HISTOGENESIS AND HISTOCHEMISTRY OF PULPAL CALCIFICATION

PERCY L. JOHNSON AND G. BEVELANDER

Department of Histology, Murray and Leonie Guggenheim Institute for Dental Research, and the College of Dentistry, Graduate School of Arts and Science, New York University, New York, N. Y.

CALCIFICATION in the pulp of human teeth occurs in the form of pulp stones, and as areas of diffuse calcifications in a relatively restricted area. Calcified structures have long been recognized and were described and commented upon by Bodecker, Salter, Black, Preiswerk, and others.

The origin of denticles has been ascribed to several different causes. Fridrichovsky[4] maintained that denticles develop from a folding of embryonic tissue which normally would form dentin. Orban[7] asserted, on the other hand, that epithelial rests trapped in the pulp tissue initiate cellular activity resulting in the formation of denticles. Hill[5] expressed the opinion that the formation of denticles is associated with certain stellate cells of the pulp which function in building an irregular dentin around a calcific deposit.

It was formerly held that the occurrence of pulpal calcifications was related to irritation, trauma, caries, etc. They were also causally associated with neuralgia and referred pain of various kinds. The current concept in regard to the origin and presence of pulpal calcifications, according to Thoma,[11] Boyle,[3] and Hill,[5] differs from the ideas referred to above in two important respects: (1) a differentiation is made between the occurrence of pulpal calcifications resulting in denticle formation and areas of the pulp considered pathologic (necrotic) which subsequently undergo calcification; and (2) referred pain is no longer ascribed to the presence of pulpal calcifications.

It is the purpose of this investigation to record our observations on the occurrence and structure of calcifications in the pulp. In addition, we shall describe, in so far as possible, the histogenesis and histochemical components of these structures. Finally, we shall compare our observations on pulpal calcifications with the histogenesis of bones and teeth.

MATERIALS AND METHODS

Human teeth obtained immediately after extraction were used in this investigation. Following extraction the teeth were bisected transversely or longitudinally and placed at once in fixing fluid. Some of these specimens were then infiltrated with paraffin prior to the preparation of thin slabs cut by means of a copper disk impregnated with diamond dust. Still other teeth were cracked in a

This investigation was supported (in part) by a research grant from the National Institute for Dental Research of the National Institutes of Health, United States Public Health Service, Bethesda, Maryland.

Received for publication April 15, 1955.

vice, fixed and the pulps removed intact, sectioned and treated as desired. Finally, in another group of teeth, the apical end of the tooth was cut off, the coronal portion then placed in the fixer, decalcified, and subsequently embedded in paraffin or celloidin. The fixing fluids used were chosen to reveal the several histochemical components identified as follows: Helly's fluid followed by 0.05 per cent toluidine blue for metachromasia, 80 per cent alcohol for the Gomori phosphatase test and the Von Kossa test for mineral deposits; Bouin's fluid for hematoxylin and eosin preparations, Carnoy's fluid for the McManus-Schiff reaction for glycoprotein, glycogen and nucleic acid demonstration. Comparison of sections prepared by means of the cutting wheel with those cut with the microtome showed relatively little distortion.

OBSERVATIONS

A. *Pulp Stones.*— The numbers and sizes of pulp stones in the pulp of human teeth were observed to vary greatly (Fig. 1). While some teeth are devoid of pulp stones, others were observed to contain from 1 to 12 or more. The size of these stones was sometimes so small as to be barely perceptible, while others consisted of large conglomerate fused masses occupying the greater part of the pulp cavity (Fig. 18). Pulp stones, according to our observations, usually occur in the pulp horns and only occasionally are they observed in the region of the root canals.

Pulp stones in the fresh state appear quite uniformly transparent in contrast to dentin and enamel. When the pulp was examined with the aid of a binocular dissecting microscope it was sometimes necessary to probe the pulp stones in order to ascertain that they were actually calcified bodies and not large vacuoles filled with transparent fluid. When subjected to decalcification with acids, they behaved like dentin and enamel in that they did not effervesce.

During the course of this investigation we have devoted considerable attention to the manner in which pulp stones arise. In so far as we have been able to ascertain, the precursors upon or around which these structures arise are cell nests (Figs. 2 and 4). Subsequently, these cells become enclosed by concentrically arranged fibers ; later this complex is impregnated with mineral salts. The pulp stone is elaborated in successive stages. Between each calcified increment there is a prominent fibrous junction. The periphery of the structure is surrounded by a thick zone of uncalcified fibers which we have ascertained to be reticular connective tissue. The fibers which are incorporated in the pulp stones are derived from the surrounding connective tissue (Fig. 5). A variation of this situation is shown in Fig. 8, which illustrates a calcified mass embedded in a condensation of collagenous fibers.

More complex arrangements of pulp stones are also of frequent occurrence. The complexity consists of increased size and fusion of one or more of the bodies. In the example just cited the fusion is effected by a fibrous union (Fig. 10).

Figs. 1 to 9.—Fig. 1. Denticles of decalcified tooth (H & E. orig. mag. X 10). Fig. 2. Denticle (McManus-Schiff, orig. mag. X 300). Fig. 3. Denticles impinging on nerve bundle (McManus-Schiff, orig. mag. X 150). Fig. 4. Beginning formation of denticle (Eosin-Azure, orig. mag. X 750). Fig. 5. Matted fibers surrounding developing denticle (H & E. orig. mag. X 200). Fig. 6. Free, embedded and adherent denticles (H & E. orig. mag. X 80). Fig. 7. Diffuse calcifications in pulp (Von Kossa. Light green, orig. mag. X 750). Fig. 8. Collagenous fibers approaching ana surrounding denticle (H & E, orig. mag. X 750). Fig. 9. Diffuse calcifications in pulp (Von Kossa, orig. mag. X 1, 200).

Figs. 10 to 20.--Fig. 10. Fusion of two denticles with fibrous matrix (H & E, orig. mag. Fig. 11. Early stages in diffuse calcification between nerves (Metachromasia. T. Blue, orig. mag. X 900). Fig. 12. Enlarged portion of dimise calcification (Metachromasia. T. Blue, orig. mag. X 1,200). Fig. 13. Fibrous component or denticle with Von Rossa positive granules oriented along fibers (orig. tuag. X 1,200). Fig. 14. Early formation of diffuse calcifications (Schiff, orig. mag. Fig. 15. Early formation of diffuse calcifications (Foote stain, orig. mag. X 900). Fig. 16. Myelinated nerve fiber with node of Ranvier (M. Blue. orig. mag. X 1.200). Fig. 17. Denticle (Metachrotnasia. T. Blue, orics. mag. X 100). Fig. 18. Denticles adherent to one another (H & E, orig. mag. X 400). Fig. 19. Embedded fibers in body of denticle (orig. mag. X 1,200). Fig. 20. Enlarged denticle with dentinal tubules about periphery (H orig. mag. X 900).

In those pulp stones which arise or lie in proximity to dentin, the presence of odontoblasts on the peripheral aspect of these bodies was frequent. Subsequently, a calcified tissue resembling dentin appears to be deposited on the periphery of the pulp stones. Such a denticle, at first free, may later become adherent and subsequently become incorporated into the dentinal tissue appearing as an integral part of the calcified dentin (Fig. 6). It is possible, as indicated above, to trace the origin and development of the incorporated denticle; the original free pulp stone can be identified as an area unlike dentin in appearance surrounded by dentinal tubules which take a different direction from the regularly arranged dentin surrounding this mass (Fig. 20).

Structures such as bloodvesselsand nerves do not, in our opinion, contribute or serve to initiate the formation of pulpal calcifications. Fig. 3, for example, illustrates a typical relationship between a group of nerve fibers and pulp stones. The fact that the pulp stones are in such intimate relation to the nerve that they encroach upon it appears to be incidental and does not implicate the nerve as a nidus for this type of pulpal calcification (Fig. 16).

B. *Diffuse Calcifications.*—Calcifications of this variety occur as unorganized masses in the apical part of the root canal (Fig. 7). They appear as multiple accretion centers separated from one another by the ground substance of the pulp. As enlargement occurs, they elongate in the direction of the long axis of the pulp. These accretions upon superficial examination appear amorphous. Examination under higher magnification, however, reveals that they consist of minute spheres or ellipsoids (Fig. 9). The ellipsoidal character of these bodies is the result of the fusion of neighboring accretions (Figs. 11 and 12). The process of fusion continues and eventually results in the formation of fluted columns of calcified cylinders attached at their sides and united by calcified material devoid of a fibrous matrix. This process results in extensive calcification of the root canal. These masses may impinge upon vessels and nerves which may in turn cause changes in structure and function. Despite the intimate association of these structures with vessels or nerves, we have found no evidence that they are in any way concerned with the origin or development of these calcified structures.

HISTOCHEMICAL OBSERVATIONS

A. *Phosphatase and Glycogen.*—Pulps of several adult teeth were examined for the presence of alkaline phosphatase by means of the Gomori method. Some of these pulps were normal, others showed retrogressive changes, and still others revealed definite pathosis. Phosphatase appeared in these pulps in one locus only, the peripheral part of the pulp cavity in the region of the odontoblasts. This observation augments our earlier studies in which we described the diminution of this enzyme in the pulp as the tooth matures. Of particular interest was the absence of phosphatase in any of the components making up or surrounding the pulpal calcifications.

Tests for the presence of glycogen using the McManus-Schiff reagent, Best's carmine and iodine methods were completely negative. Our previous

studies on embryonic teeth showed that glycogen disappears before calcification of the tooth begins. In comparing the formation of enamel and dentin with the formation of pulp stones, we find that glycogen is present before calcification, phosphatase before and during calcification of dentin and enamel, but, in the proximity of pulp stones, these substances are not present during any stage of their existence. This finding lends additional support to the concept that calcification can occur in the absence of both phosphatase and glycogen.

B. *Glycoprotein.*—The formation of pulp stones and areas of diffuse calcification takes place in an environment consisting of fibers and ground substance showing a pronounced glycoprotein staining reaction (Fig. 14). As calcification proceeds or after it has taken place, the fibers surrounding the pulp stone are intensely Schiff-positive, while the calcified area responds very slightly to this treatment, both with respect to the fibers and ground substance. Application of the Foote technic to pulp stones (Fig. 15) indicates that the peripheral fibers have the characteristics of reticular fibers. It is obvious that during calcification certain changes occur in the calcified mass which result in a marked diminution of the periodic-Schiff reaction.

C. *Nucleic Acids and Basophilia.*—In view of our previous observations[6] in which we observed a pronounced nucleic acid localization in odontoblasts and ameloblasts associated with the formation and calcification of dentin and enamel, respectively, we directed our attention to the nucleic acid content of the pulpal cells in adult teeth. Comparison of nucleic acid localization of both RNA and DNA in cells in close proximity to the pulp stones and various other cells in the pulp were essentially similar in respect to the presence of nucleic acids. The visualization of these substances was extremely weak in all of the pulp cells. In regard to nucleic acids we cannot, on the basis of our observations, implicate them in the formation or calcification of pulp stones.

D. *Von Kossa.*—Pulp stones in varying degrees of development treated according to the Von Kossa method result in a negative reaction during the early stages of development. Larger and more highly developed pulp stones show positive (black) central areas while the peripheral portion remains negative. Subsequent staining by safranin 0 or the McManus-Schiff reaction reveals that the periphery of these structures is fibrous and, as previously mentioned, when treated according to the Foote method they appear to be reticular fibers. The sequence of events in terms of calcification therefore is similar to that observed in other calcified tissues in that an organic phase precedes calcification.

Examination of areas of diffuse calcification tested by the methods mentioned in the previous paragraph shows a striking similarity. The youngest bodies reveal ground substance and reticular fibers, and the center shows the first sign of calcification; when calcification takes place, the fibers and ground substance react faintly, if at all, to the McManus-Schiff reaction.

One of the most interesting observations made on Von Kossa-treated material is shown in Fig. 13. This photomicrograph illustrates the peripheral fibers of a growing pulp stone. It further depicts the presence of minute beads of

Von Kossa positive material arranged in linear fashion along the fibers. These structures were examined with a phase contrast microscope and with the highest magnification available to us using the compound light microscope. From these observations we conclude that these mineral bodies are essentially an integral part of the fiber. Evidence for this conclusion is based upon our examination of this fiber complex which shows that internodes of organic material connect each bead of calcified material. The significance of this observation is, we believe, in the clear demonstration of the intimate association shown between fibers and calcifying material.

In diffuse areas of calcification we observed (Fig. 9) that isolated concretions of calcified globules reveal small buds extending over most of the peripheral surface. In these buds we observed no fibrous matrix, but a pronounced metachromatic ground substance in the peripheral area is, nevertheless, present.

Metachromasia.—Adult pulps treated with toluidine blue show a relatively weak or negative metachromatic response with the exceptions noted below. The region of predentin is markedly metachromatic. This is also true for those areas of the pulp which appear to be or are elaborating pulpal calcifications. In those areas in which diffuse calcifications are being formed the metachromatic ground substance extends for a considerable distance beyond the actual area undergoing calcification. In the areas where calcification has occurred, each individual center is surrounded by a marked metachromatic peripheral zone.

Metachromasia observed in pulp stone formation is in most respects similar to the situation described above. The uncalcified organic phase is intensely metachromatic; as calcification occurs the metachromasia is reduced or lost in the calcified matrix but is still prominent in the peripheral fibrous zone. Pulp stones made up of several increments show metachromasia at the interfaces of the increments and peripherally.

In the pulp calcifications we have studied, we observed that both glycoprotein and acid polysaccharides are components of the ground substance of those areas which subsequently calcify. The fact that the metachromatic substance was not removed by hyaluronidase digestion suggests that the acid polysaccharide may be chondroitin sulfate.

DISCUSSION

Pulpal calcifications, usually referred to as pulp stones, may be grouped, according to our observations, into two categories: denticles and areas of diffuse calcification. We observed the denticles to be located in the region of the pulp horns and the areas of diffuse calcification in the root canals. This is in agreement with the accepted views now generally held.

Denticles arise from a nidus of cells. Subsequently, reticular fibers surround the cells and then calcification of the initial organic matrix occurs at the same time a peripheral organic matrix is laid down. Extensive calcification of the pulp horns, sometimes observed, results from the fusion of several

individual denticles. Attached and imbedded denticles originate as free bodies. Their subsequent attachment or incorporation into dentin is the result of dentin formation on the free surface of these structures. The only denticles we observed which reveal dentinal tubules are the heterogeneous structures made up in part of concentric lamellae of calcified matrix—the free denticle, surrounded in whole or in part by dentin. We conclude from our observations that the differentiation between "true" and "false" denticles is untenable. Denticles are false, that is, they do not contain dental tubules. Subsequently, they may, in the process of attachment to, or incorporation by dentin, be surrounded by tissue containing dentinal tubules.

Areas of diffuse calcification were observed to be localized in the root canals. They were observed in early development as minute spheres which undergo subsequent enlargement due to the fusion of neighboring centers. Like the denticles, these structures also have an organic matrix composed of reticular fibers and a ground substance.

The histochemical observations made in reference to pulpal calcifications are, we believe, of more than routine interest when compared with similar studies conducted on growing calcifying tissues such as dentin and enamel. A precursor complex consisting of reticular tissue, and a ground substance containing mucoprotein and metachromatic components was observed for denticles and diffuse calcifications. This is also found in bone, dentin, and cementum. Osteoblasts, odontoblasts, and ameloblasts contain considerable amounts of phosphatase, glycoprotein, and nucleic acids during the time calcification takes place. It appears that pulp stones have an acid polysaccharide and a mucoprotein precursor in common with other regions of calcification. Unlike them, neither phosphatase nor (by implication) phosphorylase are components of the calcific structures which arise in the pulp.

Many factors relating to pulpal calcifications still remain in the realm of speculation or obscurity. It is not clear whether calcification in the pulp is correlated with retrogressive changes or pathosis, whether this phenomena is a protective mechanism or an exhibition of the multipotency of the pulp constituents. The present studies do serve, we believe, as a basis for comparison between events which happen in the pulp and in other regions which calcify, particularly those organs subject to aberrant forms of calcification.

CONCLUSIONS

1. A study of pulpal calcification in the human adult tooth has been made. According to our observations, there are basically two varieties of pulp stones: denticles consisting of a concentrically arranged lamellae and areas of diffuse calcification.

2. Denticles occur in the pulp horns. They are at first free but they may become attached or embedded. In this process they acquire tissue containing dentinal tubules. We find no indication that denticles are "true" or "false."

3. Areas of diffuse calcification are confined to the pulp canals. They appear first as minute centers which fuse with neighboring bodies, eventually giving rise to the columnar or cylindrical structures which have frequently been illustrated.

4. Histochemical tests to which the pulp was subjected reveal that reticular connective tissue fibers, mucoprotein, and acid polysaccharides were constant constituents of the loci subsequently giving rise to denticles or diffuse calcification. Nucleic acids, alkaline phosphatase, and glycogen commonly found in cells associated with the calcification of bones and teeth were not present.

5. The possible significance of pulpal calcifications is discussed and comparison is made between normal and aberrant calcifications.

REFERENCES

1. Black, G. V.: The American System of Dentistry, 1886, Vol. 1, pp. 860-880.
2. Bodecker, C. F.: Anatomy and Pathology of the Teeth, 1894.
3. Boyle, P. E.: Kronfeld's Histopathology of the Teeth, Philadelphia, 1950, Lea & Febiger.
4. Fridrichovsky, J.: Zur Histologie der Denticle, *Ztschr, f Stomatol.* 25: 124, 1927.
5. Hill, T. J.: Textbook of Oral Pathology, Philadelphia, 1949, Lea & Febiger.
6. Johnson, P. L., and Bevelander, G.: The Localization and Interrelation of Nucleic Acids and Alkaline Phosphatase in the Developing Tooth, *J. D. Res.* 33: 128-135, 1954.
7. Orban, B.: Epithelial Rests in the Teeth and Their Supporting Structures, Proc. Am. Assoc. Dent. Schools, pp. 121-133,1929.
8. *Idem:* Oral Histology ana Embryology, St. Louis, 1953, The C. V. Mosby Company.
9. Preiswerk, G.: Lehrbuch und Atlas der Zahnheilkunde, Bd. 30, München, 1908.
10. Salter, J. A.: Dental Pathology and Surgery, Baltimore, 1875, William Wood & Company.
11. Thoma, K.: Oral Pathology, St. Louis, 1941, The C. V. Mosby Company.