

Review

Calcified Tissues in the Earliest Vertebrates

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Remains of the first vertebrates — the Heterostraci — are found in Ordovician marine rocks some 500 million years old. Representatives of this group continued for 150 million years before becoming extinct towards the end of the Devonian period. The preservation of the bony armour of these primitive jawless vertebrates is such that it is possible to examine their microscopic structure in considerable detail. Indeed, palaeontologists have prosecuted such studies since the early part of the last century, although the history of this research followed two independent courses, Russian and English.

AGASSIZ (1845), working on fossil fish from Russia, noted that the main tissue of the armour of *Psammosteus* was a homogeneous substance without bone-cell spaces and hence he believed that it was more akin to dentine than to bone. Later PANDER (1857) pointed out that the external, tuberculated surface was composed of true dentine. GÜRICH (1891) noted fine tubules radiating out from the vascular canals of the spongy bonelike tissue and ROHON (1893) observed that “one can convince oneself of the presence of simple bone cells” with a spindle-shaped form and no cell processes — a view he later (1901) retracted.

Until the end of the century no-one had any idea of the affinities of *Psammosteus* but English workers had in the meantime been examining the armoured fish *Pteraspis*. HUXLEY (1858), in a classic paper on the microstructure of early fossil vertebrates, noted that the armour of this animal possessed no bone-cell spaces. LANKESTER (1868) used this character to distinguish a group of fishes, which he named the Heterostraci. His diagnosis, in fact, began “*scutae materia sine lacunis osseis*”. In the second part of his monograph (1870) LANKESTER figured, without comment, a microscopic section of a heterostracan which showed the same spindle-shaped spaces observed by ROHON.

In 1899 TRAQUAIR claimed that the Russian, and recently discovered Scottish, *Psammosteus* and *Pteraspis* should by virtue of their histology both be included in the Heterostraci, a view confirmed by subsequent work. TRAQUAIR also described the radiating tubules, first observed by GÜRICH, and he considered that this supported AGASSIZ' contention that the main tissue of the armour was closer to dentine than to bone.

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The next major advance was due to GROSS (1930) who gave a detailed and thorough account of psammosteid microstructure, especially the specialisations of its dentine. In this paper he introduced the noncommittal term *aspidin* for the tissue making up the bony armour. Later (1935) he suggested that the coarse spaces in the aspidin were the former site of Sharpey's fibres and he was followed in this interpretation by BYSTROW (1955) and ØRVIG (1958a). OBRUCHEV (1941), on the other hand, interpreted the fine radiating tubules in the aspidin of *Obruchevia* as having once housed the processes of osteoblasts (aspidinoblasts of BYSTROW), a view supported by HALSTEAD TARLO (1965). A return to ROHON's transitory stand was made by HALSTEAD TARLO (1963, 1964, 1965) when the spindle-shaped spaces were unequivocally identified as aspidinocyte lacunae — a point of view strongly contested by ØRVIG (1965, 1967, 1968) and by MOSS (1968). DENISON (1967) also contended that the coarse spaces were made by fibre-bundles but that the finer tubules in the Ordovician *Astraspis* formerly housed odontoblast processes and hence the main tissue was a type of dentine. In this same paper DENISON was able to demonstrate that the globular calcified cartilage previously recorded by ØRVIG (1951) and SPJELDNAES (1967) actually belonged to the earliest heterostracan *Eriptychius*.

In this brief review I shall describe the nature of the tissues preserved and outline some of the controversies that have arisen over their interpretations. Finally, I will discuss the relationships of these tissues to one another.

Aspidin

The main part of the dermal armour between the external dentine tubercles and the basal lamellar layers, consists of a spongy textured tissue, with the macroscopic appearance of cancellous bone (Fig. 1). This middle layer of aspidin is made up of a three dimensional scaffolding of calcified trabeculae of varying sizes. In some forms such as *Pteraspis* and *Cythaspis* the trabeculae form vertical walls enclosing polygonal spaces, although in the uppermost part of the plates, just below the dentine tubercles and also at the edges of the plates, a spongy texture prevails. In the later representatives of the Heterostraci such as *Psammolepis* and *Psammosteus* it can be seen that, as well as the initial trabeculae, there are concentric layers of lamellar aspidin surrounding the so-called vascular spaces. These, named aspidones by GROSS (1961), appear to have been laid down by the successive apposition of aspidin against the trabeculae.

The basic architecture of this tissue can thus be compared directly with that of modern bone. The tissue has been contrasted on the grounds that it did not undergo remodelling, resorption and renewal (URIST, 1962, 1964a, b) and hence was physiologically closer to dentine. GROSS (1930) believed that remodelling must have occurred, although he was unable to provide any direct evidence. That remodelling took place is evidenced by the fact that the plates of the head and anterior part of the trunk of *Pteraspis* completely fuse when the animal reaches maturity. In sections, there is no evidence of discrete plates as the large cancellae continue across the joins, whereas before fusion the edges of the plates have a fine spongy texture. This latter must have been replaced and hence it can be concluded that remodelling was possible. The first direct evidence of resorption

was described in the psammosteid *Ganosteus*, where resorption scalloping of concentric lamellae was present in the upper part of the spongy aspidin (HALSTEAD TARLO, 1963, 1964, 1965). The redeposition of secondary aspidones in previously resorbed cavities has also been described (HALSTEAD TARLO, 1963, 1964). It must be stressed that this phenomenon is not very commonly observed, although DENISON (1967) has recorded further examples from the Ordovician *Epiptychius*.

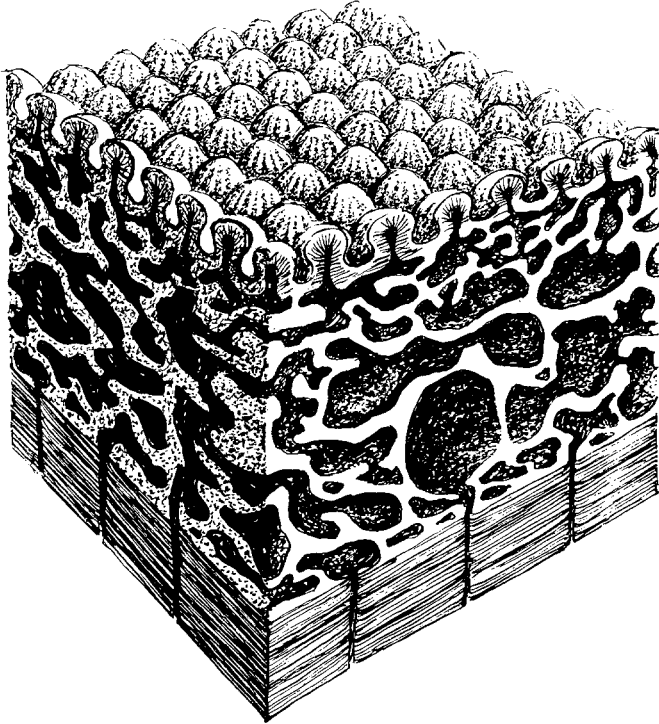


Fig. 1. Block diagram of heterostracan armour, showing superficial dentine tubercles, spongy aspidin and basal lamellar aspidin (from HALSTEAD, 1969 a, by permission of Oliver and Boyd, Edinburgh)

The recognition of resorption, as opposed to post-mortem changes brought about by geological agents, is not, as yet, very frequent, but the fact that unequivocal examples are now recognised does demonstrate that aspidin had the ability to remodel.

A further method of studying aspidin is by examining sections under polarised light (crossed nicols). In the earliest examples of aspidin this only reveals the normal extinction crosses indicating either a concentric or radial alignment of the apatite crystallites. In this instance the former alternative seems the more likely. The arrangement of the crystallites and hence by inference the collagenous fibrils of the organic matrix seems to have been comparable to that of dentine. With the later and more advanced Heterostraci such as *Psammolepis* the initial trabeculae show a black and white speckled appearance, indicating a more complex and more random organisation of the organic matrix, comparable to woven bone. In contrast the concentric lamellae of the aspidones show a well-

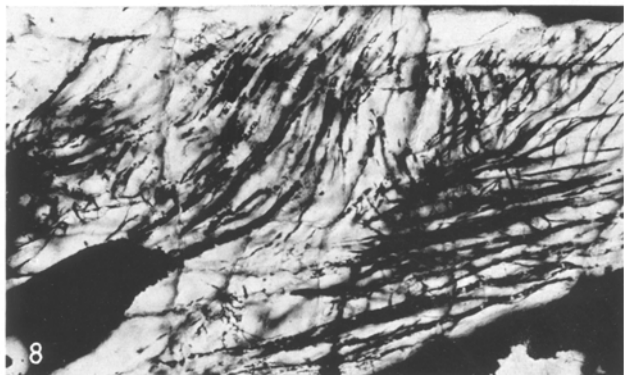
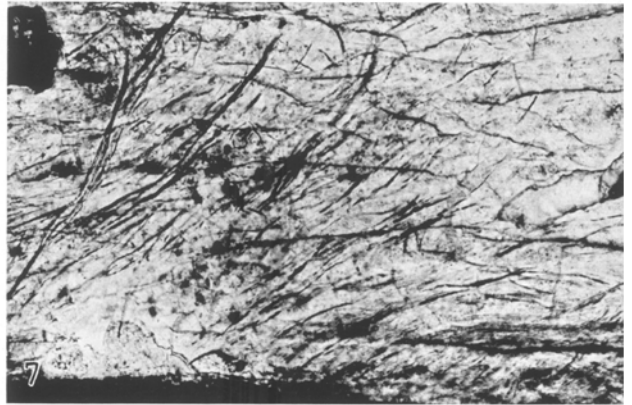
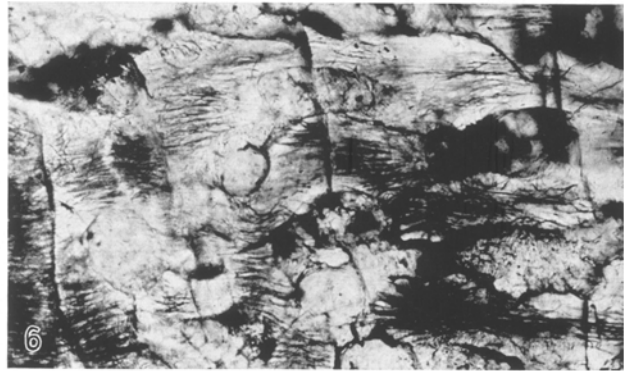
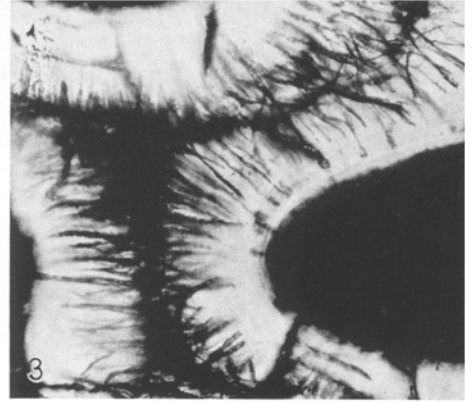
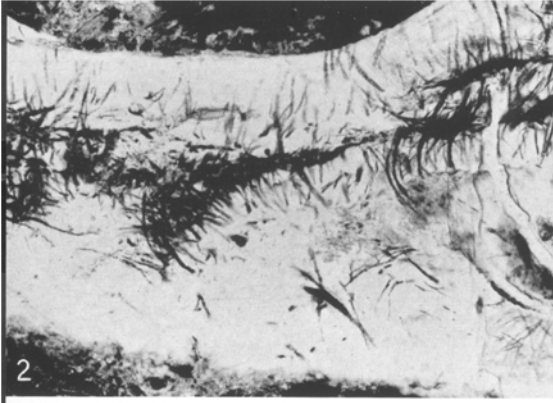
marked black and white banding (HALSTEAD TARLO, 1965, pl. 7, Fig. 3). This latter observation was first made by GROSS (1930) but not figured until 1963 (HALSTEAD TARLO). One of the last representatives of the Heterostraci in the fossil record, *Obruchevia*, reveals a narrow black and white banding, which is difficult to distinguish from that present in modern bone. During the passage of 150 million years, aspidin appears to show a gradual evolution of the organisation of its organic matrix from a condition characteristic of dentine to that of bone.

The basal layer of the dermal plates which consists of lamellar aspidin was contrasted by GROSS (1930) with that found in other vertebrates in that it did not show this black and white banding. Although this was undoubtedly true in the sections examined by GROSS, it is not always the case. Some members of the later Heterostraci do exhibit this black and white banding, so that this feature cannot be used to distinguish the two tissues from one another.

The main difference between aspidin and bone on which there has been some controversy in recent years is the nature of the fine structures preserved in the aspidin. Aspidin has generally been held to have been an acellular bone, and as such has been considered primitive by DENISON (1963) and HALSTEAD TARLO (1963). STENSIÖ (1927) and ØRVIG (1951), on the other hand, believed it to have been secondarily derived from cellular bone in the same way as the acellular bone of the modern teleosts. Latterly (1965, 1967) ØRVIG has taken up a neutral position on this question. As noted in the introductory paragraph, aspidin encloses empty spaces (Fig. 2) which have been observed by numerous authors since the last century. MOSS (1961) illustrated in decalcified sections some of the spindle-shaped spaces as "light staining streaks" and more recently (1968) claimed that these structures were simply oblique sections of uncalcified fibre-bundles which had been mistaken for spindle-shaped spaces. The thickness of the sections of fossil material is such that it is possible to trace the full extent of these structures within the section by focussing up and down. There is no doubt that the trabeculae of aspidin enclose simple spindle-shaped spaces. These have generally been interpreted as having been the former site of bundles of collagen (GROSS, 1930, 1935; BRYANT, 1936; BYSTROW, 1955; ØRVIG, 1951, 1965, 1967, 1968; DENISON, 1967; MOSS, 1968). Recently, the claim has been made that they housed the scleroblastic cells responsible for the production of the organic matrix on which calcification took place; that they represented the lacunae of aspidinocytes (HALSTEAD TARLO, 1963, 1964, 1965; HALSTEAD, 1969a), a view accepted by OBRUCHEV (1964) and NOVITSKAYA (1966).

Similar spaces make up the radiating "tubules" in the aspidin and have also been interpreted in these two contrasting manners. In many cases they can be seen to be continuations of the randomly arranged spaces in the trabeculae,

Figs. 2—8. Aspidin. Fig. 2. *Psammolepis proia* MARK-KURIK, part of trabecula with spindle-shaped spaces. Fig. 3. *Psammosteus megalopteryx* (TRAUTSCHOLD), with spaces elongated normal to lamellae of aspidin. Fig. 4. *Obruchevia heckeri* (OBRUCHEV), aspidones penetrated by fine tubules. Fig. 5. *Ganosteus stellatus* ROHON, trabeculae of aspidin with fine tubules. Fig. 6. *Tesseraspis tessellata* WILLS, with fine Sharpey's fibres running horizontally towards margin of plate. Fig. 7. *Ganosteus stellatus* ROHON, Sharpey's fibres in basal lamellar aspidin. Fig. 8. *Weigeltaspis godmani* HALSTEAD TARLO, Sharpey's fibres in basal aspidin. All photomicrographs $\times 128$



Figs. 2—8

aligned normal to the lamellae laid down by apposition (Fig. 3). At the same time they generally do not continue through to the vascular spaces but taper off. For this reason HALSTEAD TARLO (1963, 1965) interpreted them as representing extensions of the cell processes of the aspidinocytes, in an endeavour to maintain contact with the source of nutriment in the vascular spaces. In view of the nature of the collagenous matrix, as inferred from polarised light studies, it seems unnecessary to postulate a further organisation of fibre bundles. It also makes difficult an explanation of comparable spaces aligned parallel to the lamellae.

A further set of structures have been observed in aspidin, first by OBRUCHEV (1941) in his description of the late psammosteid *Aspidosteus* (= *Obruchevia*, Fig. 4). Fine tubules radiate from the vascular spaces and OBRUCHEV considered that they were canaliculae which had housed the cell processes of retreating osteoblasts (Figs. 4, 5). BYSTROW (1955), on the other hand, claimed that they were exactly the same as the much coarser tubules previously observed. OBRUCHEV's interpretation was supported by HALSTEAD TARLO (1965) in his study of other psammosteid genera, which he compared to the aspidin of the Ordovician *Astraspis* in which the fine tubules of the aspidin were of the identical bore to those in the dentine tubules (Fig. 9). DENISON (1967) also accepted that the tubules in the aspidin and tubercles of *Astraspis* were made by cell processes retreating as they produced the matrix. As the tubercles were dentine, he considered that the main tissue of the plates should be interpreted as trabecular dentine. ØRVIG (1951) considered the tissue in the tubercles to be dentine but later changed his mind and identified it as aspidin (1958a). If DENISON's view were to be accepted, it would mean that aspidin could readily change into trabecular dentine. This is evidenced from the histology of *Ganosteus* which evolved from an animal with typical aspidin to one with typical "trabecular dentine". A much more reasonable interpretation has been argued by HALSTEAD TARLO (1965), who suggested that the difficulties in interpretation could be readily overcome by recognising that, at the very beginning of the evolutionary history of the vertebrates, the tissues aspidin and dentine were hardly to be distinguished. Needless to say, this view has been vigorously opposed by ØRVIG (1967).

The last structures in aspidin to be considered are Sharpey's fibres. Until recently it was the general practice to identify as such all the structures discussed above. This usage was attacked by HALSTEAD TARLO (1963) on the grounds that Sharpey's fibres are not components of internal systems but are rather means of anchoring one tissue to another. ØRVIG (1966) defended the use of this term as a general one applied to the lower vertebrates, but conceded "that it might be advantageous ... to use the terms *extraneous* [i.e. Sharpey's fibres proper] and *intrinsic* fibres ... those which are integral parts of the bone tissue matrix."

True Sharpey's fibres of varying calibre are commonly found obliquely aligned in the basal layers of the heterostracan plates (Figs. 7, 8). They are also found at the edges of plates and presumably held the latter firmly in the dermis. The growth of small polygonal plates or tesserae in such genera as *Tesseraspis* shows the characteristic development of such bundles of collagenous fibre bundles (Fig. 6). These were erroneously interpreted as "elongated aspidinocyte spaces" by HALSTEAD TARLO (1965).

The spaces enclosed in the tissue aspidin can be interpreted as having once housed bundles of collagenous fibres (ØRVIG), bundles of collagen and cell processes (DENISON) or bundles of collagen, cell processes and cell bodies (HALSTEAD).

Dentine

Although it has been recognised since the time of PANDER (1857) that the outermost part of the dermal armour was made up of the tissue dentine, a detailed study was not carried out until GROSS (1930, 1935) published his definitive accounts, which were mainly concerned with the psammosteids. In all cases the dentine tubercles or ridges are penetrated by tubules which open into pulp cavities and by comparison with modern dentine it is reasonable to believe that their formation was similar.

In the Ordovician heterostracans there appears to be two different types of dentine. In *Astraspis* there is a glassy, sculptured cap to the tubercles that has been identified as enamel (BRYANT, 1936), but which ØRVIG (1958a) showed contained minute tubules and hence could be identified as dentine. DENISON (1967) suggested that this capping was enameloid. Its appearance and scalloped inner margin was due to "mineralization of the outer zone beginning at the surface and proceeding centripetally some distance towards the centre of the tubercle" (Fig. 9). The main part of the tubercles has fine tubules radiating from the pulp cavity and this led ØRVIG (1951) to identify this tissue as dentine (Fig. 9). Later (1958a) he retracted this view and claimed that it was aspidin and that the tubules had housed collagenous fibres as previously suggested by BRYANT (1936). HALSTEAD TARLO (1965) considered that this tissue showed similarities to both dentine and aspidin, on the grounds that comparable tubules were present in some of the later psammosteids. He concluded that "the calcified tissue in the tubercles is probably nearer to dentine than to aspidin itself" (Fig. 9). He claimed that the tubules "marked the former site of the processes of cells forming the tissue, much as normal dentine tubules indicate the presence at some time of processes of odontoblasts". This view was supported by DENISON (1967) who claimed that this tissue was indeed dentine and noted that some of the tubules could be traced into the tissue forming the enameloid capping. At the same time, since similar tubules pervaded the entire tissue, he interpreted it as dentine. This similarity was noted by BRYANT (1936) and by HALSTEAD TARLO (1965) and this tissue would seem to be best interpreted as intermediate between dentine and aspidin, perhaps marking the point at which dentine and aspidin differentiated. This interpretation is, however, contested by ØRVIG (1967).

With the contemporary genus *Eriptychius*, the dentine tubules are coarse and well separated (Fig. 10); furthermore, they show the characteristic lateral and terminal branches of true dentine (BRYANT, 1936; DENISON, 1967). Towards the pulp cavity, the tubules are so wide that HALSTEAD TARLO (1965) considered that they represented extensions of the pulp cavity — a view disputed by DENISON. In the later Lower Devonian genera *Tesseraspis* and *Weigeltaspis*, the tubercles contain anastomosing pulpal canals to which the dentine tubules run (Figs. 11, 13). This is perhaps best seen in *Weigeltaspis* (Fig. 13). It is not difficult to envisage this condition having been derived from that found in *Eriptychius*.

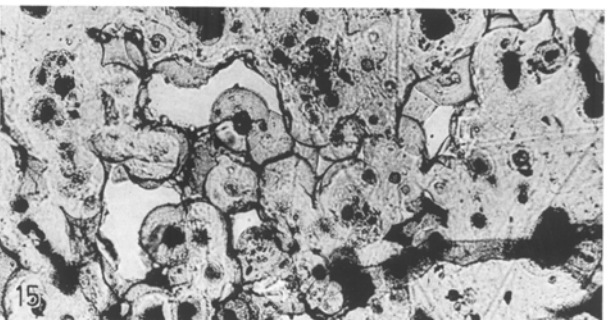
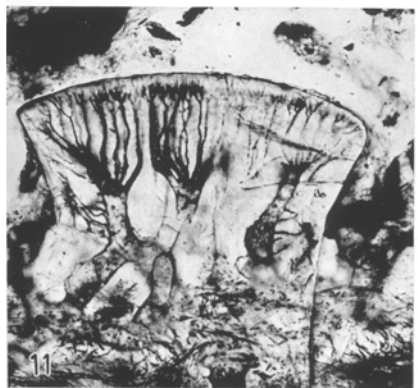
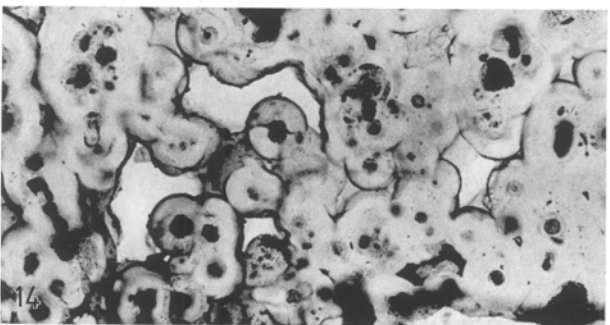
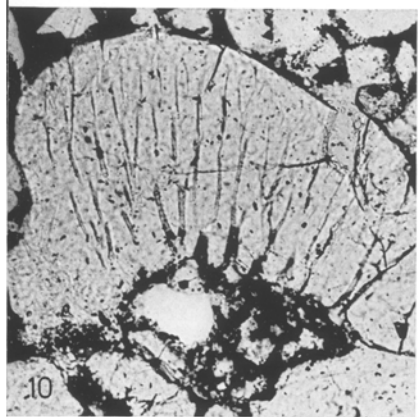
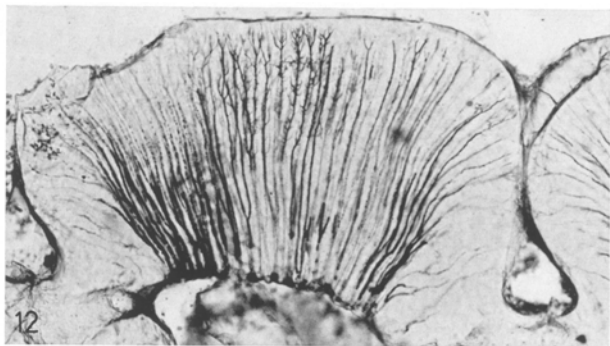
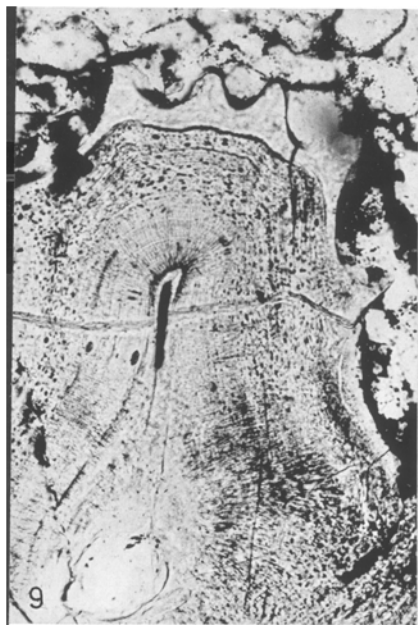
In the majority of the later heterostracans the dentine tubules are notable for their modernity. The psammosteid *Tartuosteus* has tubules with fine lateral and terminal branches that are very reminiscent of human dentine (Fig. 12). The presence of tubules in dentine has been related by TARLO (1965) to the probable original role of dentine as a tissue primarily concerned with sensitivity.

Several authors (PANDER, 1857; ROHON, 1893; WILLS, 1935) have suggested that the dentine tubercles were surmounted by an enamel capping. However, in well preserved sections, it can be seen that this layer is simply where the terminal branches of the dentine tubules are situated. On the other hand, when sections are examined under polarized light, in for example *Tesseraspis*, the outer part stands out as a thick bright layer indicating a difference in the organisation of the apatite. There is no indication of this differentiation when the sections are viewed in ordinary light (HALSTEAD TARLO, 1965, pl. 8, Figs. 3—6). This is in contrast to the situation in *Astraspis*, where the enameloid cap is in optical continuity with the underlying tissues.

A notable feature of the dentine tubercles first noted by ROHON (1893) was the presence of successive generations. By analogy with tooth succession, he considered that the underlying tubercle was an unerupted secondary tubercle, as later did WILLS (1935). However, GROSS (1930, 1935) clearly demonstrated that the new generations were positioned on top of the old. He described the situation where new tubercles were positioned on top of the old surface and in which the intervening space was filled by a mass of dentine, through which meandered numerous long dentine tubules. For this infilling material he introduced the term "Wucherdentin". BYSTROW (1955) figured similar material which differed from that described by GROSS in that the dentine tubules did not stop at the original surface of the plate but instead passed down into the underlying vascular spaces of the aspidin. Further sections were published by TARLO and HALSTEAD TARLO (1964, 1965) and by HALSTEAD TARLO (1965) which showed the formation of new aspidin between the two layers of dentine tubercles, as well as the resorption of the summits of the underlying tubercles (see Fig. 16b). This confirmed GROSS's contention that the second generation tubercles were the more superficial. DENISON (1963) figured a section of *Pycnaspis* (= *Astraspis*) in which three generations of tubercles were present. HALSTEAD TARLO (1965), TARLO and HALSTEAD TARLO (1965), and OBRUCHEV and MARK-KURIK (1966) illustrated specimens of *Ganosteus* in which new tubercles were situated in resorbed cavities on the sides of older tubercles — again confirming the "way-upness" of the different generations (Fig. 16d). OBRUCHEV and MARK-KURIK (1966) have demon-

Figs. 9—13. Dentine. Fig. 9. *Astraspis desiderata* WALCOTT, dentine tubercle with sculptured enameloid cap, dentine and underlying aspidin penetrated by fine tubules. Fig. 10. *Eriptychius americanus* WALCOTT, dentine tubercle with coarse well spaced tubules. Fig. 11. *Tesseraspis tessellata* WILLS, dentine tubercle with tubules radiating from anastomosing pulpal canals. Fig. 12. *Tartuosteus maximus* MARK-KURIK, dentine tubercle with simple pulp cavity, showing detail of lateral and terminal branches of tubules. Fig. 13. *Weigeltaspis godmani* HALSTEAD TARLO, detail of tubercle to show coarse dentine tubules and complex anastomosing pulpal canals

Figs. 14 and 15. Cartilage. *Eriptychius orvigi* DENISON, globular calcified cartilage viewed with different light intensity. All micrographs $\times 128$



Figs. 9—15

strated that on the laterally-produced branchial plates of the psammosteids, worn tubercles were surmounted by new globular tubercles, which could only have been produced secondarily. HALSTEAD TARLO (1966) described branchial plates, in which sheets of new dentine have spread over the old tuberculated surface from the margin suffering abrasion. In all these instances, it is evident that the production of new dentine was in response to damage. HALSTEAD TARLO (1966) recorded remains of animals which had suffered the attentions of predators, where the damaged parts of the armour had been sealed off by the formation of dentine tubercles. This is seen at the edges of bitten branchial plates and in cases where the armour had fractured during the life of the animal (OBRUCHEV and MARK-KURIK, 1966, Fig. 16a). From such evidence HALSTEAD (1969a) has concluded that the production of second generation dentine tubercles evolved initially as a healing mechanism. This view has been opposed by ØRVIG (1968) on the grounds that such regeneration can occur where there is no evidence of abrasion or damage. This is particularly well seen in the formation of so-called "blisters" (HEINTZ, 1957; STENSIÖ, 1958, 1964; ØRVIG, 1961; HALSTEAD TARLO, 1965, 1968a; OBRUCHEV and MARK-KURIK, 1966). Although these "blisters" appear sporadically in parts of the armour that could not have been subject to abrasion, it is a singular fact (MARK-KURIK, personal communication) that in certain localities specimens of all species present exhibit these "blisters", while in nearby areas they are completely absent. Such a localisation of this phenomenon would seem to support the contention of GROSS and HALSTEAD that they were a response to some sort of irritation, perhaps chemical. ØRVIG (1968) is of the opinion that such superposition "was the common property of dermal elements of lower vertebrates in general". This is undoubtedly true but it is here suggested that this initially arose as a simple physiological response. Such a type of regeneration is surely to be expected in the outer layer of an animal. The skin of an organism must be capable of healing, if the animal is to be viable. The formation of new tubercles is simply a reflection of this fundamental property of skin.

Where the formation of secondary dentine tubercles is demonstrably a response to damage, this must of necessity have involved the proliferation of soft tissues around the lesion. In the more advanced heterostracans, the psammosteids, which achieved sizes of up to five feet in length, such processes could hardly have been possible on those areas of the carapace in contact with the substrate and hence subject to constant abrasion. The first response to serious abrasion was illustrated in *Rohonosteus* by KIAER (1915) where small plugs of dentine were present in between the dentine tubercles, where the vascular spaces opened to the exterior. Naturally, such openings must have been covered by soft tissues during life. It seems evident that at the beginning of wear the soft tissues, surviving in a reticular network around the bases of the tubercles, induced the formation of odontoblasts which produced dentine. Further abrasion removed the original superficial layer of dentine tubercles, but the infilling or pleromic¹ dentine continued to invade the vascular spaces of the aspidin so that there was always a minimum thickening which kept pace with the rate of abrasion (Fig. 16c).

1. The term *pleromic* was coined by TARLO (see HALSTEAD, 1969a).

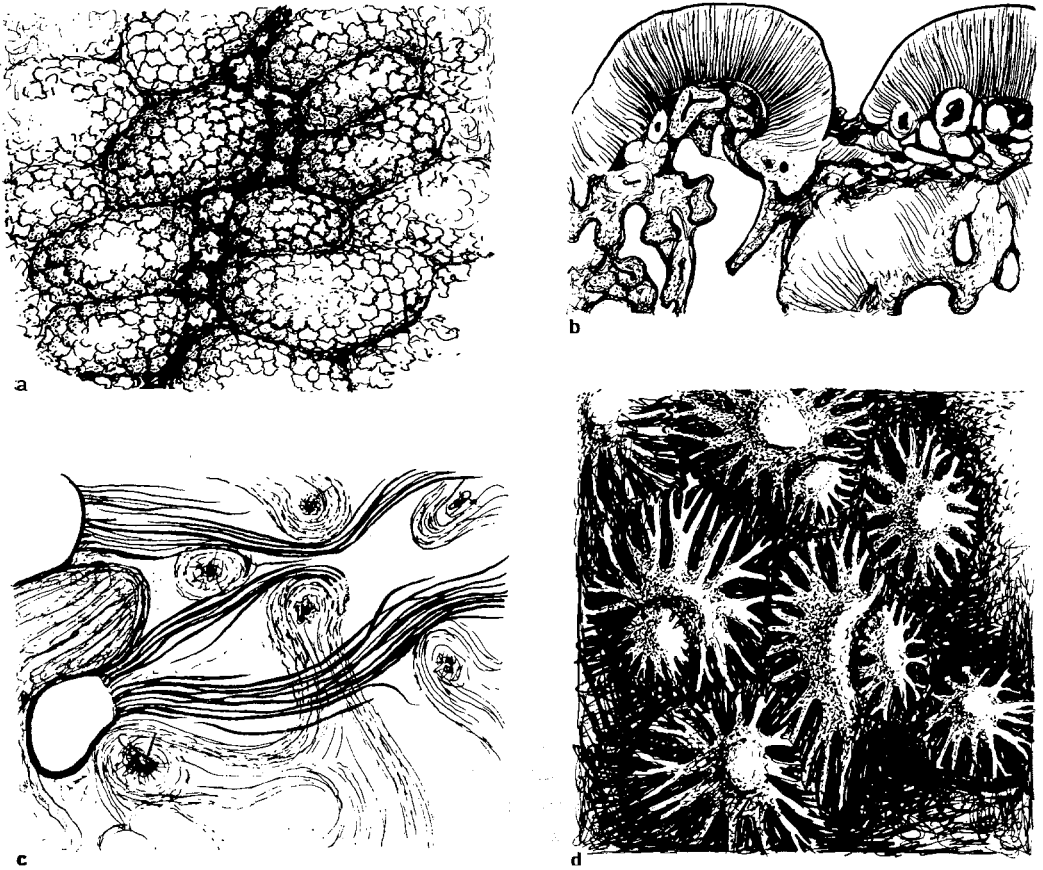


Fig. 16. a *Psammosteus megalopteryx* (TRAUTSCHOLD), ornamentation of superficial tesserae, with fracture healed by line of new dentine tubercles. b *Ganosteus stellatus* ROHON, second generation dentine tubercles, developing aspidin and resorption of summits of first generation tubercles. c *Tartuosteus maximus* MARK-KURIK, pleromic dentine. d *Ganosteus stellatus* ROHON, ornamentation of dentine tubercles with second generation tubercles situated in resorption cavities (from HALSTEAD, 1969a, by permission of Oliver and Boyd, Edinburgh)

This phenomenon was first recognised and carefully analysed by GROSS (1930, 1935) who described such strengthened armour as “massive Spongiosa”. BYSTROW (1955) also described this specialisation and figured specimens which, HALSTEAD TARLO (1965) considered, demonstrated transitional stages between the formation of second generation tubercles, “Wucherdentin”, and pleromic dentine. The relationship of pleromic dentine to the formation of secondary dentine in modern teeth was also discussed recently (HALSTEAD TARLO, 1965; HALSTEAD, 1969a). Whereas, in the normal development of pleromic dentine, there was no evidence of activity on the part of the pulpal cells in the tubercles, HALSTEAD TARLO (1965, pl. 12, Fig. 4) described a section where secondary dentine was produced in the pulp cavity. The dentine tubules of the tubercle could be followed across the region of the former cavity, passing laterally into the spaces in the aspidin.

Evidently in this instance the cells of the pulp had been stimulated to produce secondary dentine comparable to that now occurring in modern teeth.

A situation in which no direct stimulation was involved was recorded by OBRUCHEV (1941) who described the remarkable genus *Aspidosteus* (= *Obruchevia*) in which the entire dorsal plate of the carapace (the only material known at the time) was strengthened by pleromic dentine. These plates had a sculpturing of grooves and pits and hence there could have been no possibility of this formation having been a response to abrasion. HALSTEAD TARLO (1966) noted that, in the closely related genus *Traquairosteus*, where the dentine was restricted to isolated tubercles on the top of pustules of aspidin, there was an incipient development of pleromic dentine. A reduction in the ornamentation of dentine tubercles seemed to be directly related to the formation of pleromic dentine as a normal process. Nevertheless, there is no doubt that originally pleromic dentine arose as a direct response to wear.

ØRVIG (1967) has developed the concept of pleromic dentine, given by HALSTEAD TARLO (1965), and has introduced "pleromic hard tissues" as a major subdivision in his classification of vertebrate calcified tissues.

Cartilage

The first record of cartilage is in the Ordovician and was given by ØRVIG (1951) who published a sketch showing the spherulitic nature of its calcification. Unfortunately at that time there was no indication of the animal to which this tissue belonged. SPJELDNAES (1967) attempted to ascertain the provenance of this tissue but was unable to do so, although the inference that it was derived from either *Astraspis* or *Eriptychius* could hardly be avoided. This problem was finally resolved by DENISON (1967), who established that this tissue belonged to the genus *Eriptychius*. This tissue is only known from this very first heterostracan; it is not present in any later form, nor does it occur in the contemporary *Astraspis*. From DENISON'S photographs the globules of this tissue are reminiscent of the initial formation of aspidin which took place in the "blisters" of the psammosteids (Fig. 16b; see also HALSTEAD TARLO, 1965, pl. 11, Fig. 5, 6). From this observation I came to the conclusion that this was perhaps a spherulitic form of aspidin (HALSTEAD reported in ANON, 1968). Dr. R. H. DENISON generously sent me a specimen of this tissue, which I have sectioned (Figs. 14, 15). In comparison with undoubted examples of globular calcified cartilage in modern elasmobranchs and with fossil material (ØRVIG, 1951), I am now of the opinion that this tissue is calcified cartilage.

In the plates formed of this tissue, there is an outer rim or shell delimiting the definitive size of the plate. Here the calcification is of the spherulitic variety but in wave-like fronts. Within this defining shell, the calcification is in globules (Figs. 14, 15). ØRVIG (1968) has described comparable structures in other primitive vertebrates (cephalaspids and antiarchs) which he interprets as "spheritic" calcification of the bone. Such spherulitic calcification is well known in dentine and is perhaps most dramatically seen in the pea-sized spherulites in the ivory of elephants' tusks. It is even present in the dentine of *Astraspis* tubercles (ØRVIG, 1951, identified as aspidin 1968).

To my mind the globular calcified tissue in *Eriptychius* (as well as in the cephalaspid *Alaspis*, and the antiarch *Bothriolepis*) can still best be interpreted as cartilage. Through the courtesy of Dr. H. Sissons, of the Institute of Orthopaedics, I have examined sections of fracture callus. The sequence of events in the healing of bone fractures involves a type of fibrocartilage which subsequently induces the formation of osteoblasts and hence bone. The latter normally replaces the cartilage (see McLEAN and URIST, 1968). MOSS (1964) has proposed that cartilage and bone are part of a spectrum of skeletogenic tissues and that there is an induction system leading from the former to the latter. The development of fracture callus would tend to confirm this. At the same time, as ØRVIG (1951) has cogently argued, the marked differences between cartilage and bone are not so marked when the lower vertebrates are considered.

In view of the fact that healing mechanisms and pathological conditions frequently reveal primitive evolutionary patterns, there seems to be a *prima facie* case for interpreting the most primitive spherulitic tissue as calcified cartilage. It is worth noting that at the end of evolutionary lineages, the primitive patterns frequently reappear and this would explain the presence of globular calcified cartilage in the last cephalaspid and late antiarchs.

Inter-Relationships

Aspidin and Bone. STENSIÖ (1927) and ØRVIG (1951) have argued that aspidin was derived from a cell-bearing tissue. This view was opposed by DENISON (1963) and by HALSTEAD TARLO (1963), who contended that aspidin was likely to have been a primitively acellular tissue. ØRVIG (1957) indicated the way in which the acellular bone of modern teleosts was derived from cellular bone. Although CURREY (1961) has criticised the data ØRVIG used, there is no doubt that the acellularity of teleost bone is secondary. As this is uncontested, ØRVIG inclined to the view that aspidin originated in a comparable manner. Subsequently, ØRVIG (1965, 1967) conceded that the acellularity of aspidin might be primitive. Recently however (1968) he has returned to his earlier premise that "there is reason to conclude that the possession of osteocytes is indeed a primitive condition in bone tissue of lower vertebrates in general".

The view of HALSTEAD (1969a) that aspidin gradually became cellular has been opposed by ØRVIG (1965, 1967, 1968), DENISON (1967) and MOSS (1968). On the other hand the gradual evolution of the organisation of the organic matrix from dentine-like to bone-like (HALSTEAD TARLO, 1963, 1964, 1965; HALSTEAD, 1969a) has not yet been challenged. Even if the case for cell lacunae being in aspidin is not accepted, there seems every reason to suppose that an acellular tissue preceded a cellular tissue (DENISON, 1963; HALSTEAD TARLO, 1963). Aspidin, in many features that are not in dispute, shows an approach to bone and in this respect it is clearly primitive. Hence it would appear reasonable not to consider this tissue as a secondary derivative of bone.

Dentine and Aspidin/Bone. ØRVIG (1958b, 1968) has argued that dentine evolved from a cellular calcified tissue. He has demonstrated that in the evolutionary history of the cephalaspids, the canaliculae of osteocytes became elongated at the outer margin of the tubercles. This tissue he termed *mesodentine*. In

the arthrodires, primitive jawed vertebrates, he noted that the odontoblasts frequently became enclosed in the matrix, so that by the presence of lacunae of "odontocytes" the tissue has similarities to bone — this he termed *semidentine*. He concluded that from a bone-like tissue it was possible to evolve dentine *via* mesodentine and semidentine or directly *via* mesodentine.

This view was not accepted with regard to the heterostracans (HALSTEAD TARLO, 1965). He argued that the most primitive type of hard tissue was aspidin in which the apatite crystallites were laid down in association with collagen fibrils in successive lamellae. In fact, in the organisation of the organic matrix there is no distinction between primitive aspidin and dentine. Dentine differs only in its possession of tubules. In the earlier heterostracans, it is not possible to identify the boundary between these two tissues, since at the margins of the dentine tubercles the dentine becomes devoid of tubules and grades into aspidin. In *Astraspis* both tissues are penetrated by comparable tubules as already noted above. Later forms such as *Ganosteus* show a clear differentiation only under polarised light. Occasionally, as well as having dentine without tubules, it is found that dentine tubules can pass into aspidin (HALSTEAD TARLO, 1965, pl. 8, Figs. 1, 2). The characteristic bone-like black and white banding reveals the tissue in question to be advanced aspidin. If it is necessary to postulate a genetic relationship between dentine and aspidin, it might be suggested that the former arose from the latter by the development of tubules which acted as a routeway of sensation as proposed by TARLO (1965), the organisation of the matrix remaining unchanged.

This suggestion has been attacked with remarkable vigour by ØRVIG (1967) on the grounds that the fine tubules in aspidin are likely to have contained collagenous fibres and not cell processes. As discussed above, ØRVIG himself (1951), HALSTEAD TARLO (1965) and DENISON (1967) interpreted such tubules in *Astraspis* as having been made by cell processes, hence it is difficult to understand why ØRVIG (1967) characterises such a derivation of dentine as "this remarkable theory."

Cartilage and Bone. Comparative anatomists and embryologists have long contended that cartilage was primitive and bone secondary and they have pointed to endochondral ossification and the cartilaginous nature of the skeleton of living lower vertebrates. On the other hand, palaeontologists have stressed the antiquity of bone and have demonstrated that there was a general trend towards the reduction of bone and its replacement by cartilage (STENSIÖ, 1927). Nevertheless, as WHITE (1946) stated, the early vertebrates did not "spring forth like Pallas Athene fully armed onto an unsuspecting world". This conflict of evidence was apparently resolved by ROMER (1942, 1963, 1964, 1968) who put forward the concept that cartilage was an embryonic adaptation and that its survival in primitive living vertebrates was an example of neoteny.

Recently the discussion has centred around two main points: 1. that there is no direct relationship between cartilage and bone in evolution (HALSTEAD TARLO, 1964, 1968b; HALSTEAD, 1969a; MOSS, 1968) and 2. that cartilage is primitive (HAM, 1953, 1965; DENISON, 1963; MOSS, 1964; HALSTEAD in ANON, 1968; HALSTEAD, 1969b). HALSTEAD-TARLO (1968b) stated that "bone was primarily a chemical store [a view retracted in 1969b], then a protection and

only later in evolution an internal skeleton; cartilage was primarily an internal supporting material. Bone and cartilage arose independently of one another to subservise different functions". This opinion is echoed by MOSS (1968) who stressed that "by differentiating between dermal and endoskeletal sites the problem is capable of resolution. ROMER, in this sense, is right. Cartilage did not precede bone in the dermal skeleton. DENISON is right also. Cartilage did precede bone in the endoskeleton."

DENISON (1963) favoured a cartilaginous tissue as a likely evolutionary precursor to bone, whereas HAM (1953, 1965) suggested in passing that bone evolved to replace calcified cartilage — a view which was dismissed equally casually by HALSTEAD TARLO (1964). There seems every reason to believe that cartilage is primitive, following the excellent survey published by ØRVIG (1951) and the suggestion of MOSS (1964) that these skeletal tissues "form a modulating series interconnected by common inductive mechanisms" as seen in fracture callus (MCLEAN and URIST, 1968).

HALSTEAD-TARLO (1968b) and MOSS (1968) have stressed that the production of either cartilage or bone was dependent on whether the cells took up a deep or superficial position. This observation, following the early work of HOLMGREN (1940) and elaborated by JARVIK (1959), does not necessarily imply a direct evolutionary relationship between cartilage and bone. However, a recent study on primitive arthropods by WESTOLL (1967) leads to the inference that during evolution dermal bone can sink in to become cartilaginous. MOSS (1968) remarked "no one has ever suggested, nor do I, that a cartilaginous dermal (exo-) skeleton ever existed in any form." As a consequence of DENISON'S (1967) discovery of calcified cartilage in the Ordovician *Eriptychius* such a suggestion can now, in fact, be proposed.

The final problem to be considered is the nature of the most primitive vertebrate calcified tissue. HALSTEAD TARLO (1964, 1965) considered that the most primitive tissue was aspidin in which the apatite crystallites were seeded onto collagen fibrils — the "inotropic" calcification of ØRVIG (1967). Such a method of initial calcification was proposed by MOSS (1964, 1968). In contrast to this approach, ØRVIG (1967, 1968) has stressed the antiquity of "spheritic" calcification. "More precisely, of the two kinds of calcification, the spheritic may represent the more primitive which in early phyletic stages was responsible for hard tissue formation, and the inotropic the more advanced, that is a later acquisition which, as it evolved, eventually came to predominate in most hard tissues, at any rate those of the dermal skeleton" (ØRVIG, 1968). Although in personal discussions with Dr. T. ØRVIG, I have argued against this interpretation, I now find myself entirely convinced by his thesis, which DENISON'S discovery seems to confirm.

I visualise the initial calcification being spherulitic (ØRVIG) and also a direct evolutionary relationship between cartilage and bone (MOSS). By analogy with living tissues, the original spherulitic calcification can be designated a type of cartilage. This is clearly the simplest method of depositing mineral. Once this process became more organised, in that the mineral was directly associated with collagen fibrils, the tissue aspidin was produced. Thereafter, the evolution to bone proper is not difficult to envisage (HALSTEAD, 1969a).

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