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BIOPHYSICAL STUDIES ON DENTAL CALCULUS FROM GERMFREE AND CONVENTIONAL RATS

by

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INTRODUCTION

For the formation of dental calculus, microorganisms are supposed to play an important role. The bacteria are thought to be of significance not only for the organization of the dental plaque, which seems to be the first stage of calculus formation, but also for the attachment of the calculus to the surface of the tooth. In addition, it has been suggested that the bacteria, through their growth and metabolism, produce conditions leading to the precipitation of certain specific inorganic salts which enter into the structure of the dental calculus. However, it has recently been found that germfree animals also show calculus-like deposits (*Baer & Newton,* 1959; *Fitzgerald et al.,* 1960; *Gustafsson & Krasse,* 1962) indicating that the formation of dental calculi can occur in the absence of bacteria. This observation does not exclude bacteria from taking an active part in the ordinary process of calculus deposition. The possibility exists that another calculus forming mechanism is operating under sterile conditions compared to that underlying deposition in the presence of the oral microflora. In such a case, however, one would expect the structure and composition of dental calculi laid down in the

presence or absence of microorganisms to be quite different. The scope of the present investigation is therefore to obtain information about the constitution of tooth deposits from germfree and conventional rats. Such a knowledge should be of importance when evaluating the role of bacteria in dental calculus formation.

MATERIAL AND METHODS

A detailed description of the methods used for the rearing of the germfree rats used in this investigation has been given elsewhere (*Gustafsson & Krasse,* 1962). Therefore only a short review of these methods is given here.

The germfree rats were reared according to the methods of *Gustafsson* (1948, 1959). They were given the semisynthetic diet D 7 (*Gustafsson,* 1959) and water ad libitum, and the animals were maintained on this diet from weaning for various periods of time. Two groups of conventional rats were included in the study. The first group (C I) was given the same sterilized diet as the germfree rats. By autoclaving, the diet formed a cake which was rather tough and required more masticatory effort than the same non-sterilized diet given to the second conventional group of rats (C II). The germfree and conventional rats which were fed the sterilized cake-type diet had less calculus than the conventional rats, which were given the soft, sticky non-sterilized diet. After sacrificing the rats, the heads were autoclaved and the jaws dissected. Calculi appeared mainly on the mesial and buccal surfaces of the maxillary first molars. From each group of rats three to four molars from different animals were randomly taken for examination in the light microscope (transmitted and polarized light) and for microradiographical studies. For this purpose plano-parallel sagittal ground sections of the teeth were made, having a thickness of about 80 μ. Contact microradiograms were recorded on Kodak Maximum Resolution plates using V-filtered Cr-radiation from an x-ray tube operated at 20 kV and 20 mA. The use of Cr-radiation made it possible to determine very small absorption differences within the specimens.

X-ray crystallographic examination was performed on small fragments of calculi dissected from the main tooth deposit. Five

to six of these specimens were investigated from each group of rats using a micro x-ray diffraction camera. Ni-filtered Cu-radiation was used in every case. In addition, spot test analyses were made within the ground sections from conventional rats in order to get information about the compositional homogeneity of the calculi.

RESULTS

In Fig. 1, representative ground sections of dental calculi attached to the first maxillary molars from germfree and conventional rats (C II) can be seen in transmitted light together with their x-ray absorption pictures. The regular internal structure characterizing deposits from germfree rats is apparent from Fig. 1 a. The calculus is built up of thin parallel lamellae (about 0.5—3 μ) extending parallel to the enamel surface and following the contours of the latter fairly well. The distribution of mineral salts is seen from Fig. 1 b. Taken as a whole the degree of mineralization is slightly lower than that of the dentin and thus amounts to about 40 per cent by volume. However, striae having a somewhat higher mineral content are observable and correlate with the lamellar structures seen in transmitted light. From these findings it is clear that the calculi from germfree rats here examined are composed of lamellar sheets of varying mineral content running approximately parallel to the enamel surface.

Calculi from both groups of conventional rats show essentially the same basic internal structure as those of germfree rats. The lamellar structure is always present but seems to be less regular and occupies the outer part of the deposit (Fig. 1 c). The central or innermost part of the calculi examined are composed of structureless masses surrounded by a network of organic material as shown by its very low x-ray absorption. The overall mineral content is about the same as for calculi from germfree rats and, within the lamellar part of the deposit, striae having a higher mineral content are also observable (Fig. 1 d). Again there is a good correlation between the lamellar structure seen in the microradiogram and that in transmitted light. Compared to calculi from germfree rats, the mineralization pattern is less uniform. It is noteworthy that a narrow zone bordering the

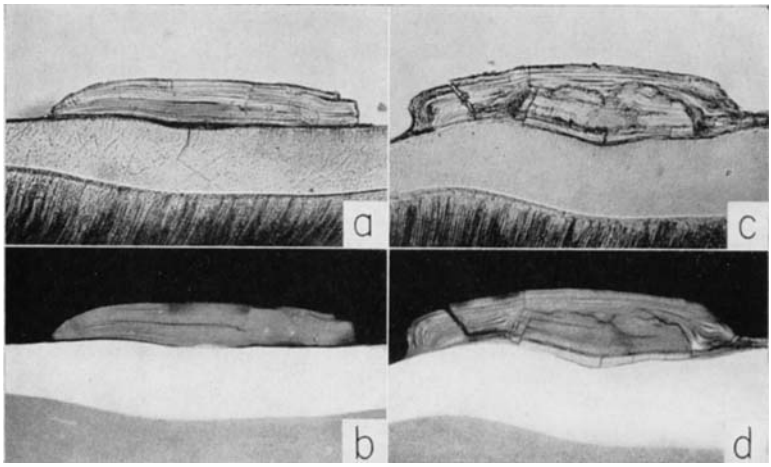

Fig. 1, a—d. Light microscope and x-ray absorption pictures of ground sagittal sections of dental calculi attached to the first maxillary molars of germfree (a, b) and conventional (c, d) rats. Note the regular internal structure of the dental calculus of a germfree origin, × 130.

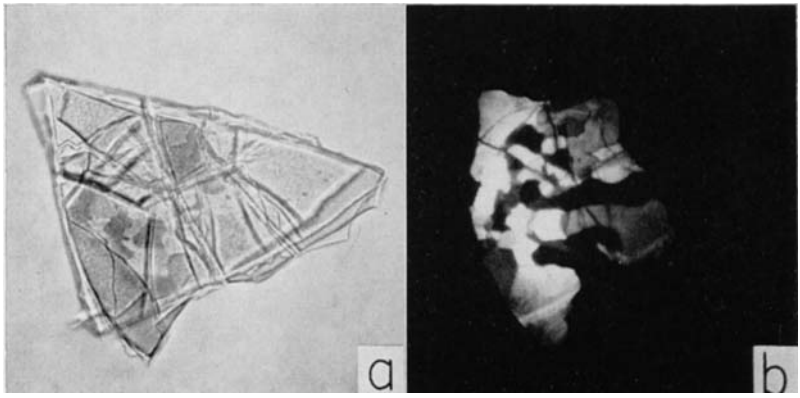

Fig. 2. Flat dental calculus fragment from a conventional rat seen perpendicular to its surface between parallel and crossed polars. Thin flakes of calcite are observable to the left in the specimen (b), due to their strong birefringence. × 500.

enamel surface within the calculus shown in Fig. 1 d has a slightly higher mineral content than the deposit as a whole. This finding might be associated with the fact that the deposits from conventional rats seemed to be more firmly attached to the enamel surface than the calculi of a germfree origin.

The x-ray diffraction examination of intact calculi specimens from germfree and conventional rats showed the presence of poorly crystalline apatite in all cases. As judged from the diffraction patterns the apatite crystallites had about the same dimensions as in bone and dentin, i.e. thin rod-shaped particles a few hundred Å in length. Using the micro x-ray diffraction technique, efforts were made to demonstrate orientation of the crystallites within the lamellar structure of the specimens. However, no preferred orientation could be observed. Examination of small regions within the central parts of the section seen in Fig. 1 c and d also gave an apatite pattern thus demonstrating the compositional homogeneity of the calculus.

One of the deposits from a conventional rat showed a somewhat different structure (Fig. 2 a, b). Under the polarizing microscope strongly birefringent crystals were found within a rather small region of the calculus. The crystals were composed of very thin flakes and showed distinct extinction positions indicating that they were single crystals. This region also showed a distinct border towards the microcrystalline apatite part of the deposit. X-ray data revealed that these crystals were composed of calcite ($CaCO_3$).

DISCUSSION

The fact that dental calculus formation can occur in the absence of bacteria seems to indicate that microorganisms are not essential in the process of calculus deposition. Other findings which lend support to this idea are the observations that the frequency of the deposits is roughly the same in germfree and conventional rats fed exactly the same diet (*Gustafsson & Krasse,* 1962) and that a change in the oral microflora by means of broadspectrum antibiotics does not exert any effect on calculus deposition (*Fitzgerald et al.,* 1960). The inorganic fraction of tooth deposits from germfree rats have previously been shown

to be composed of apatite (*Fitzgerald & McDaniel,* 1960) and the results from the present investigation thus confirm this finding. Dental calculi from conventional rats also contain apatite, perhaps occasionally in conjunction with macrocrystalline calcite which was found in only one case in this investigation. The more or less constant finding of apatite as the single crystalline phase in dental calculi from conventional rats is noteworthy considering the various mineral components frequently found in combination with apatite in calculi of human origin (e.g., whitlockite, octocalciumphosphate, brushite). However, this finding might be explained in part by the fact that pH of rat saliva is considerably higher than that of human saliva (pH 8—9 compared to 6.5—7.5) and seldom falls below 7.0 even after feeding a cariogenic diet (*Haldi et al.,* 1960). This would favour the precipitation of basic calciumphosphate. Consequently, the expected inorganic calciumphosphate to be found in calculi from germfree rats is apatite, especially since the possible interaction from acid bacterial metabolites is completely eliminated.

On a microscopical level, dental calculi from germfree rats show a very well-ordered internal structure as visualized by the examination of thin ground sections in transmitted light. The microradiographic observations are in agreement with this finding. The depositional mechanism underlying this phenomenon is poorly understood. However, it seems as if the process is of a continuous character followed by a rhythmical apposition of fresh calculus material in the form of thin lamellar sheets. In relation to the internal structure of dental calculi laid down in the presence of microorganisms differences do exist. Reference has already been made to the less regular lamellation and mineral distribution and certain structural details probably associated with the metabolism and growth of incorporated bacteria within the deposit. However, taken as a whole the structure and composition of dental calculi produced under germfree conditions and in the presence of bacteria show remarkable similarities which do not suggest a different mode of deposition under germfree conditions. This also indicates the subordinate role of microorganisms in dental calculus formation in the rat.

SUMMARY

Dental calculi from germfree and conventional rats were investigated by means of light microscopy, microradiography and x-ray diffraction. Deposits of a germfree origin showed a well-ordered internal structure of thin lamellar sheets of varying mineral content running approximately parallel to the enamel surface. Calculi from conventional rats had essentially the same basic internal structure but differed in having less regular lamellation, mineral distribution and microscopical appearance. The inorganic fraction of calculi from the different groups of rats were found to be apatite in all cases.

RÉSUME

ÉTUDES BIOPHYSIQUES SUR LE TARTRE DENTAIRE DE RATS DÉPOURVUS DE MICRO-ORGANISMES, ET SUR LE TARTRE DENTAIRE DE RATS ORDINAIRES

Du tartre dentaire de rats dépourvus de micro-organismes et du tartre de rats ordinaires ont été examinés par microscopie optique, microradiographie et diffraction de rayons X. Les dépôts tartriques provenant d'animaux dépourvus de micro-organismes présentaient une structure interne régulière consistant en minces enveloppes lamellaires à contenu minéral variable, disposées à peu près parallèment à la surface de l'émail. Le tartre provenant de rats ordinaires présentait essentiellement la même structure interne de base, mais se distinguait par une lamellation moins régulière, ainsi que par une moindre régularité de la répartition minérale et de l'aspect microscopique. On a constaté que la fraction inorganique des dépôts provenant des différents groupes de rats était de l'apatite dans tous les cas.

ZUSAMMENFASSUNG

BIOPHYSISCHE STUDIEN ÜBER ZAHNSTEIN BEI BAKTERIENFREIEN RATTEN UND KONTROLLRATTEN

Zahnstein von bakterienfreien Ratten und Kontrollratten ist mit lichtmikroskopischer Technik, mit Mikroradiographie und mit Röntgendiffraktionstechnik untersucht worden. Zahnstein der

bakterienfreien Ratten zeigte eine ganz gute innere Orientierung von dünnen Lamellen mit einem wechselnden Gehalt an Mineralien. Die Lamellen waren mit der Oberfläche des Zahnes ungefähr parallel. Zahnstein der Kontrollratten hatte ungefähr dieselbe innere Struktur. Die Lamellen, die Verteilung der Mineralien und das mikroskopische Aussehen waren aber nicht so regelmässig. Der anorganische Bestandteil des Zahnsteines war in allen Gruppen Apatit.

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