Zeitschrift für Zellforschung 82, 558—576 (1967)

The Structure and Development of Marsupial Enamel Tubules

A. Boyde and K. S. Lester*

Anatomy Departments, The London Hospital Medical College, University College and King's College, University of London

Received May 19, 1967

Summary. The development and structure of marsupial enamel tubules has been studied in a number of species by a variety of microscopical techniques. The results were as follows.

1. The undoubted continuity of dentinal and enamel tubules could be traced in all species examined.

2. The tubules leave more residue than the surrounding enamel when decalcified.

3. The tubules are permeable to dyes in extracted teeth.

4. The dyes methyl blue and trypan blue did not reach the enamel tubules from the pulp or blood-stream in *in situ* adult teeth of *Metachirus nudicaudatus*.

5. The tubular nature of the tubules is well demonstrated in scanning electron micrographs and replicas of fractured enamel and also in replicas of argon-ion beam eroded *Macropus* molar enamel surface.

6. The tubules are situated within the enamel prisms.

7. The tubules may be recognized in electron micrographs of developing enamel as regions in which crystallites do not develop.

8. The study of enamel tubule development revealed no special features of the ameloblasts or of the nature of the first secreted enamel.

Introduction

John Tomes (1849) discovered that in the marsupials with the sole exception of the wombat "the greater number, if not all, of the dentinal tubes are continued into, and constitute a considerable portion of the enamel". The variety of views expressed since that time concerning the position and origin of the tubules in marsupial enamel are summarised in Table 1. It will be noted that whereas the majority of previous workers have been convinced of the continuity of the enamel and dentinal tubules, opinion has been equally divided on the question of whether they originate from the dentine or are of purely enamel origin. Very little developmental

* The Cambridge Instrument Co. "Stereoscan" was provided by the Science Research Council, the Siemens Elmiskop I by the Wellcome Trust, and the Hilger and Watts Stereometer by Mr. R. V. Ely. We also wish to acknowledge the considerable help we have received from Miss Susan Perry who typed the manuscript.

Explanation to Table 1.

A) "Indeed in all teeth the enamel fibre is at an early stage of formation partially tubular". (Tomes, 1849).

B) v. Ebner invoked a resorption of the first formed dentine to account for the continuity with the dentinal tubules. He also described the positive birefringence of marsupial enamel for the first time.

C) Noted that the "honeycomb" of developing enamel is of greater thickness in marsupials.

D) Canals not bound to individual prisms.

E) "Tubules" are ground section artefacts.

F) "Kittsubstanz" of many earlier authors is equivalent to prism sheath substance of modern terminology, rather than to interprismatic regions.

Table 1. *Summary of previous views on the position and origin of the tubules in marsupial enamel*

		e = enamel origin d = dentinal origin	− = no continuity with dent. tubules + = continuous with dent. tubules	p = in prisms i = in interprismatic regions s = in prism sheath substances F	*Name* adopted (*Canal* = Kanälchen, canal, or canaliculus)	= developing tissue studied +	Other remarks	
A)	J. TOMES (1849, 1856)	e	+	p	*tube* and *fibril*		note A	
B)	v. EBNER (1890)	e	+	s	*canal*		note B	3—5 round each prism
C)	C. S. TOMES (1897, 1904)	e	+	p	*fibre* and *fibril*	+	note C	1 in every 4 prisms
	RÖSE (1893, 1897)	d	+	s	*canal*			
	PAUL (1896)			s	*tubule*			
	WILLIAMS (1897, 1923 and cit. by CARTER, 1920)	d	+	p and s	*fibril* *canal*			
D)	WALKHOFF (1898)			note D	*canal*		denied existence of "Kittsubstanz"	
	MUMMERY (1914, 1915, 1919)	d	+	s	*tube* and *fibril*	+	penetration of dentinal fibril	
	ADLOFF (1914)			s	*canal*		largely substitute for prism sheath substance	
	CARTER (1917, 1920, 1922)			p and s	*tube* and *fibril*	+	direction not dependent on prisms	
	WEIDENREICH (1926)	d	+	pis	*canal*		part of same system as tufts and lamellae	
	MUNCH (1929)			—	*canal*		parallel prism direction	
	SPRAWSON (1930)		+	i	*tube*		stain penetration d.ts. → e.ts.	
	MARCUS (1931)	d	+	p	axial *fibril*			
	HÄUSELE (1932)			p				
	SKUES (1932)	e		p	*fibril*	+		
	MCCREA and ROBINSON (1935/36)		+	—			stain penetration d.ts. → e.ts.	
E)	MOSS and APPLEBAUM (1963)	e	—	p	*fibre*	+	≡ to tufts, (note E) uncalcified rods	
	BOYDE and LESTER (1967)	e	+	p	*tubule*	+	deficient crystallite formation locally	

Explanation see p. 558

material has been studied and there have been no reports of the investigation of this problem with modern techniques for elucidating ultrastructure. Further, although the permeability of the enamel tubules has been demonstrated *in vitro* (SPRAWSON, 1930; MCCREA and ROBINSON, 1935/36), no previous attempts have been made to demonstrate a physiological continuity of the dentinal and enamel tubules *in vivo*.

Materials and Methods

The marsupial species studied and the method of their study are listed in Table 2.

Table 2. *Materials and methods*

Reference numbers*	Species studied	Type of Examination				
		Electron microscopy of tubule development	Electron microscopy of adult structure	Light microscopy of tubule development	Light microscopy of adult structure	Dye diffusion experiments
B 61	*Metachirus nudicaudatus*	+	+	+	+	+
B 64	*Didelphys marsupialis*			+	+	
B 119	*Vombatus ursinus*		+		+	
B 151	*Trichosurus vulpecula*	+	+	+	+	
B 154	*Acrobates pygmaeus*			+		
B 169	*Antechinus swainsoni*			+		
B 177	*Pseudocheirus convolutor*			+		
B 215	*Protemnodon rufrogrisea*			+		
	Macropodidae (various species)		+	+	+	+

* (See "The Mammals" — Desmond Morris).

Electron Microscopy of Tubule Development

Tooth germs were fixed in Palade's and Dalton's fixatives and embedded in methacrylate. Approximately 500 Å thick sections were cut on a Porter-Blum microtome and examined at 60 kV in a Siemens Elmiskop I. $^1/_2$ μ thick sections were stained with crystal violet and basic fuchsin for light microscope controls.

Electron Microscopy of Adult Structure

Single-stage carbon replicas of fractured whole adult teeth were prepared by a method described by BOYDE (1967). Stereo-pair micrographs (tilt angle usually 8° 40″) were taken at 40 kV.

Fractured whole teeth and teeth extracted for prolonged periods with hot 1:2 diaminoethane were examined in a scanning electron microscope (Cambridge Instrument Company "Stereoscan"). A conducting coat of *ca.* 200 Å carbon and 300 Å gold was applied by vacuum evaporation with the specimens rotating so as to ensure good coverage of the surface to be examined. Stereo-pair electron micrographs (tilt angle usually 10°) were taken at 10 kV.

One transverse ground section of a *Macropus* molar was bombarded with 5 keV argon ions in the specimen chamber of a scanning electron microscope (BOYDE and STEWART, 1962).

Light Microscopy of Tubule Development

Ground sections were embedded in Canada balsam and examined by ordinary transmitted light.

Dye Diffusion Experiments

The pulp chambers of extracted *Macropus* and *Metachirus* molars were filled with solutions of crystal violet and basic fuchsin and allowed to stand for 1 or 2 days until dry. The external surfaces of these teeth had been covered with wax to prevent centripetal access of spilt solution.

One 1 year old 3.5 kg opossum (*Metachirus nudicaudatus*) was given a series of four intraperitoneal injections of 1% aqueous trypan blue over a period of one week (total 40 mls 1% solution injected).

Cavities which exposed the pulp were prepared in the buccal surfaces of some of the cheek teeth of a second 1 year old opossum and an adult female opossum of unknown age. Dry methyl blue was "teased" into these cavities with a probe; moistened; and the cavity sealed with quick setting zinc oxide and eugenol cement.

For the first experiment, the teeth were extracted after an interval of one month following the final injection. The teeth were extracted after an interval of one week in the case of the intrapulpal cavities. Ground sections were prepared in both instances.

Results

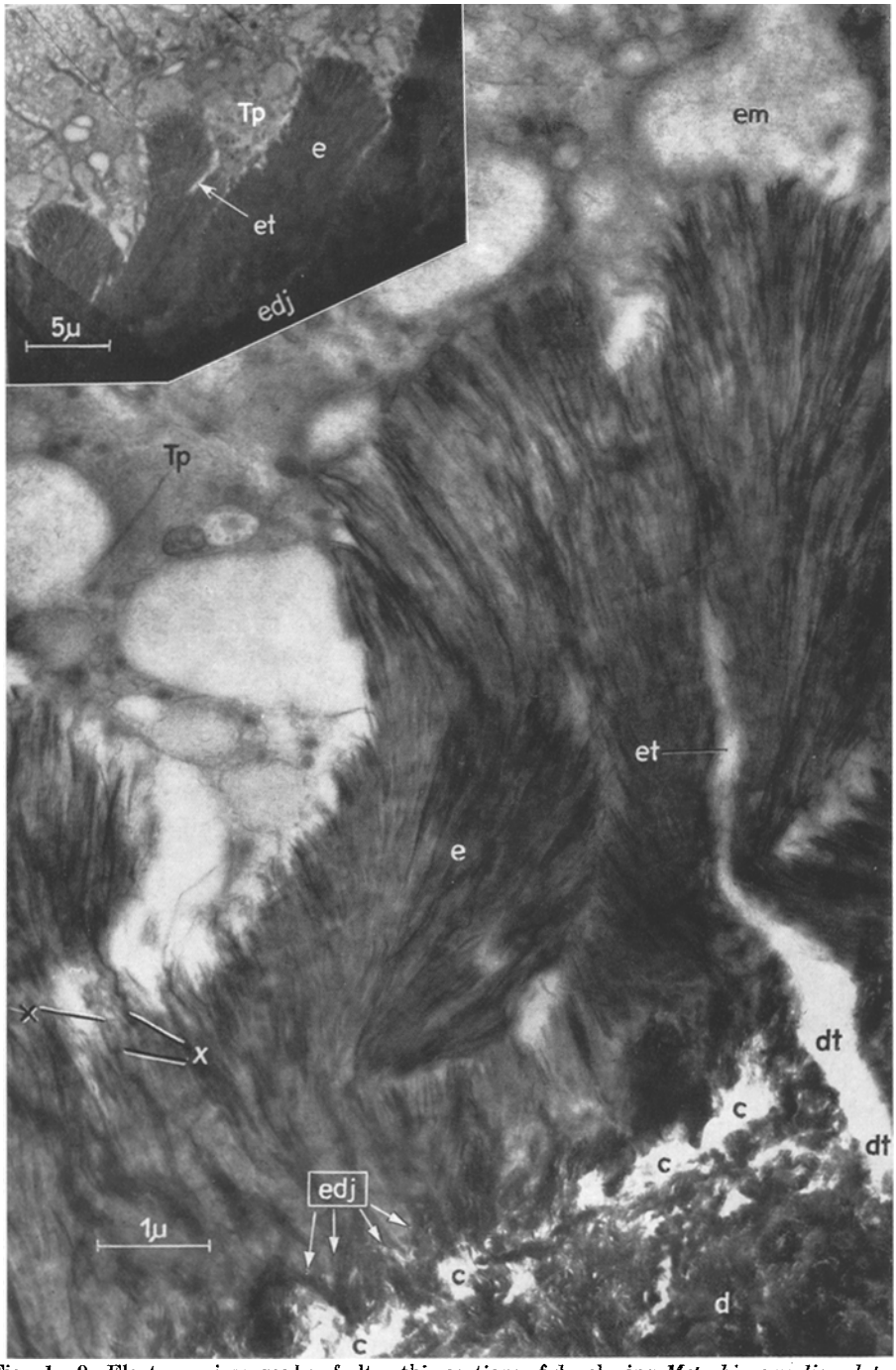
Electron Microscopy

Marsupial enamel was found to bear a close resemblance to the ungulate pattern, with longitudinal rows of prisms separated by longitudinal inter-row sheets ("Pattern 2", BOYDE, 1964). It is characteristic of this pattern that the crystallites within prism domains diverge less from the prism direction than those in "Pattern 3" prisms. The orientation of the prism crystallites, following the overall cuspal or incisal inclination of the prisms, thus contrasts with that of the inter-row sheet crystallites which incline slightly cervically. The main difference between marsupial and ungulate enamels observed in this study was the more common occurrence of a joining of prism-sheaths between members of the same row of prisms in ungulate enamel, related to a greater prominence of the inter-row ridges at the developing surface.

The main interest centred on the identification of the so-called enamel tubules. Some trouble was experienced in establishing reliable criteria for their identification in ultra-thin sections, since they were only seen as defects in the generally even distribution of crystallites in the developing enamel and were difficult to distinguish from artefact tears in the sections. The tubules were eventually positively identified by tracing the same pattern of defects in serial sections. Fig. 6 shows a small part of a pattern of tubules that was identified in all of 35 serial sections.

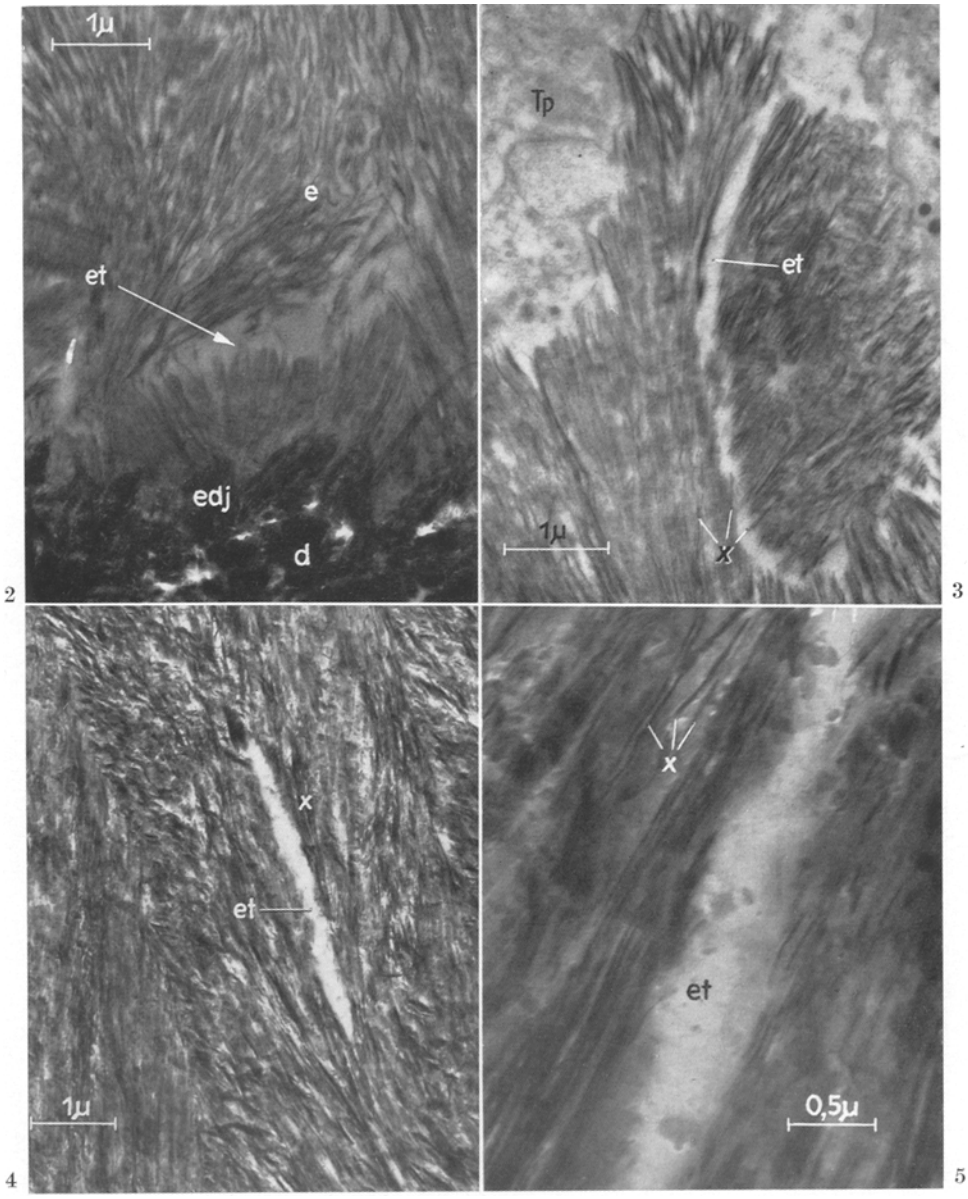
The enamel tubules were found to develop and to be propagated at the mineralising front of the enamel as areas in which there was a failure to form crystallites (Figs. 1, 3). Their site of development in the cervical slopes of the depressions in the mineralising front, rather than in relation to the interrow ridges, determines their intraprismatic localisation. These discontinuities in the otherwise intact mineralising front were not matched by any apparent abnormality in the surface of the Tomes' processes of the ameloblasts or in the extracellular granular material (the enamel matrix gel). The tubules were found to be located anywhere within prism domains, even in positions confluent with the boundary between a prism and an adjacent inter-row sheet, but not at the prism sheath separating two prisms in the same row (Figs. 7, 8, 9, 19, 20, 21).

The only morphologically identifiable contents of the tubules were the occasional isolated crystallites which passed through or across them (Figs. 3, 4, 5). The



Figs. 1—9. Electron micrographs of ultra-thin sections of developing *Metachirus nudicaudatus* enamel

Fig. 1. Longitudinal section of early developing enamel. Note one "tubule" crossing from dentine to enamel. The inset figure shows the orientation of the section, and also a "tubule" at the mineralising front. *e* enamel, *d* dentine, *edj* enamel-dentine junction, *et* enamel tube, *dt* dentinal tube, *x* enamel crystallites, *a* ameloblasts, *Tp* Tomes' process, *em* enamel "matrix", *ptd* peritubular dentine, *lb* lateral branch of dentinal tubule, *tb* terminal bar apparatus, *irs* inter-row sheet, *p* enamel prism, *c* crack, *f* fold in replica film, *sz* surface zone



2 3 4 5

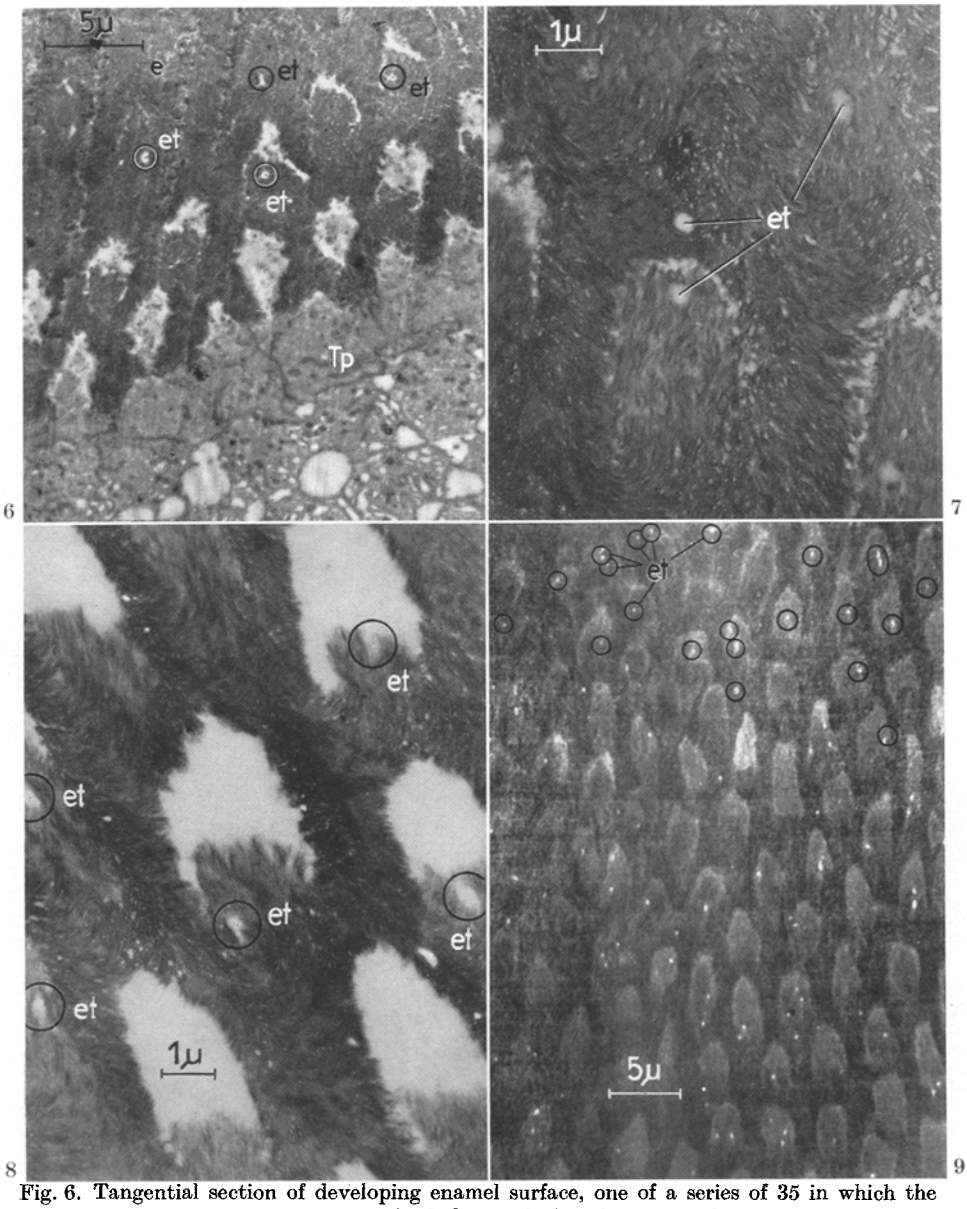
Fig. 2. A bend and bulge in an enamel tubule close to the enamel-dentine junction, showing the projection of crystallites into the "tubule lumen"

Fig. 3. A tubule continuous from the mineralising front for some distance into the enamel

Figs. 4 and 5. Tubules in more mature enamel

material within the tubules appeared entirely similar to the rest of the organic matrix throughout the enamel.

The undoubted continuity of dentinal and enamel tubules could be traced in all the marsupial species we have examined (Figs. 1, 10, 11, 12, 16, 17, 18 and 27), except in *Vombatus ursinus* [wombat, the one marsupial reported by Tomes (1849)



6 7 8 9

Fig. 6. Tangential section of developing enamel surface, one of a series of 35 in which the same pattern of tubules marked could be traced

Figs. 7 and 8. Transversely sectioned prisms near the developing front showing intraprismatic location of tubules

Fig. 9. Low power survey showing distribution of tubules, often several per prism

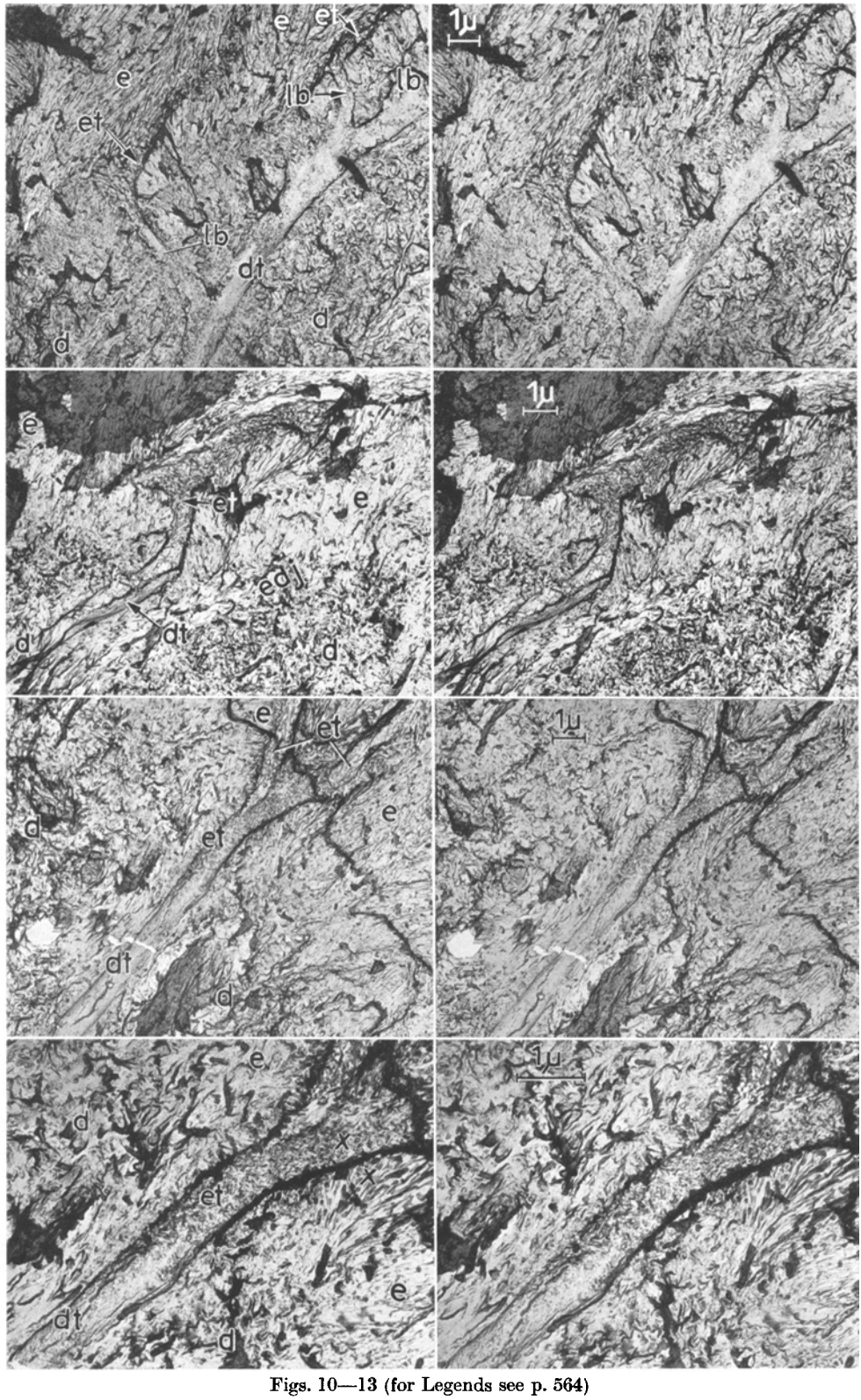
Figs. 10—15. Stereo-pair electron micrographs of carbon replicas of fractured enamel and dentine (10—14 *Macropus*, 15 *Sus*)

Fig. 10. Lateral branches of terminal part of dentinal tubule crossing enamel-dentine junction at right-angles and continuous with enamel tubule

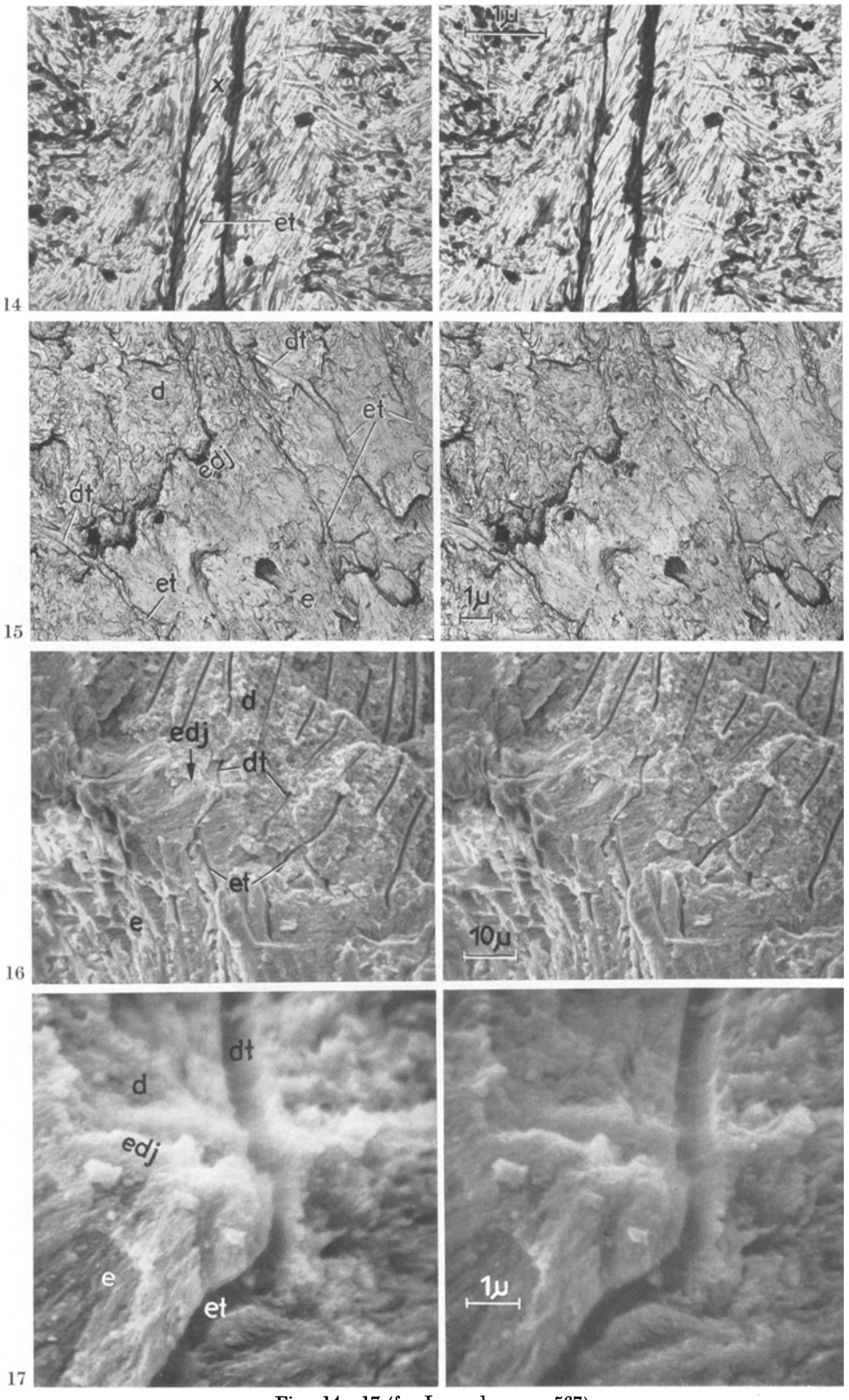
Fig. 11. Dentinal tubule bending abruptly to cross enamel-dentine junction at right-angles and the corresponding enamel tubule continuing parallel to its original course

Fig. 12. Single dentinal tubule continuous across enamel-dentine junction with two enamel tubules

Fig. 13. Enlargement of enamel-dentine junction region shown in Fig. 3 to show different nature of wall of dentinal and enamel tubule



Figs. 10—13 (for Legends see p. 564)



Figs. 14—17 (for Legends see p. 567)

not to have tubular enamel] where the dentinal tubules do not reach the enamel-dentine junction. This we have found to be associated with the presence of large "von Korff" fibre bundles oriented parallel to the enamel-dentine junction (LESTER and BOYDE, in press).

The majority of dentinal tubules reached the enamel-dentine junction without the branching and diminution of diameter normally a feature of tubules in this region. It was often observed in instances where there was terminal bifurcation of the dentinal tubule, that both the resulting branches were continuous across the enamel-dentine junction with tubules in the enamel. Single, unbranched dentinal tubules could often be traced in continuity with a tubule branching within the enamel.

The tubules always crossed perpendicular to the enamel-dentine junction, regardless of the course of the dentinal tubules and the enamel prisms away from the junction, the two usually lying in the same straight line. The tubules are thus approximately parallel with the enamel crystallites closest to the junction, since these first formed crystallites develop perpendicular to a relatively flat mineralising front: the ameloblasts have no Tomes' process projections into the enamel matrix at this stage. There was often a bulge in the enamel tubules where they bent until almost parallel with the enamel-dentine junction before straightening out to proceed with the prisms. This bulge, which was first described by TOMES (1849) but first figured by OWEN (1845), could be identified in many instances in the electron microscopic material. At the region of the bend and bulge the crystallites lay at a considerable angle to the tubules and even projected into the tubule "lumen" to some extent (Fig. 1, 2, 11 and 13). Thereafter, the tubules ran within clearly definable prism domains.

The enamel tubules could be distinguished from prism sheaths (with which they might possibly be confused in longitudinal sections or fracture profiles of the prisms) on the basis of the orientation of the crystallites surrounding them. Prism sheaths or boundaries are planes at which there is an abrupt change in crystallite orientation and they develop along lines at which there is a sharp change in the orientation of the mineralising front in the nature of a concavity towards the enamel. Enamel tubules on the other hand, were found to develop in the convex cervical floors of the depressions and, with one exception, were surrounded by crystallites having basically the same orientation even if this orientation was not parallel with the long axis of the tubule (Fig. 14). The one exception applied in the case of tubules which were confluent with the lateral part of a prism boundary, when the crystallites of the inter-row sheet forming one wall of the tubule obviously made a large angle with those in the other three-quarters of the wall and which belonged to a prism domain.

The structure of the walls of the dentinal tubules showed no differences from those of the placental mammals that we have studied (BOYDE and LESTER, 1967).

Fig. 14. Enamel tubule at some distance from the enamel-dentine junction showing parallel crystallites exposed as its wall

Fig. 15. Stereo-pair transmission electron micrograph of carbon replica of fractured tooth surface of *Sus domesticus*. Enamel-dentine junction region shows dentinal tubules continuous with minute enamel tubules

Fig. 16. Low power survey showing a number of tubules crossing the enamel-dentine junction

Fig. 17. Single tubule at junction

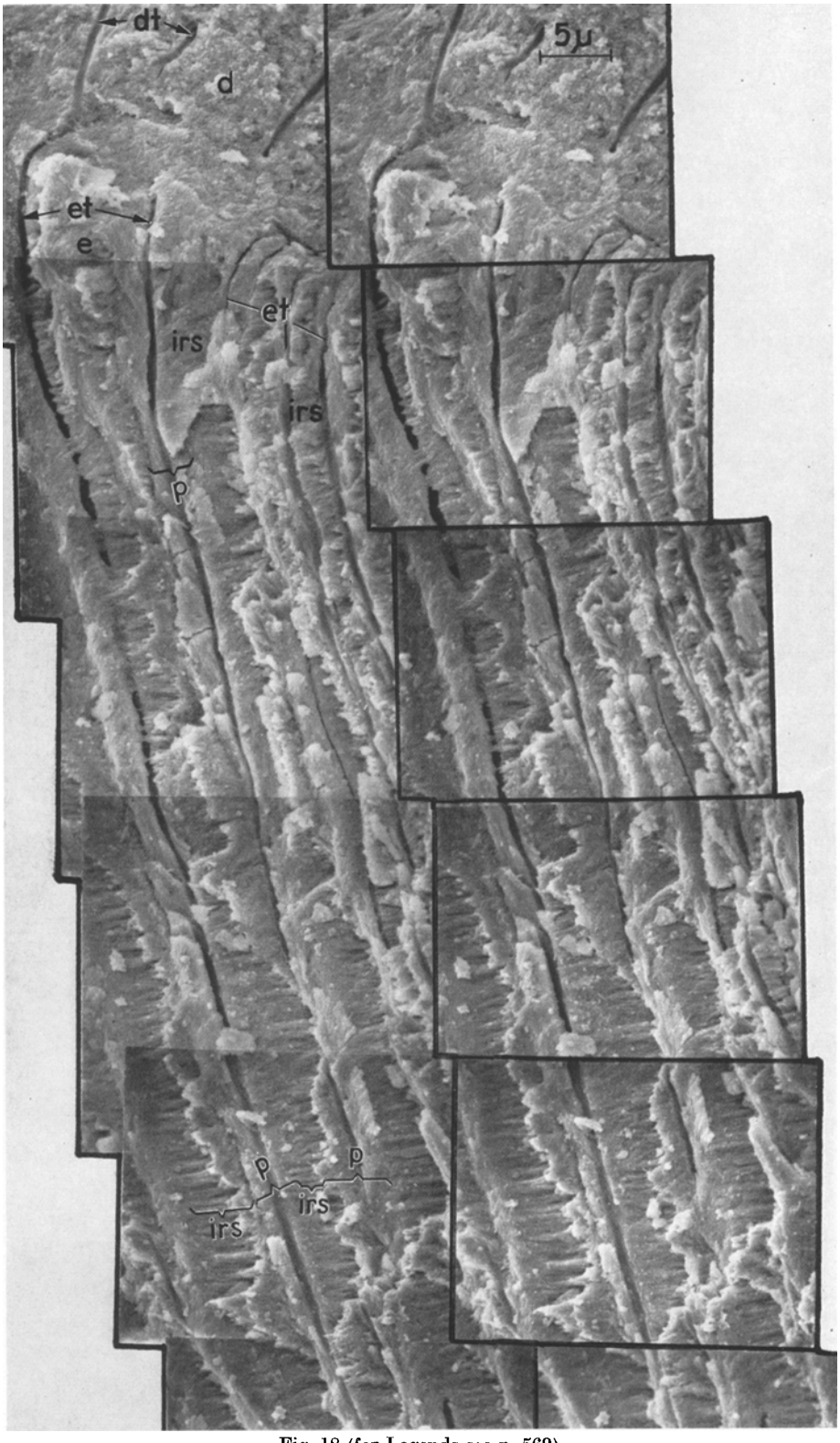


Fig. 18 (for Legends see p. 569)

Argon Ion Etching

The etch structure on argon ion-bombarded human enamel surfaces (BOYDE and STEWART, 1962) was mainly referable to the differences of crystallite orientation associated with its division into prisms. Macropus enamel, however, etched in a very different and characteristic way. Each enamel tubule was revealed by the formation of a deep etch pit (Figs. 22, 23, 24) surrounding its opening at the polished surface of the section: they could not be seen at all before bombardment.

The absence of differential etching in the surface zone enamel of *Macropus* (Fig. 25) first drew our attention to the thickness of this non-prismatic layer in this species.

The more rapid erosion of intertubular dentine led to a differential etching effect which left peritubular dentine standing proud of the surface. Fig. 23 shows that the peritubular dentine of *Macropus* reaches right up to the enamel-dentine junction.

Light Microscopic Observations

The enamel tubules were preserved as basophilic fibres in both the developing and mature decalcified enamel of all the marsupial species examined (Figs. 27, 28). In the mature enamel they were the only elements to be retained after decalcification and their relation to the original prism architecture could not be ascertained. In the "transitional" stage of maturation (CHASE, 1935) the basophilic fibres were well differentiated from the pale staining, acidophilic "matrix" (Fig. 26). The basophilic fibres were generally not distinguishable amidst the intense basophilia of the whole "matrix" in "young" enamel. It was not possible, therefore, to determine the relationship of the "fibres" to the honeycomb developing front of the enamel in this material.

Dye Diffusion Experiments

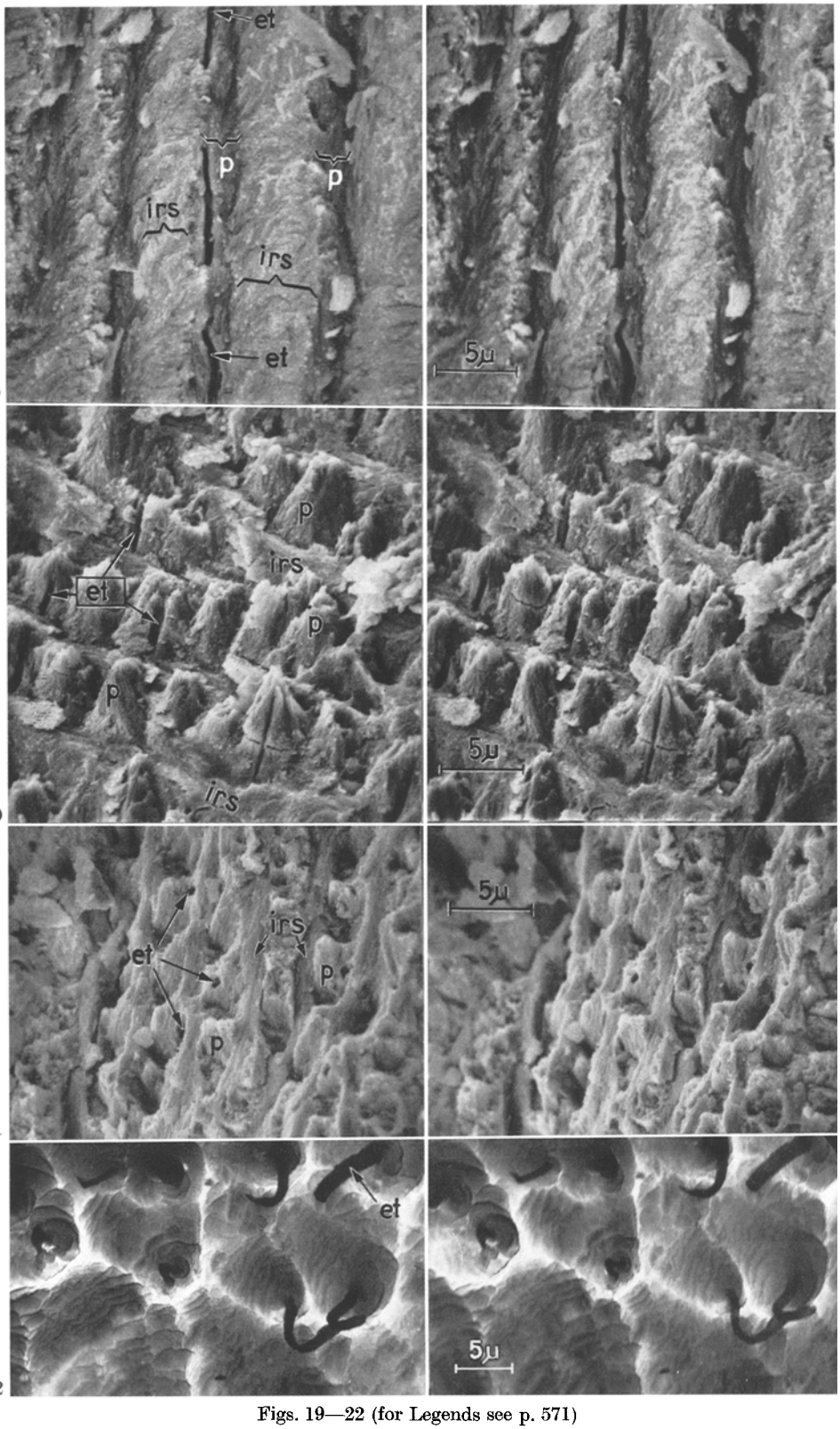
In vitro dye diffusion experiments on extracted *Macropus* molars showed that both the dyes used freely entered the dentinal tubules and thence the enamel tubules, to which they remained more or less confined. Some general diffusion of the dyes through the inner layers of the enamel did occur, but the enamel tubules could be seen more clearly because of their dye content.

No trace of penetration of trypan blue into either dentine or enamel was found in the opossum given vital injections of this dye six weeks previously and this in spite of the fact that the animal developed an overall blue colour which could be seen through the skin and oral mucous membrane.

The methyl blue dye inserted into the pulp of the cheek teeth 1 week beforehand had entered the dentinal tubules and penetrated some one-half to two thirds of the way towards the enamel-dentine junction. No trace of the dye could be found in enamel tubules. Tetracycline antibiotics were administered to some of these adult opossums, but no sign of the characteristic yellow fluorescence under ultraviolet irradiation was ever found in the enamel.

Figs. 18—21. Stereo-pair scanning electron micrographs of fractured *Macropus* enamel and dentine

Fig. 18. Part of montage showing one tubule crossing from the dentine and continuing into the enamel to the limit of the field. Inter-row sheet crystallites roof over the tubule in several places



Figs. 19—22 (for Legends see p. 571)

Discussion

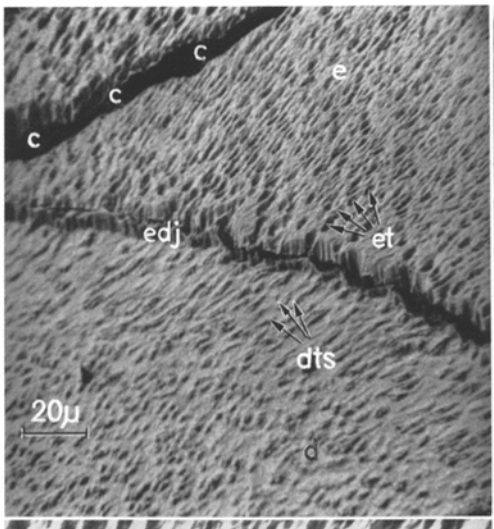
The present results lend support to both "tubular" and "fibrillar" concepts of the structure of the enamel "tubules". We have adopted the name "tubule" on the rather arbitrary basis that it is more suggestive of their nature as defects in a more or less continuous phase of "enamel". The tubules are "defects" in the sense that they contain fewer crystallites than the surrounding enamel. Use of the term defect is not meant to exclude the possibility that a factor causing the absence of the mineral component may reside in the organic matrix in the first place and it is not meant to imply that the genesis of the tubules is necessarily accidental. To state that the tubules are regions of the enamel which do not "mineralise" fully is only to summarise their nature and does not begin to provide an explanation of the mechanism of their development or of their adult function.

The present study provides no answer to the question of why the tubules develop in marsupial enamels. Features related to the enamel tubules have been described in rodent enamels: both actual tubules in the *Muridae, Sciuridae,* (von Ebner, 1890) and more amorphous spaces in the *Hystricomorpha* (Tomes, 1850; Korvenkontio, 1934—35). These structures are continuous with neither the enamel-dentine junction nor the enamel surface. The fact that these rather similar regions in rodent enamels (i. e. the "cells" of Tomes, 1850) and the tubules in some marsupial enamels (e. g. in the cervical region of *Petaurus* molar enamel — Tomes, 1849; von Ebner, 1890) are not continuous with the dentine tubules, would suggest that the *predisposing* cause of the development of these defects does not lie in connection with the surface of the dentine. In the development of those enamels in which the dentine and enamel tubules are continuous, we must consider that a predisposing cause is present at the earliest stage of formation of any given increment of enamel, that is to say when the first enamel crystallites develop on the surface of the dentinal collagen matrix. However, it is quite probable that some extra *initiating* factor in enamel tubule development resides at the future enamel-dentine junction in marsupials, and that this combined with a predisposition to the development of "defects" in crystallite formation determines the very high frequency of the tubules "crossing" the enamel-dentine junction in the teeth of marsupials.

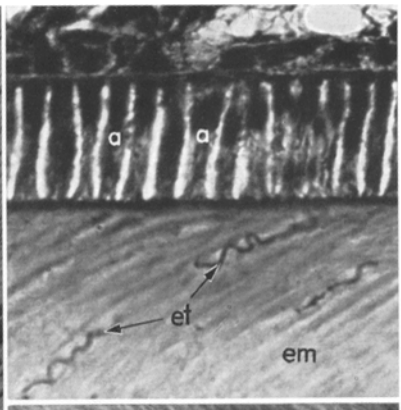
The fact that a high proportion of the enamel tubules appear to be in direct continuity with the dentinal tubules might suggest that their presence may depend in some way on an "influence" from the dentinal tubules (the odontoblast processes). It must then be determined why the odontoblast processes persist so near to the enamel in the groups that possess tubular enamel with continuous enamel and dentine "tubules" and what the postulated "influence" is. There is no doubt that the so-called "enamel tubules" are purely enamel formations. There is no evidence for their containing a morphologically identifiable dentinal component, for example, collagen fibres. However, it is possible that the growth of the first enamel crystallites at the enamel-dentine junction depends, in some way, upon the presence of an underlying substrate of dentine, such as would exist if the odontoblast processes actually attain the amelo-dentinal contact plane.

Figs. 19—21. Fractures in various planes showing intraprismatic localisation of tubular defects

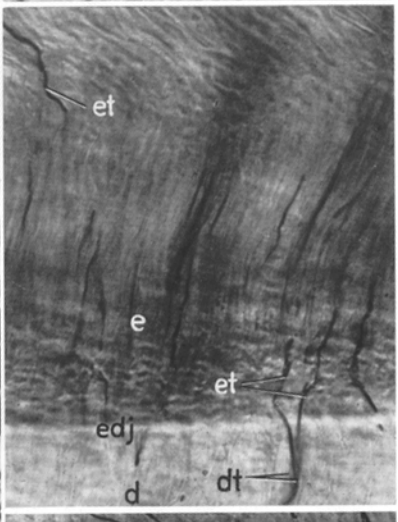
Fig. 22. Stereo-pair electron micrograph of formvar replica of argon ion etched *Macropus* enamel. — Scanning electron micrograph of formvar replica of ion etched *Macropus* molar showing long processes deriving from the tubules related to the bottom of ion etched pits



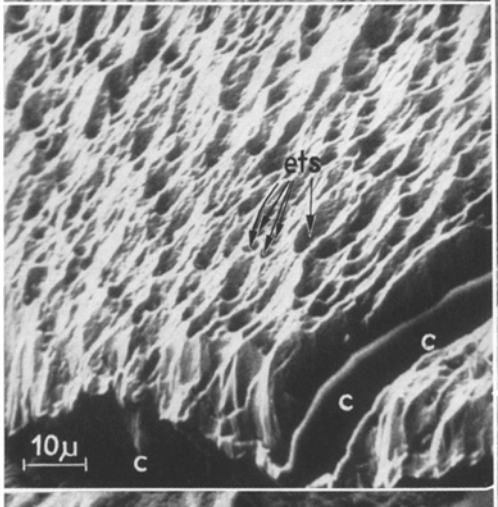
23



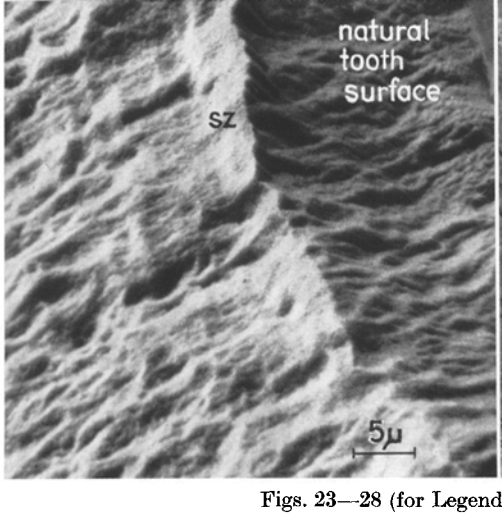
26



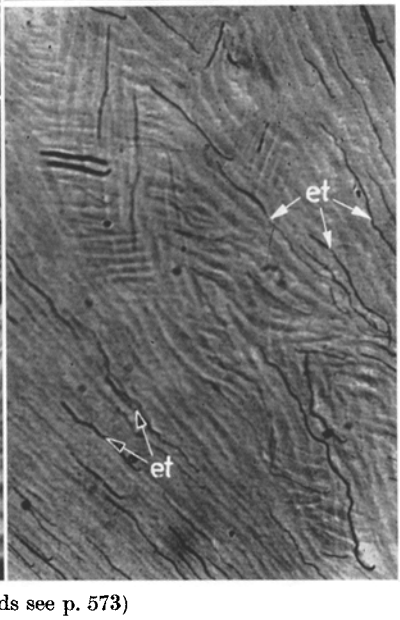
27



24



25



28

Figs. 23—28 (for Legends see p. 573)

It is conceivable that the initial nucleating factor which starts *enamel* crystallite formation at the enamel-dentine junction resides in the collagen fibres of the dentine matrix. Since these collagen fibres lie predominantly perpendicular to the surface of the dentine, and apatite crystallites are known to form on (or in) the surface of collagen fibres, an epitactic mechanism here might explain the degree of preferred orientation observed even in the first formed layer of enamel. Deficiencies in the surface of the dentine would result in "nucleation deficiences" for the enamel crystallites. Defective territories in which crystallites did not commence to grow (i. e. the "tubules") could only be propagated in regions in which the crystallites were parallel and essentially at right angles to the dentine surface, that is to say mainly within the prisms.

The observation that the enamel "fibres" only appear as structures differentiated from the remainder of the decalcified enamel matrix in the "transitional" (Chase, 1935) stage of maturation suggests that the "tubules" (fibres) acquire their high organic content as a result of "maturation". This would make their acquisition of an increased organic content analogous to the development of the "prism-sheaths", the organic matrix being forced into these regions during the growth in diameter of the enamel crystallites (Boyde, 1964). The enamel "tubules" are thicker than prism sheaths and could contain a greater bulk of organic material: this would explain the greater mechanical stability of the content of the tubules (i. e. the "fibre" of Tomes, 1856) after acid decalcification.

It seems very probable that the development of marsupial enamel tubules and rodent enamel "cells" (Tomes, 1850) is associated with the extremely rapid deposition of these tissues; and is, as it were, an accidental effect of this cause. The rate of deposition of rodent incisor enamels at approximately 16 μ per day contrasts strongly with that in man, at an average of 4 μ per day (Massler and Schour, 1946). Carter (1917) stated that — "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". Moss and Applebaum (1963) also — "suggest that the apparently very rapid rate of enamel matrix formation may play a role in the production of this type of enamel". A surprising difference was found

Figs. 23—25. Scanning electron micrographs of ion-etched *Macropus* molar

Fig. 23. Enamel, showing ion etched pits related to the enamel tubules and, in dentine, the prominence of the peritubular dentine and that it extends to the enamel-dentine junction

Fig. 24. Enamel, showing more detail of the distribution of the etch pits

Fig. 25. Enamel, ground section surface to the left, showing lack of differential etching in the surface zone enamel

Fig. 26. Light micrograph of haematoxylin and eosin stained section of developing *Protemnodon rufrogrisea* enamel and ameloblasts. — Trichrome stained decalcified coronal section of *Protemnodon rufrogrisea* incisor. Close to final stage of amelogenesis. The enamel "fibres" are the dark spirals within the lighter staining enamel matrix

Fig. 27. Ground section of *Macropus* molar. — Longitudinal ground section of *Macropus* molar showing continuity of two air-filled tubules across enamel-dentine junction

Fig. 28. Decalcified section of developing *Protemnodon rufrogrisea* enamel. — Decalcified, trichrome stained section of *Protemnodon rufrogrisea* incisor showing decussation of enamel prisms together with their associated "fibres"

in the amount of enamel formed in the dentition of two sibling pouch young *Metachirus nudicaudatus* used in the present studies, the one killed 7 days after the other. If the length of the Tomes' process is in any way an indication of the secretory activity of the ameloblast then the greater length of the marsupial Tomes' process (C. S. Tomes, 1897, 1904) might indicate a greater secretion pressure, in the sense of a greater rate of activity, of the marsupial ameloblasts.

We cannot agree with the opinion of Moss and Applebaum (1963) that the enamel tubules in *Macropus* are in fact uncalcified enamel rods and that they are therefore equivalent to the tufts in other mammalian enamels, since tuft regions are only under-mineralised, not unmineralised, and are several prisms wide (Boyde, 1964). Furthermore, enamel tubules are situated within perfectly normally mineralised enamel prisms.

Moss and Applebaum also believe that there is no continuity between the enamel and dentine "tubules". They state that ... — "considering the abundance of both dentinal tubules and of enamel fibers in the Marsupial, it is little wonder that the illusion of continuity between them is achieved with the use of relatively thick ground sections" and also (loc. cit) that — "the enamel "tubules" are an artifact of ground section preparations, and, further, that *in vivo* they contain uncalcified enamel matrix rather than the continuation of any odontoblastic process". There is certainly no justification for considering the tubules as artefacts in the sense in which we have used the term. Their conclusion that the tubules contain uncalcified enamel matrix is in agreement with our own findings.

The enamel spindles of other mammalian enamels cannot be regarded as structures analogous to the enamel tubules, since there is good evidence that the spindles are, or have, dentinal components (Frisbie, 1952; Schlack, 1940). They are also much thicker, being greater in diameter than the prisms, and much shorter, reaching only some 50—100 μ from the dentine surface. The presence of spindles, or that of similarly shaped structures in a similar situation, has been noted between the ameloblasts just before the commencement of amelogenesis (Lams, 1920; Chase, 1948) whereas the tubules develop during enamel development as an absence of crystallite growth in limited territories within otherwise normal domains. Frank (1965, personal communication) has studied spindles with the electron microscope and confirms that they may contain an odontoblast process.

Enamel "tubules", rather than the spindle-formed dilatations that are found in human enamel, are also found in certain members of the Orders *Rodentia* (e.g. the jerboa-Tomes, 1849; von Ebner, 1890), *Insectivora* (Tomes, 1849 — e.g. hedgehog, mole, shrew), *Primates* (Carter, 1922 — N. B. the *Lemuroidea*), and *Cheiroptera* (Löher, 1929). The marsupials are a special group in respect of their possessing "enamel tubules" in that only the one exception noted by Tomes (1849) has ever been reported (the wombat does not have enamel tubules).

We have examined the ground sections of cheiropteran teeth in the Tomes' collection of the Royal College of Surgeons of England and can confirm that tubules are present in some species, e.g. *Pteropus poliocephalus*, *Magaderma lyra*, and *Barbastellus communis*. Our preliminary electron-microscope studies of the teeth of a number of placental mammals — calf (*Bos bovis*), pig (*Sus domesticus*), dolphin (*Delphinus delphis*) and man (*Homo sapiens*) — have revealed the presence of very narrow structures in the enamel which are continuous across the enamel-dentine junction with dentinal tubules (Fig. 15). Although these structures almost

certainly do not extend through the entire thickness of the enamel, it is difficult to regard them as enamel spindles because they exhibit no extension of dentinal matrix, no dilatation along their length, and because their average diameter of ca. 0.4 μ approaches the limit of resolution of the light microscope. Further, their ultrastructure is in every way similar to the enamel tubules of metatheria. Although the presence of these minute enamel tubules has only been observed in these four eutherian species to date, there seems little reason to doubt their more widespread existence[1].

Ion etching seems to have been a particularly suitable way of preparing the enamel surface for the purpose of visualising the distribution of enamel tubules. The tubules always arose from the bottom of an ion etched pit, and this is why they can be seen in the formvar replicas (Fig. 22), but not in the shadow of the bottom of the pits in the scanning electron micrographs (Figs. 23 and 24). STEWART (personal communication, 1967) has suggested that the formation of these etch pits may be related to the surrounding surface charging and focussing the ions into the openings of transversely sectioned enamel tubules, thereby leading to a locally increased rate of bombardment and sputtering.

References

ADLOFF, P.: Zur Frage der Kittsubstanz der Schmelzprismen. Dtsch. Mschr. Zahnheilk. **32**, 454—461 (1914).

BOYDE, A.: The structure and development of mammalian enamel. Thesis, University of London (1964).

— A single-stage carbon-replica method and some related techniques for the analysis of the electron-microscope image. J. roy. micr. Soc. **86**, 359—370 (1967).

—, and K. S. LESTER: An electron microscope study of fractured dentinal surfaces. Calc. Tiss. Res. **1**, No 2 (in press) (1967).

—, and A. D. G. STEWART: A study of the etching of dental tissues with argon ion beams. J. Ultrastruct. Res. **7**, 159—172 (1962).

CARTER, J. T.: The cytomorphosis of the marsupial enamel-organ and its significance in relation to the structure of the completed enamel. Phil. Trans. Roy. Soc. Lond. Ser. B. **208**, 271—305 (1917).

— The microscopical structure of the enamel of two sparassodonts, cladosictis and pharsophorus, as evidence of their marsupial character together with a note on the value of the pattern of the enamel as a test of affinity. J. Anat. (Lond.) **54**, 189—195 (1920).

— On the structure of the enamel in the primates and some other mammals. Proc. Zool. Soc. Lond. 1922, p. 599—608.

CHASE, S. W.: The nature of the enamel matrix at different ages. J. Amer. dent. Ass. **22**, 1343—1352 (1935).

— The development, histology and physiology of enamel and dentine — their significance to the caries process. J. dent. Res. **27**, 87—92 (1948).

EBNER, V.: Strittige Fragen über den Bau des Zahnschmelzes. S. B. Akad. Wiss. Wien, math.-nat. Kl. Abt. 111, **99**, 57—104 (1890).

FRISBIE, H. E.: Terminal dentinal tubules and tubules appearing to cross the dentinoenamel junction (enamel spindles). J. dent. Res. **31**, 466 (Abst.) (1952).

HÄUSELE, F.: Zur Phylogenie der Schmelzprismen. Z. Zellforsch. **12**, 395—429 (1932).

KORVENKONTIO, V. A.: Mikroskopische Untersuchungen an Nagerincisiven unter Hinweis auf die Schmelzstruktur der Backenzähne. Histologisch-Phyletische Studie. Ann. zool. Soc. zool.-bot. fenn. "Vanamo" **2**, 1—274 (1934/35).

[1] A number of authors have purported to show either enamel "capillaries" or enamel tubes in human teeth by light microscopy (BEUST — Dent. Cosmos, 1914, 1924; KATO — Dent. Cosmos, 1929, 1930; ALLEN — Dent. Cosmos, 1930; FORHUFSVUD — D.Z.M.U.K.H., 1942; Acta Odont. Scand. 1946, 1947).

LAMS, H.: Histogénèse de la dentine et de l'émail chez les mammifères. C. R. Soc. Biol. (Paris) **83**, 800—802 (1920).

LESTER, K. S., and A. BOYDE: The question of VON KORFF'S fibres in mammalian dentine (in press).

LÖHER, R.: Beitrag zum gröberen und feineren (submikroskopischen) Bau des Zahnschmelzes und der Dentinfortsätze von Myotis myotis, Zahn-Studie I. Z. Zellforsch. **10**, 1—37 (1929).

MARCUS, H.: Zur Phylogenie der Schmelzprismen. Z. Zellforsch. **12**, 395—429, (1931).

MASSLER, M., and I. SCHOUR: The appositional life span of the enamel and dentin forming cells. J. dent. Res. **25**, 145—150 (1946).

MCCREA, M. W., and H. B. G. ROBINSON: Dentinal projections in marsupial enamel. J. dent. Res. **15**, 313—314 (Abst.) (1935/36).

MORRIS, D.: The mammals. London: Hodder and Stoughton 1965.

MOSS, M. L., and E. APPLEBAUM: The fibrillar matrix of marsupial enamel. Acta anat. (Basel) **53**, 289—297 (1963).

MUMMERY, J. H.: On the nature of the tubes in marsupial enamel, and its bearing upon enamel development. Phil. Trans. Roy. Soc. Lond. Ser. B. 205—313 (1914).

— Calcification. Brit. dent. J. **36**, 69—75 (1915).

— The microscopical anatomy of the teeth. London and Oxford: Medical Publ. 1919.

MUNCH, G.: Beitrag zur Frage der Vitalität des Schmelzes. Vjschr. Zahnheilk. **45**, 532—542 (1929).

OWEN, R.: Odontography, vol. I text, vol. II illustrations. London: Baillière 1845.

PAUL, F. T.: Some points of interest in dental histology. The enamel organ. Dent. Rec. **16**, 493—498, (1896).

RÖSE, C.: Contributions to the histogeny and histology of bony and dental tissues. Dent. Cosmos **35**, 1189—1202. (Transl. by R. HANITSCH 1893).

— Über die verschiedenen Abänderungen der Hartgewebe bei niederen Wirbeltieren. Anat. Anz. **14**, 33—69 (1897).

SCHLACK, C. A.: Investigations into the etiology of enamel spindles in human teeth. J. dent. Res. **19**, 195—213 (1940).

SKUES, K. F.: The development of dental enamel in marsupials. Aust. dent. J. **36**, 187—196, 232—245, 282—294 (1932).

SPRAWSON, E.: On the histological evidence of the organic content and reactions of marsupial enamel, with a note on human enamel. Proc. roy. Soc. B. **106**, 376—387 (1930), same in Brit. dent. J. **51**, 1028—1036 (1930).

TOMES, C. S.: On the development of marsupial and other tubular enamels, with notes upon the development of enamel in general. Phil Trans. Roy. Soc. Lond. ser. B **189**, 107—122 (1897).

— A manual of dental anatomy. Human and comparative, 636 p. London: Churchill 1904.

— On the minute structure of the teeth of creodonts with special reference to their suggested resemblence to marsupials. Proc. Zool. Soc. Lond. 1906, p. 45—58.

TOMES, J.: On the structure of the dental tissues of marsupial animals, and more especially of the enamel. Phil. Trans. Roy. Soc. Lond. **139**, 403—412 (1849).

— On the structure of the dental tissues of the order Rodentia. Phil. Trans. Roy. Soc. Lond. **140**, 529—567 (1850).

— On the presence of fibrils of soft tissues in the dentinal tubes. Phil. Trans. Roy. Soc. Lond. **146**, 515—522 (1856).

WALKHOFF, O.: Beiträge zum feineren Bau des Schmelzes und zur Entwicklung des Zahnbeins. Dtsch. Mschr. Zahnheilk., trans. in J. Brit. dent. Ass. **19**, 757—771 (1898).

WEIDENREICH, F.: Über der Schmelz der Wirbeltiere und seine Beziehungen zum Zahnbein. Z. Anat. Entwickl.-Gesch. **79**, 292—351 (1926).

WILLIAMS, J. L.: A contribution to the study of the pathology of enamel. Dent. Cosmos **39**, 169—196, 269—301 and 353—374 (1897).

— Disputed points and unsolved problems in the normal and pathological histology of enamel. J. dent. Res. **5**, 27—107 (1923).

Dr. A. BOYDE
Anatomy Department, University College, University of London
Gower St., London W. C. 1