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SECTION MEETINGS

IOWA	
State University of Iowa	November 24, 1959
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University of Washington	November 14, 1959
PACIFIC COAST	
University of California Medical School	December 16, 1959
SOUTHERN	
Louisiana State University	December 15, 1959

Mineralization of Dental Calculus.* (25479)

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Most previous studies on human dental calculus have dealt with gross morphology, chemical composition, mechanisms of deposition, and pathological effects upon adjacent tissues. The microscopic structure of calculus has been investigated almost exclusively in demineralized material(1). In recent studies of structural characteristics of calculus as revealed in ground sections, we observed that various configurations recur consistently in all specimens. An impression has been gained that calculus may be organized according to a specific pattern, in a sense resembling calcified tissues. It was quite obvious that through use of experimental technics information could be obtained which bears both upon formation and structural organization of calculus and upon deposition of mineral in organic matrices in general.

Material and methods. Sandblasted polyester strips (Mylar, DuPont) were fastened to the lingual surfaces of 73 lower incisors in 16 subjects who were known to form calculus rapidly(2,3). At various intervals, from 1 day to 4 weeks, the strips, with the deposit which had formed on them, were removed and processed for either optical or electron microscopy. Material to be examined optically was fixed in 10% neutral formalin, and embedded in celloidin. Serial sections (12μ) were stained with hematoxylin and eosin, alizarin red S, and by the Gram, von Kossa, and periodic acid Schiff (PAS) methods. For electron

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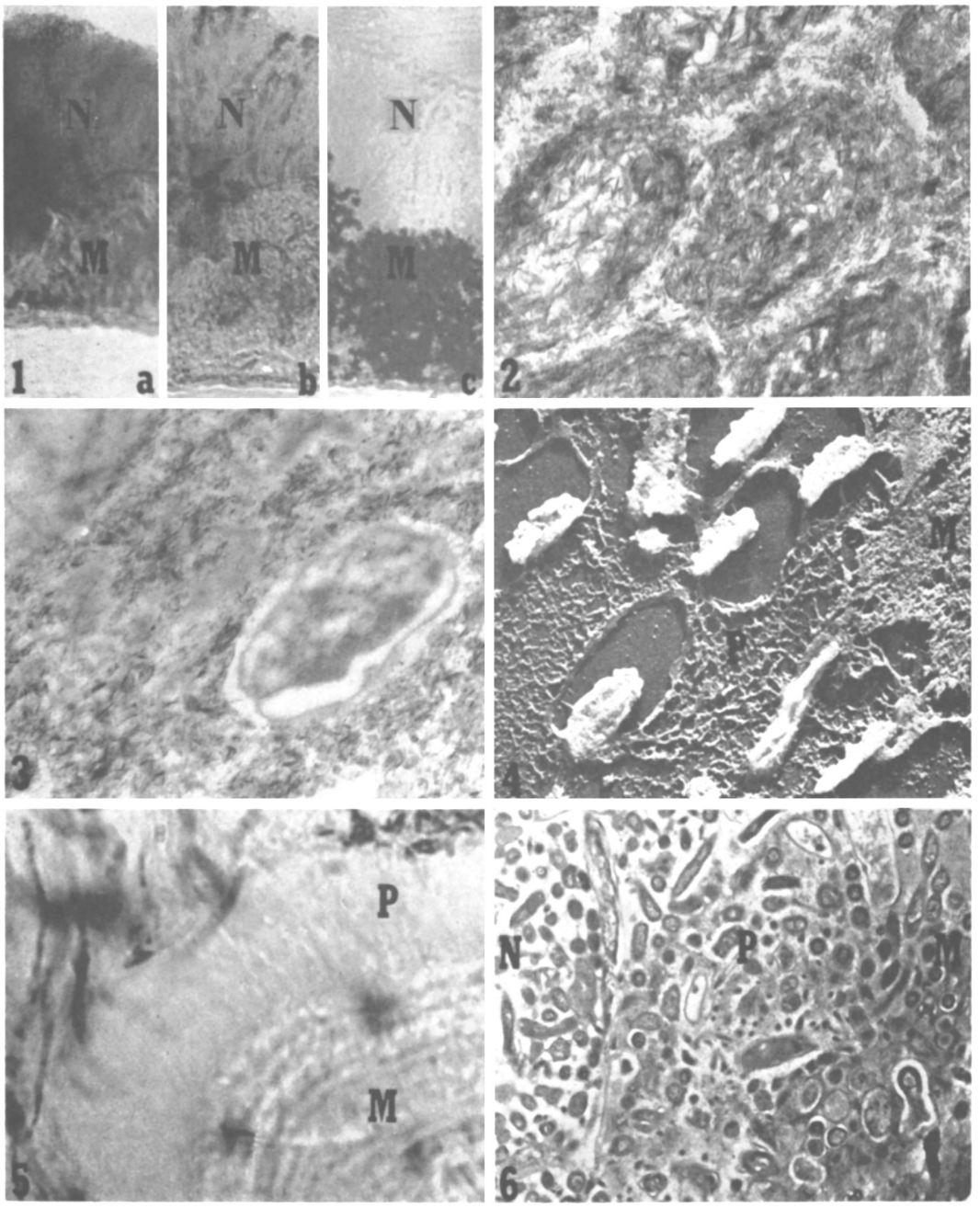

FIG. 1. Four-wk deposit showing mineralized region (M) and non-mineralized region (N) stained by H & E (a), Gram (b) and von Kossa (c) methods. Polyester strip is at bottom. Photomic., 510×.

FIG. 2. Needle-like crystals within and between microorganisms in a region of advanced mineralization. Electron mic., 25,000×.

FIG. 3. Crystals only between and on surfaces of microorganisms in a region of beginning mineralization (M). Electron mic., 25,000×.

FIG. 4. Organic intermicrobial material (P) immediately external to zone of beginning mineralization (M). Electron mic., 18,500×.

FIG. 5. The same 2 regions as in preceding figure. Photomic., 1790×.

FIG. 6. Differences in density in intermicrobial material in regions of recently formed (N), solidified (P) and mineralized (M) deposit. Electron mic., 4000×.

microscopy the specimens were fixed in an osmium tetroxide-potassium dichromate mixture and embedded in a mixture of methyl and butyl methacrylates. Sections were cut serially with a Porter-Blum microtome equipped with glass and diamond knives.

Results. The first signs of mineralization were observed in 3-day-old specimens. Areas adjacent to the polyester strips appeared clearly black with von Kossa stain, red with alizarin red S, were PAS negative and took H and E only faintly. Only a few microorganisms were brought out by the Gram stain. At 4 weeks the mineralized regions were considerably larger and more numerous, but retained the same staining properties (Fig. 1 a-c, area M). Under the electron microscope, the outlines of microorganisms could actually be discerned in these masses. Large numbers of needle-like crystals were seen throughout, both between and inside the microorganisms (Fig. 2). These crystals appeared as fine needles and gave electron diffraction patterns typical of apatite. Toward the outer border of the mineralized areas, where calcification was less advanced, apatite crystals were found on the surfaces of and between the microorganisms, but not within them (Fig. 3). When the plastic embedding material was removed from such sections and the preparations were shadowed, a fine fibrillar matrix was evident throughout the interbacterial region. An even more copious amount of this material was seen in the immediately surrounding areas, where mineral deposition had not yet begun (Fig. 4). Examined optically, the latter zone did not take alizarin red S or von Kossa stain, stained light blue with H and E, and was slightly PAS positive (Fig. 5 P). Still farther from the mineralized zone, the most recently deposited material was made up primarily of Gram positive microorganisms (Fig. 1b, area N); it stained deep blue with H and E, and was strongly PAS positive. Under the electron microscope the most striking feature of this area was the relative lack of density and definition of the interstitial substance (Fig. 6).

Discussion. From our observations it might be deduced that the sequential process of mineralization in calculus is the same as in calcified biological tissues. Thus, the microorganisms and intermicrobial material serve as the organic "matrix" which subsequently becomes impregnated with mineral crystals. The apparent increase in quantity and density of the intermicrobial substance shortly before inception of mineralization seems to be a counterpart of the solidification that occurs in the dentinal matrix during the same developmental period(4). The progressive changes in staining properties also indicate that basic alterations occur in composition of the initially deposited matrix, and the positive PAS reactions suggest that, as in tooth and bone, polysaccharide plays a role in the mineralization process. The pattern in which the apatite crystals are laid down is undoubtedly unique for calculus but it is of interest that the microorganisms themselves ultimately become calcified.

The most important aspect of this study seems to us to be the demonstration that calculus can be made to serve as a useful test object in basic studies of mineralization. Its ready accessibility and rapidity of its development present promising experimental possibilities. At the same time, extension of the work will also provide further information related directly to the composition and microstructure of calculus itself. One of the most challenging problems lies in identification of the organic intermicrobial substance and the tracing of its source.

Summary. Experimental studies have been made of mineralization in developing human dental calculus, formed *in vivo* on polyester strips attached to the teeth. The findings suggest a close parallelism between calcification process in this exogenous deposit and in calcified tissues. The microorganisms and intermicrobial substance first laid down serve as the organic matrix, which undergoes a series of chemical and morphological changes prior to mineralization. Calcification within this matrix follows a definite pattern, crystals first being laid down between and on surfaces of the bacteria, and later inside them.

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Prevention of Levarterenol Necrosis in Rabbits by Use of Levarterenol-Phentolamine Mixtures.* (25480)

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Levarterenol (Levophed), administered by slow intravenous drip, is the vasopressor drug most frequently employed in treatment of shock. Accidental extravasation may produce such intense local vasoconstriction that ischemic necrosis of the skin may ensue. Vasoconstriction can be reversed by prompt injection of the anti-adrenergic drug phentolamine (Regitine) into the area of extravasation (1,2). The method is not effective when extravasation has been overlooked for several hours and irreversible tissue damage has occurred. Neglect of an extravasation might not be serious if phentolamine could be given intravenously with levarterenol. Preliminary clinical studies indicate that levarterenol-phentolamine mixtures may be used effectively(3). The purpose of our study is to determine the least amount of phentolamine that will inhibit the local vasoconstrictor action of doses of levarterenol usually employed in treatment of shock.

Methods. The method described by McGinn and Schluger(4) was used for producing sloughs on rabbit abdomen. Solutions were prepared in 1000 cc of 5% glucose in water. They were administered through 22 gauge 1½ inch needles inserted subcutaneously at center of shaved abdomen and directed caudally. The drip was adjusted so that 250 cc were infiltrated during 2 hour period. The needles were then removed. Experiments were carried out on 36 adult rabbits, divided into 6 groups of 6 rabbits each. Group A served as control receiving only levarterenol.† Of this group, 2 were injected with 8 mg/l levarterenol, 2 with 16 mg/l, and 2 with 32 mg/l. Each of the other 5 groups was similarly subdivided. However, the levarterenol solutions for these animals contained increasing amounts of phentolamine‡: Group B, 0.5 mg; Group C, 1 mg; Group D, 2.5 mg; Group E, 5 mg and Group F, 10 mg.

Results. Group A: Subcutaneous infiltration of levarterenol solutions without phentolamine produced a well-demarcated swelling. After 24 hours, there was a mottled, warm area of induration. After 72 hours, necrosis with scaling and crusting occurred. Size of slough varied directly with concentration of levarterenol. The smallest measured 3 x 4 cm and the largest 12 x 20 cm.

Group B: The 2 rabbits that received the mixture with 8 mg levarterenol developed no necrosis. The 2 injected with 16 mg and the 2 with 32 mg developed areas of necrosis approximately the same size as those in corresponding animals in Group A.

Group C: No necrosis was observed in 2 rabbits that received the mixture with 8 mg levarterenol or in one with 16 mg. A 1 x 1 cm slough developed in the second rabbit of latter group. Necrosis appeared in both rabbits given the mixture with 32 mg levarterenol. One measured 2 x 2 cm and the other 0.5 x 2 cm.

Groups D. E. F: Necrosis did not develop in any of the animals.

Discussion. Experiments have confirmed

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‡ Phentolamine (Regitine) was furnished by Ciba Pharm. Products, Summit, N. J.