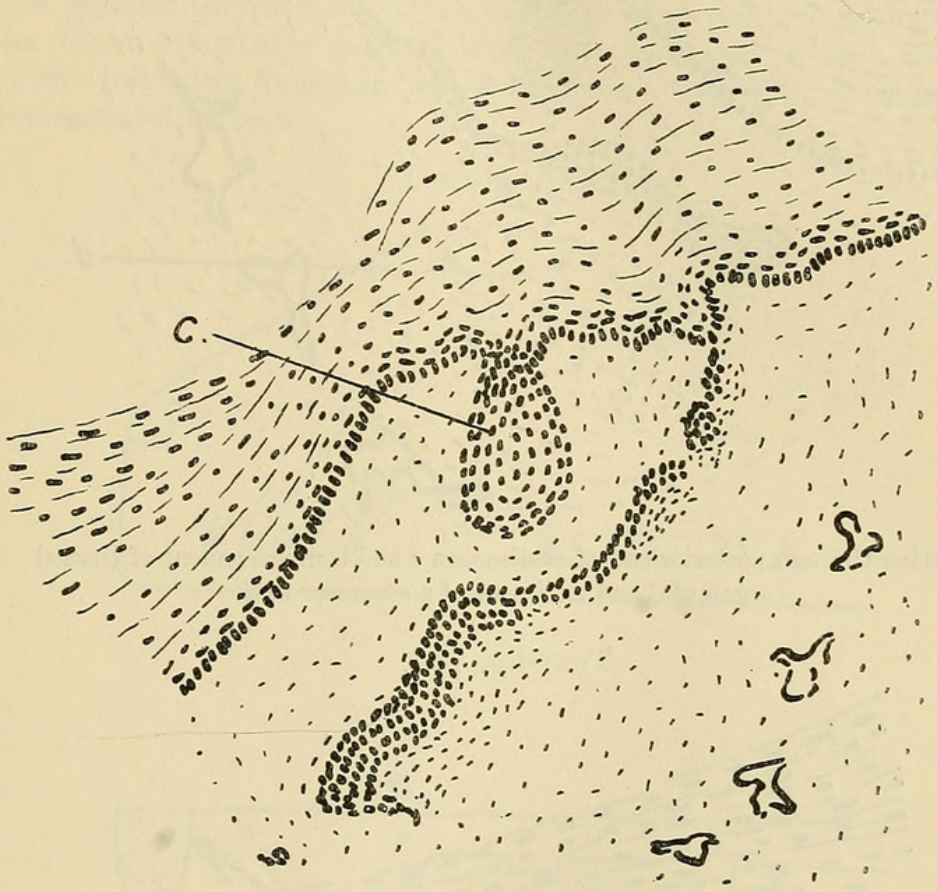
Lower Jaw.—Near to the mandibular symphysis is a well-defined involution of the oral epithelium into the subjacent mesoblastic tissues. The cells, both peripheral and central, of this tubular downgrowth have precisely the same characters as those covering the alveolar margin. From the appearance of these cells and from the inclination of the downgrowth (downwards and towards the middle line) being the same as that of undoubted tooth-germs situated more posteriorly in the jaw, the opinion that it is a vestigial tooth-germ (*a*) seems to be justified. Very closely following upon this structure and placed at a deeper level in the jaw, is a small well-calcified tooth (*b*) showing both dentine and enamel. The appearance of this tooth suggests degeneration, and it may safely be asserted that this tooth, though calcified, is not the one which will ultimately become the functional incisor.

The difficulty in identifying the exact relationships of tooth-germs close to the symphysis is always great, due in part to a certain amount of crowding, accentuated in the Macropodidæ by the very large size of the functional incisor and in part to the difficulty of correct orientation. We at first thought that these two structures stood to each other in the morphological relationship of predecessor and successor. From a careful consideration of all the facts we believe that this is not the case, but that they are the representatives of two separate teeth and that the close approximation to each other is due to the crowding caused by the large functional incisor. The appearance of *a* suggests a tooth

which is attempting but failing to develope; it has not the "concentric" appearance of a vestigial tooth-germ such as one so often sees as the last evidence of existence of a predecessor to a calcified tooth.

Rapidly succeeding the calcified tooth *b* is a well-defined flask-shaped dental rudiment lying in a very superficial position in the jaw. This, though close to, is quite independent of any of the other tooth-germs here present and will in the meanwhile be referred to by the letter *c* (text-fig. 184).

Text-fig. 184.

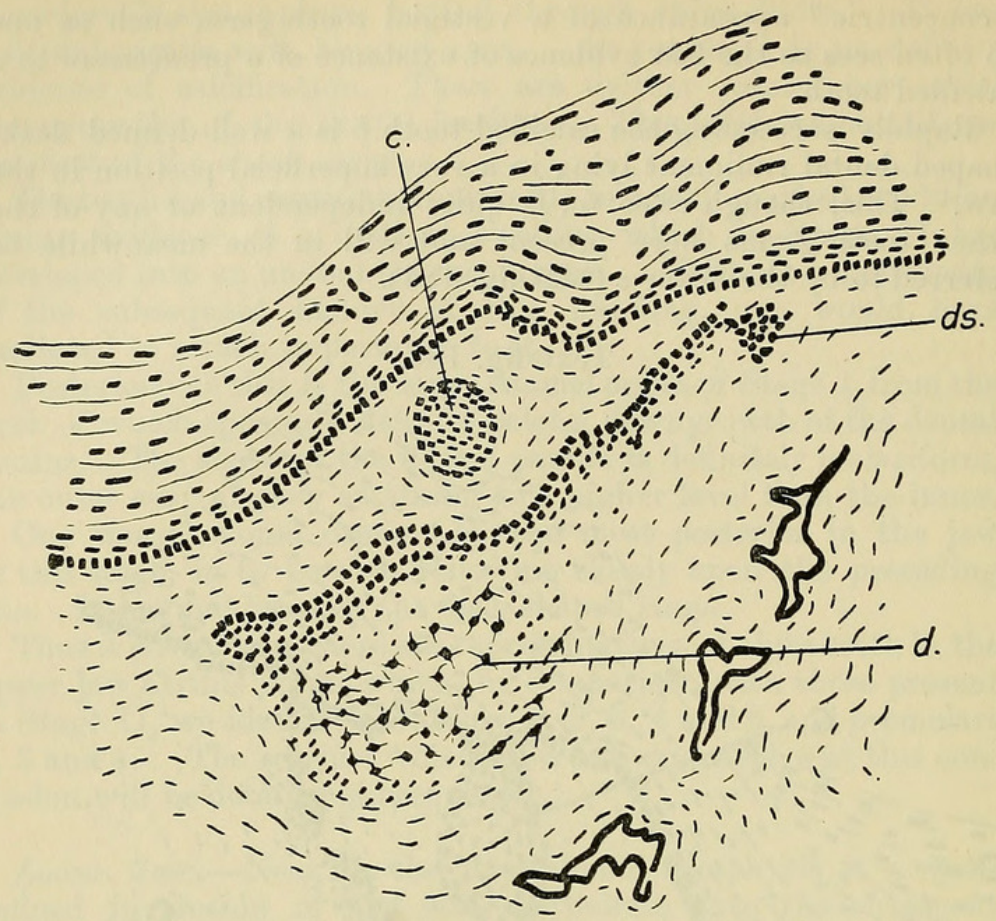


Section showing flask-shaped rudiment of tooth-germ *c*.

At this point the dental lamina runs horizontally inwards, as in the upper jaw. It forms a thickened band of cells lying some distance below the tooth-germ *c*. Tracing this band backwards it quickly becomes connected with the upper part of the enamel organ of a large tooth (*d*) in which the dental tissues are differentiated and which extends through several slides. In relation to the neck of this enamel organ is a lingually situated downgrowth of the dental lamina indicating a potential successor (*ds*) to the tooth *d*. From this point the tooth-band vanishes for some distance (text-fig. 185).

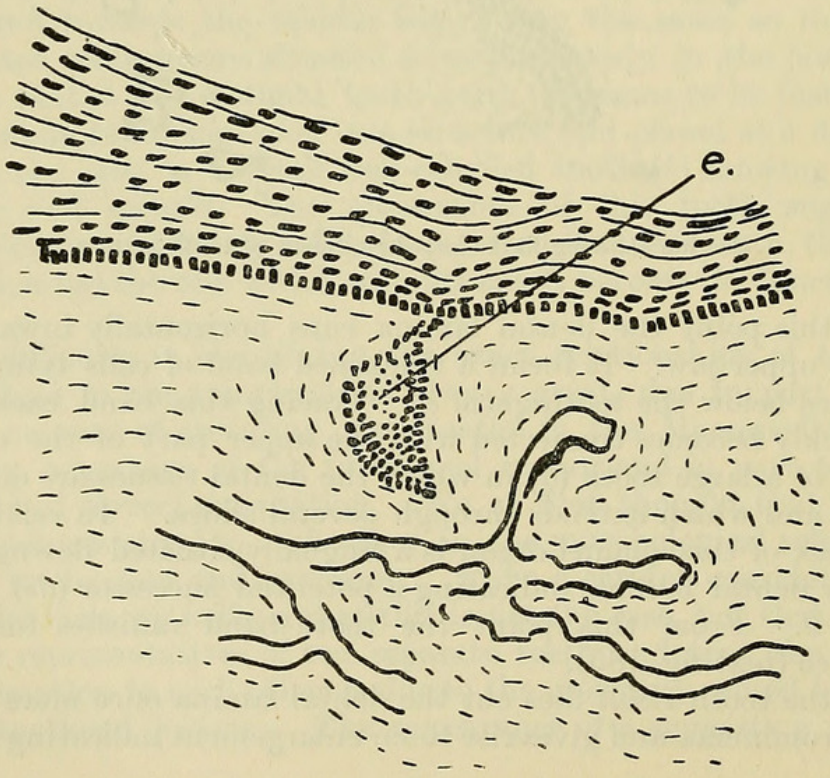
As the tooth itself dies out the dental lamina once more comes into prominence and gives rise to an enlargement indicating in our

Text-fig. 185.



Section through posterior end of tooth-germ *c* and commencement of enamel organ of *d* and rudiment of a successor to *d*.

Text-fig. 186.



Section showing the enlarged downgrowth of the dental lamina indicating the vestige of the tooth-germ *e*.

opinion a tooth vestige (*e*). It lies close beneath the alveolar margin (text-fig. 186).

Then occurs a further interval through which the tooth-band persists without giving rise to any further dental enlargements. Three further teeth (*h*, *i*, and *j*) of premolariform pattern, follow in the cheek region. Of these the middle one is considerably the most extensive, the posterior one being quite small. To the two anterior teeth there are indefinite indications of lingual downgrowths of the dental lamina.

As in the upper jaw there are indications of eight teeth, five antemolars and three maxillary teeth.

A general impression of the number and relative positions of the dental structures in both jaws may perhaps be best realised by the following diagram, which has been drawn to scale in the horizontal direction.

Text-fig. 187.

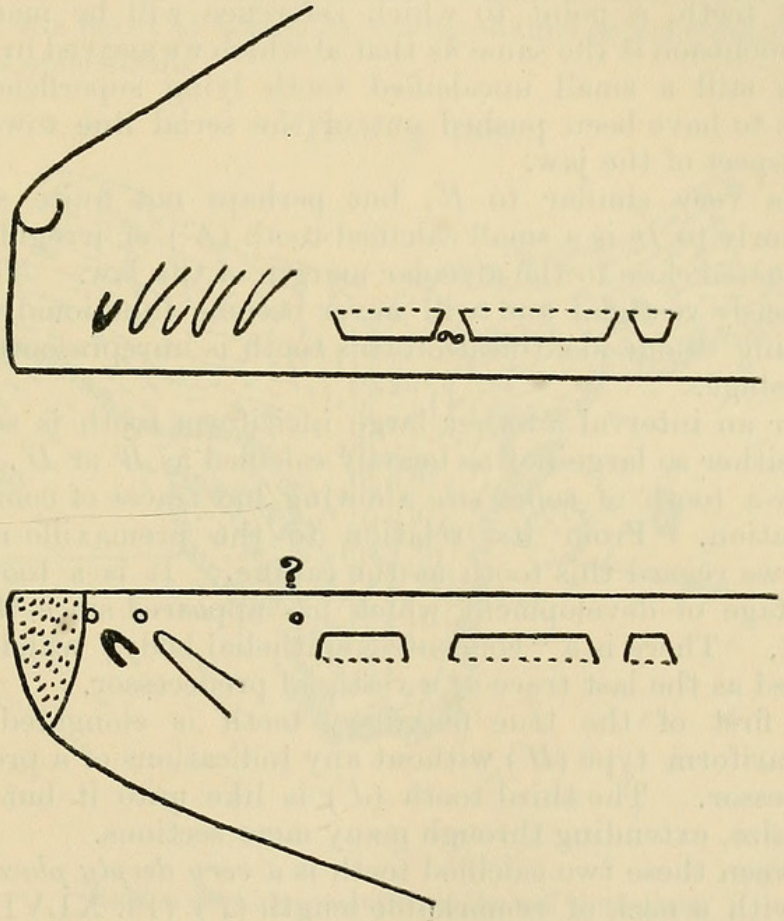


Diagram showing number and relative positions of the tooth-germs in the jaws of Stage I. Calcified germs more heavily outlined.

Stage II.

The difference in size between this embryo and that of Stage I is very considerable, and as a consequence a number of additional teeth have appeared in both jaws and those which were present in the earlier stage have developed very materially. It is unfortunate that we have not had at our disposal an embryo of

intermediate size, as this would have rendered the precise interpretation a matter of less difficulty and of greater certainty.

Without going into details we will summarise as briefly as possible the conditions found in the later stage.

Upper Jaw.—Anteriorly is a small calcified tooth (*A'*) occupying a position similar to the tooth found in the previous stage and is undoubtedly the same tooth still persisting. It shows no signs of being erupted, neither has it undergone any further development. That it will ultimately become absorbed without attaining functional activity seems to be certain.

Behind this lies a large incisiform tooth already heavily calcified. This tooth (*B'*) is the largest of the anterior series. All traces of the tooth-band between *A'* and *B'* have become lost, and several sections intervene between the disappearance of *A'* and the commencement of the enamel organ of *B'*. Thus we are of the decided opinion that these are two morphologically distinct teeth, a point to which reference will be made later. This conclusion is the same as that at which we arrived in Stage I.

C' is still a small uncalcified tooth lying superficially, and appears to have been pushed out of the serial line towards the outer aspect of the jaw.

D' is very similar to *B'*, but perhaps not quite so large. Posteriorly to *D'* is a small calcified tooth (*E'*) of irregular shape and situated close to the alveolar margin of the jaw. This tooth is obviously vestigial and will never become functionally active. According to our identification this tooth is unrepresented in the earlier stage.

After an interval another large incisiform tooth is seen (*F'*). It is neither so large nor so heavily calcified as *B'* or *D'*.

G' is a tooth of some size showing but traces of commencing calcification. From its relation to the premaxillo-maxillary suture we regard this tooth as the canine. It is a tooth in an early stage of development which has appeared since the age of Stage I. There is a "concentric epithelial body" which is to be regarded as the last trace of a vestigial predecessor.

The first of the true maxillary teeth is elongated and of premolariform type (*H'*) without any indications of a predecessor or successor. The third tooth (*J'*) is like unto it but of much larger size, extending through many more sections.

Between these two calcified teeth is a *very deeply placed* tooth-germ with a neck of remarkable length (*I'*) (Pl. XLVII. fig. 1). There is no evidence of calcification. This is evidently the second maxillary tooth which, crowded between the large calcified first and third, has been forced into the abnormally deep situation, besides being pushed from the linear series towards the palatal side of the jaw.

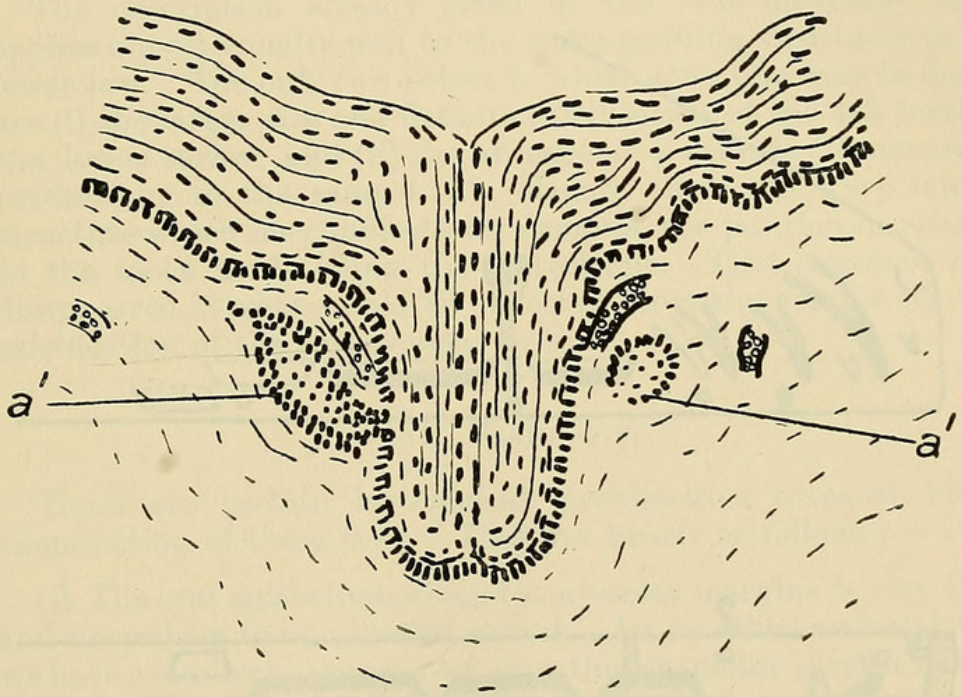
The fourth maxillary tooth (*K'*) is large and in it calcification is but just commencing. From the neck of the enamel organ springs a marked labial downgrowth of the dental lamina, connected with which is a "concentric epithelial body," the vestige

of a predecessor (Pl. XLVII. figs. 2 & 3). The importance of this will be discussed later. The last tooth of the series (L') is uncalcified and at an earlier stage of development than the maxillary teeth in front of it. It lies much nearer to the alveolar margin than do the others, and there is this further point of interest, that the anterior end of this tooth overlaps superficially the posterior end of the tooth in front, which is distinctly not the case with the more anteriorly situated teeth.

According to our identification we recognise at this stage six incisors, one canine and five maxillary teeth.

Lower Jaw.—The difficulty of interpreting the appearances in the region of the mandibular symphysis is even greater than in the earlier stage. The enormous development of the lower functional incisor, extending as it does through thirty slides *, has disarranged all the parts, more particularly anteriorly where the tip of the tooth becomes more superficial and where it now reaches well in front of the point at which its young enamel organ connected with the oral epithelium.

Text-fig. 188.



Section showing involution of the oral epithelium at the mandibular symphysis.

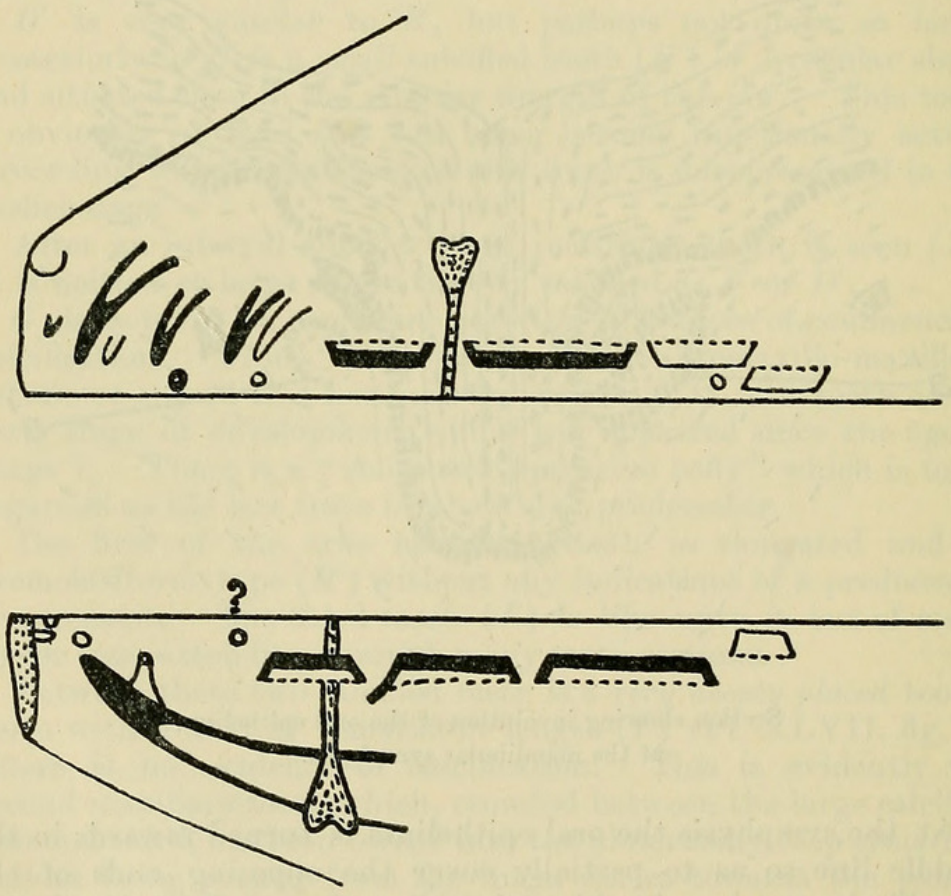
At the symphysis the oral epithelium is turned inwards in the middle line so as to partially cover the opposing ends of the mandibular rami. At this point there is a definite involution of the epithelium into the subjacent tissues. The section being cut at this point in a plane parallel with the anterior surface, the

* A rough estimate may be obtained by stating that a slide carries an average of twelve sections, each section being $\frac{1}{100}$ mm. in thickness.

direction of this digitiform involution is inwards and slightly upwards. There is an involution on either side of the symphysis and they are quite symmetrical. The position makes the interpretation somewhat doubtful, otherwise we should have but little doubt in ranking them as tooth rudiments. Regarding the origin, connection, and histological characters as of more importance than mere position, we shall regard it as such and provisionally indicate it by the letter *a'* (text-fig. 188).

Close to the middle line, but on the alveolar margin of the mandible, is a more globular involution containing cells commencing to be arranged as a concentrical epithelial body such as several authors now recognise as being tooth vestiges. This will be referred to as *b'*. This also is a bilaterally symmetrical structure and extends through several sections. Immediately above this is a fissure in the oral epithelium containing some deeply stained fibrous-looking material. The appearance suggests the possibility of this marking the situation where the minute calcified tooth, present in the jaw in this position in the earlier stage, may have been erupted. There is otherwise no trace of the calcified vestige.

Text-fig. 189.



Showing the relative positions of the teeth in Stage II.
Calcified teeth more heavily outlined.

A broad bulbous involution of the oral epithelium is seen close to the middle line (*c'*) which soon loses its connection with the

alveolar epithelium, remaining as an isolated spherical mass. It probably is the same structure described in Stage I, and is naturally of larger size.

Lying at a deeper level but entirely independent of it, is the anterior extremity of the large calcified functional incisor. That these two structures have no morphological relationship with each other is made clear, not only by their relative position but also by the fact that the connection of the enamel organ with the surface is definitely visible in a section farther back, together with a "concentric" vestige of a predecessor and an indication of a potential successor. This large functional incisor we shall indicate as *d'*.

Then follows an interval of thirteen slides without any trace of teeth, and throughout the greater part of this interval the dental lamina is scarcely visible. At length a definite "concentric epithelial mass" occurs embedded in the dental lamina. It is impossible to say whether this is the vestige of a canine or of an outer incisor. From its position relative to that of the opposing teeth, it is probably the vestige of the latter tooth. The point in favour of its being a vestigial canine is its propinquity to the first true post-canine tooth. However, it is a point of quite minor importance.

The description already given of the true maxillary teeth applies almost equally well to the corresponding structures in the lower jaw. The only two points to which attention may be drawn are (i) the larger size and definite calcification of the 4th tooth of the lower series; and (ii) the absence of a definite "concentric" predecessor to the same tooth, though there is a very minute structure which may indicate its remains, the position in relation to the tooth itself being the correct one. That it should have disappeared is what would be expected from the greater size and calcification of the successional tooth.

III. Histology.

There are certain histological peculiarities revealed by an examination of these jaws. They are briefly as follows:—

(i) The oral epithelium along the alveolar margins is very thick and heaped up to an unusual extent. In no other mammal that we have examined have we ever seen this character carried to such excess. This is the "Zahnwall" of the German writers. It is said to be a marked character in the Ungulates, and in our experience it is certainly not of common occurrence among mammalia generally. Of course no morphological significance is implied by the comparison.

(ii) The somewhat unusual compactness of the stellate reticulum and the definiteness of the stratum intermedium of the enamel organ.

(iii) The precocious development of the enamel which seems to calcify as soon as the dentine, and even in some cases to

precede it. Spherules of calco-globulin are clearly seen in many of the ameloblasts.

(iv) The abundant evidence of blood-vessels within the enamel organ is a very striking feature (Pl. XLVII. fig. 5). The fact of the vascularity of the enamel organ is one upon which there are contradictory statements. Wedl, Magitot, Legros, Sudduth and Paul deny the existence of blood-vessels in this situation. On the other hand, Prof. Poulton and the late Prof. G. B. Howes have mentioned their presence in the enamel organs of the Rat.

In *Macropus* there is no doubt whatever. They can be seen entering the enamel organ apparently at more than one point on the surface, and are often of sufficient size to clearly recognise the blood corpuscles within them. They can be traced to a point about midway between the outer and inner enamel epithelium, but we have not seen them proceeding farther, neither have we found them in the stratum intermedium, the position in which they were described by the authors above mentioned.

The fact of the vascularity of the enamel organ is one of importance in connection with the various theories held as to the processes of the calcification and enamel formation and of the functions of the stellate reticulum. It is extremely probable that the unusual vascularity of the enamel organs in this animal is correlated with the precocious development of the enamel, to which reference has just been made.

(v) Evidence of the fusion of enamel organs. There appears to be some evidence of the possibility of the occurrence of such fusion though the evidence is not decisive. Pl. XLVII. fig. 4, shows the subdivision of an enamel organ into two parts by an epithelial septum passing from the outer enamel epithelium to the inner where the latter lies over the apex of the dentinal papilla. This occurs in more than one cheek-tooth and may be seen on both sides. We have never met with anything of the kind before. That this septum is not a small capillary running directly across the stellate reticulum we are assured. We can offer no other suggestion than that it is a double enamel organ taking part in the formation of a single tooth.

This is a point of considerable morphological importance as bearing upon the question of the evolution of the molar patterns. Fusion is presupposed to have occurred by all writers who believe in any form of concrescence, but little satisfactory evidence has been forthcoming in the mammalia and little weight can be given to the evidence derived from the disintegration of the Cetacean molars.

Amongst the lower vertebrates the evidences of fusion seem to be conclusive. In one family of upper Palæozoic Sharks, the Cochliodontidæ, there is a fusion into plates not only of adjoining teeth of one series, but also of successional teeth of several series. Semon (**4**) has also shown that a fusion of individual cusps takes place in *Ceratodus*, and Röse has seen the cusps in the process of fusion in the teeth of a chameleon (**3**). Woodward (*loc. cit.*) speaks

of a supernumerary incisor in the upper jaw of an adult *Perameles* in the teaching collection of the Royal College of Science in which there are " indications of at least three fangs, and is obviously a fusion of (these) teeth." The possibility of tooth fusion in the mammalia is therefore by no means improbable. The matter must remain unsettled until more satisfactory evidence is forthcoming, but the condition figured would seem to point in that direction and we can only repeat that we can suggest no other explanation.

IV. General Considerations.

A. *Serial homologies of the Teeth.*

Upper Incisors.—Assuming our identification of the teeth to be correct, we have *six* incisors present in the upper jaw, of which the 5th is unrepresented in the earlier stage and has attained but a slight degree of development in Stage II. This excessive number of upper incisors confirms the original statement of M. F. Woodward (**11**), as the result of his observations on *Petrogale penicillata* and *Macropus giganteus*. That author is obviously aware of the difficulty in accepting so large a number, for he writes (p. 465): " The discovery of six pairs of incisors, although an absolute fact, is in many respects an unfortunate one, as we know of no adult mammal with so many, and even amongst Reptiles, many Lizards and Crocodiles have the number of teeth in each premaxilla restricted to five." He then proceeds to discuss various possible explanations such as the late calcification of the functional incisors in *Macropus* and the greater space in the premaxillary region in *Petrogale* as the possible determining causes. A reference to our text-figures 187 & 189 (pp. 933 & 936) will show that Woodward has possibly found the correct explanation, both factors appearing to be present in our specimens.

The difficulty is, however, not fully stated by a mere reference to recent forms, for Osborn (**2**) in his paper, " On the Structure and Classification of Mesozoic Mammalia," considers that the dental formula of the primitive heterodont mammal should be taken as i. 4, c. 1, pm. 4, m. 8.

In a paper published three years later (**13**) Woodward appears to have modified his former opinion, for in spite of having previously spoken of the presence of six upper incisors as " an absolute fact," he writes " I am now disposed to regard the three minute teeth which I described in the upper jaw of several genera belonging to this family " (*i. e.* Macropodidæ) " as the premilk predecessors of the three functional incisors." He gives no reasons for the alteration in his opinion, as in his previous paper he discusses and discards the interpretation which he appears later to have adopted. He further makes no reference to the alteration in the identification of the homologies of the functional incisors which such a change of opinion would involve.

We have naturally therefore paid particular attention to these

views, and we agree with Woodward's first statement that the presence of six upper incisors, in spite of all the difficulties which it involves, is "an absolute fact." The one point upon which we join issue with Woodward is his view of the homology of the first functional incisor which he regards as being i. 1, whilst we hold that it is i. 2. The small calcified vestige in both our stages is clearly anterior to and independent of the first large tooth which is obviously the first functional incisor. Our disagreement is due to differences of interpretation of the conditions present in a very puzzling region, *i. e.* close to the median symphysis; and though we hesitate to put ourselves in opposition to so accurate and experienced an observer, we nevertheless are compelled to adhere to the opinions already stated. We are, however, quite in accord with him in regarding the outer functional incisors as being the 4th and 6th, the 3rd and 5th disappearing.

We, like Woodward, are therefore at variance with the opinion of Oldfield Thomas (**5**), who regards the missing incisors as being the outermost ones of the series. Though Deppendorf (**1**) does not appear to have examined the jaw of *Macropus*, he has made interesting observations on the tooth-genesis in many other marsupials. In *Æpyprymnus* he identifies the three functional incisors as the 2nd, 3rd, and 5th. Thus he agrees with us in the identification of the 1st functional teeth, but differs from both Woodward and ourselves in the identification of the two outermost functional premaxillary teeth. It is unfortunate that there should thus be four different versions given on this subject. Possibly the conditions described by Deppendorf may be really different and not merely a difference in interpretation, as he carried out his researches in other genera, in which the relative proportions of the bone may be somewhat different and thus produce different results.

Lower Incisors.—In the lower jaw we believe there are evidences of five ante-molar teeth. Whether the most posterior one is the vestige of a canine or of an outer incisor, it is impossible to determine. Of the remaining four the first three are vestigial, so that according to our interpretation the functional incisor of the adult is i. 4. This statement is at variance with Woodward's conclusion as to the homology of this tooth which he regards as i. 2. In his illustration he figures two vestiges which obviously correspond with our b' and c', his first incisor in *Petrogale* being a minute calcified tooth just as is the germ b'. The morphological 3rd incisor of *Petrogale* is "more vestigial than the first," just as c' is more vestigial than b'.

The discrepancies between our interpretations probably depend on the following facts. (1) Woodward seems to have examined the jaw on one side only, and to do so would have divided it through at the symphysis. In doing so he would most certainly destroy the minute vestige a' which we have found lying practically within the symphysis. (2) He regards the posterior vestige as being posterior to the large functional incisor, whereas we regard it as being anterior. We both agree in stating that it lies close to the