From the Department of Sosiodontia,
State University of Groningen, Netherlands
Head: Prof. J. G. de Boer

DECALCIFICATION IN INITIAL DENTAL CARIES

A preliminary report

by F. VON BARTHELD, Research Associate

Summary

Decalcification of dental enamel in initial caries has been studied. A hypothesis is presented which is in accordance with clinical observations and is corroborated by the results of laboratory experiments. The initial carious lesion is described as a result of ionic distribution of the DONNAN type. The same mechanism is held responsible for the protective action of saliva above a critical pH.

Introduction

Dental enamel consists of an organic ground substance impregnated by a mineral component. The chemistry of both, and their interrelationship are but little known. In a physico-chemical sense enamel is a water-containing gel permeable for small molecules and ions. This fact, and its high content of easily soluble calcium salts render it susceptible to decay. According to most workers carious dissolution starts in characteristic areas of the enamel surface as a white opaque spot without perceptible loss of substance. Beginning at the surface, the opacity extends slowly into the enamel until the dentin is reached, which is subsequently attacked.

A carious dissolution of the cementum is also possible when it has become exposed in the oral cavity. However, neither dentin caries nor caries of the cementum play an important role in the etiology of dental caries, because dentin and cementum are exposed to the oral environment only after the removal of their respective protective coverings, viz. the enamel and the gingiva. It is generally agreed that caries of dentin and cementum

are secondary to the elimination of the enamel cap, which may be due to primary enamel caries, gingival retraction etc.

As it is felt that knowledge of its aetiology and chemistry would greatly benefit all facets of caries research, the white spot has been the subject of numerous investigations. These have resulted in a great many clinical and histological observations. The normally translucent enamel becomes porous and opaque, chalky white in incident light, dark brown in transmitted light. X-ray and polarized light investigations have demonstrated a partial demineralisation, obviously caused by a process of dissolution and diffusion. The white spot shows up most clearly when the tooth is dry, water having disappeared from the submicroscopic spaces that cause the opacity. While it is impossible to damage the surface of sound enamel with a probe, the opaque mass crumbles easily, being of a chalklike consistency. Nevertheless it has retained its histological aspect: in ground sections RETZIUS' lines, SCHREGER's bands, enamel prisms and their transverse striation are as clearly visible as in sound enamel, or even more distinctly. The enamel surface also has remained unchanged, having retained its glossy appearance and the normal aspect of perikymata and prism endings.

LEBER and ROTTENSTEIN ascribed this partial dissolution of the enamel to a combined action of micro-organisms and acids. MILLER and BLACK developed this concept to the well-known chemico-parasitic theory of caries aetiology, the acids being described as intermediary products of microbial metabolism. The micro-organisms exist mainly in protected areas, forming a tenacious film on those parts of the tooth surface that escape cleansing. Certain micro-organisms in this layer, known as the bacterial plaque, are capable of breaking down carbohydrates, the glycolytic process resulting in an accumulation of acid compounds and a drop of the pH in the area. For the sake of convenience we will consider lactic acid as representative of the whole complex mixture of acids that is being formed and transformed continually. As until recently no other compounds but acids were known to be able to dissolve the mineral tooth substance, it is easily understood that lactic acid was regarded as the direct cause of enamel opacity in caries. This view has persisted until today, notwithstanding the fact that the typical white spot of carious decalcification has never been produced in vitro by the direct action of lactic acid or otherwise. In all concentrations organic and mineral acids cause a superficial etching of the enamel, which is completely destroyed, while right under the surface the enamel remains undisturbed until reached by the acid (fig. A). While it seemed clear that the acids of carbohydrate fermentation

are somehow involved in enamel decalcification, clinical and experimental observations failed to establish the character of this involvement. The facts suggest a more complex mechanism than the simple action of acids. It remained unexplained why the acids of fermentation cause rampant caries in one mouth and not in another. The lack of correlation between caries susceptibility and oral hygienic conditions remained an unsolved problem. Impressed by these and other considerations many workers felt the necessity for a better explanation. They may be divided into two groups, one searching for accessory conditions that might increase or weaken the effect of acids, the other rejecting that effect altogether and looking for some other decalcifying mechanism. However, no modified or supplemented acid theory, nor any other hypothesis advanced thus far has been substantiated by experimental evidence.

No other connection between acids and caries has been demonstrated, than the constant finding of a low pH at sites of carious decay. It has been shown that a considerable „degree of acidity" can occur on the tooth surface under a plaque. These low readings were only found intermittently, but the lowest readings were consistently found in caries-susceptible subjects. As pH measurements of this kind are extremely difficult, we owe much to workers in this field. *However it must be emphasized that a low pH does not mean that a large amount of acid is present, nor that much acid is being produced at the moment of measuring the pH.* The pH as such discloses only in an easy logarithmic form the concentration of $H^{\cdot}$-ions as compared to pure water at 22° C. It informs us about the intensity of acidity regardless of the amount of acid present or its chemical nature. It should be clear, that only a titration of the plaque material would inform us about the potential acidity; a pH measurement has no quantitative meaning at all. A solution of NH_4Cl, for instance, will show a low pH reading because of hydrolysis, the compound being a salt of a rather feeble base and a strong acid. Yet the solution is quite unable to decalcify enamel because the potential acidity, the amount of acid actually present, is utterly small. A certain pH at a certain area only means that at the moment equilibrium exists between pH lowering and raising factors. If the first mentioned are minimal, the second need also be only slight to maintain the pH at that level, and vice versa. These facts, much too often overlooked, are of the utmost importance in evaluating all biochemical reactions. In this case they point to a mechanism having a decalcifying effect in correlation with a low pH, but independent of the potential acidity of the environment. A process of this kind has been described by DONNAN and co-workers.

For a complete description of the work of DONNAN we must refer the reader to the literature. An outline of the principles involved is taken from TENDELOO.

DONNAN studied the ionic concentrations in solutions separated by a semipermeable membrane. When one of the solutions contains an ion large enough to be arrested by the membrane, interesting diffusion phenomena occur. Let us consider a salt Na·E′, completely dissociated in water on the left side of the membrane, while on the right side a solution of Na·Cl′ is present.

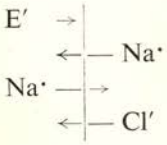

fig. 1

With the exception of E′ all ions can diffuse freely through the membrane. Nevertheless it can be deduced on thermodynamical grounds, that the salt Na·Cl′ will not disperse evenly over the total amount of water, because of the presence and concentration of the non-diffusing ion E′ on the left side of the membrane. If, for instance, the concentration of Na·E′ is many times that of Na·Cl′, practically all Na·Cl′ will stay in the right compartment; a little NaCl added to the left side will be „excreted" nearly completely through the membrane towards the right side.

Under simple experimental conditions it proved to be possible to predict the concentrations after equilibrium would be attained. In other experiments DONNAN studied the influence of non-diffusing ions on the ionic distribution of pure water in the opposite compartment.

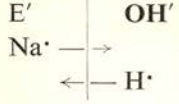

fig. 2

Under conditions as pictured in fig. 2., the Na·-ions can diffuse to the right, but they will only do so if E′-ions go with them (which however is impossible) or if a H·-ion diffuses to the left in exchange for each Na·-ion. Experiments have shown that this exchange actually takes place, until a state of equilibrium has been reached, which of course is influenced by the concentration of Na·E′. The fluid in the right compartment then contains OH′-ions in excess of the remaining H·-ions, in other words, its reaction is alkaline, while the solution on the left side has turned acid. Reverse conditions, viz. when the non-diffusing ion has a positive charge, will result in a low pH in the right compartment and a high reading on the left side. In this case an exchange

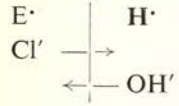

fig. 3

of Cl′ and OH′-ions will occur, again resulting in an equilibrium dependent on the concentration of E·Cl′. The right compartment then contains some HCl, or some other acid as the case may be. DONNAN has pointed out that in this way a concentration of

H·-ions as high as that of gastric juice may be attained. It must be kept in mind, however, that the low pH reading on the right does not indicate the presence of large quantities of HCl. Here again a high actual acidity is combined with a very low potential acidity. When however any of the ions concerned are continually removed as a result of some chemical reaction in either compartment, the transport of OH′ and Cl′-ions through the membrane will be greatly accelerated, notwithstanding the fact that no equilibrium can be reached and therefore the difference in pH between both sides will be less pronounced.

The possibilities outlined above will serve to show that under appropriate conditions ionic distribution causing a pronounced change of pH is possible as the result of membrane equilibria of the type described by DONNAN. We will now apply the DONNAN theory to the subject of this report.

As enamel may be regarded as a water-containing gel of very dense structure, it will, in its normal environment, contain H· and OH′-ions equally distributed. Because of its density it will only admit small molecules and ions from the oral fluid, but it will be quite impenetrable by macromolecules such as proteins and carbohydrates.

We propose to consider the enamel both as the membrane in the sense of DONNAN's theory, and at the same time as one of the „compartments", the other being the oral cavity. The fluid bathing the tooth surface may be a film of saliva, or the fluid contents of a bacterial plaque, etc. In the immediate proximity of the enamel surface we assume the presence in solution of positively charged ions E·, too large to diffuse into the enamel. Even when the solution containing these ions is near the neutral point, a DONNAN ionic distribution will take place, the saliva becoming slightly alkaline, while in the enamel a drop of the pH will occur (fig. 3.). The enamel will „excrete" OH′-ions in exchange for any small anions, e.g. Cl′, present in the surrounding fluid. The buffering action and the flow of the saliva will remove the OH′-ions, thereby accelerating their „excretion" and increasing the acidity in the enamel. Here an increasing excess of H·-ions develops, the enamel being decalcified under conditions that are exactly opposite to decalcification by immersion of a tooth in an acid, as illustrated schematically in fig. 4.

Acid	Enamel	Solution of positively ionized macromolecule	Enamel
pH = 5	pH = 7	pH = 7	pH = 5

fig. 4

It will be shown that this particular kind of acid decalcification in the enamel is responsible for the development of the characteristic „white spots" of initial dental caries.

As the excess of H·-ions and the Cl′-ions will be removed in the process of decalcification, the pH drop will be small, *but the process will continue as long as positively charged E·-ions are present on the enamel surface.*

If the fluid on the tooth surface has an acid reaction instead of being neutral, even if no significant amount of free acid is present, the decalcification will nevertheless continue as before, if only E· stays positively charged.

A negatively charged E′-ion will cause the reverse situation, the enamel becoming slightly basic and the saliva receiving H·-ions (fig. 2), even if it is acid already.

As most macromolecular compounds that form positive ions are very feeble bases, their soluble salts showing hydrolysis in solution, it was difficult to conduct an experiment at neutral pH. A solution of quinine hydrochloric however was found to have a pH of 6.7 owing to the fact that only one of its two hydroxyl groups has been replaced by Cl′. Its molecular weight is 324, and therefore it can be assumed that it will not enter sound enamel. A 3% solution in water at 37° C. proved to decalcify enamel as expected. Freshly extracted teeth were surface-dried and covered with a layer of hard paraffin (melting point 60°), leaving a window on the enamel surface. They were submerged in a 3% solution of quinine hydrochloric at body temperature. After two weeks the enamel exposed by the window showed all the characteristics of initial carious decalcification. A typical „white spot" had developed, extending to some depth into the enamel, without any break in the surface (fig. B and C).

We felt justified in regarding these results as provisional proof of the correctness of the theoretical deductions outlined above.

Discussion

The only compounds always present in the mouth that are able to cause a DONNAN distribution between enamel and saliva are ionized protein molecules. All other substances in solution are either too small (anorganic salts, acids of fermentation) or not ionized (carbohydrates and their degradation products including sugars). Fatty acids from fat decomposition are slightly ionized and of some size, but they occur only in very low concentrations in saliva and bacterial plaques and their presence is probably of short duration.

The proteins present are salivary mucin and those derived from animal

and vegetable food debris. They form colloidal solutions in water, mucin being responsible for the viscosity of the saliva. Because of their typical protein structure they possess both basic ($—NH_3OH'$) and acid ($—COO\ H\cdot$) groups in watery solution, every molecule having a considerable number of either, as proteins have a very large molecular weight. The $H\cdot$-ion is split off more easily than the OH'-ion, most pure proteins therefore possessing acid properties in water. A solution of pure gelatin has a pH of 4.7, which is its isoelectric point. It shows its lowest degree of viscosity at this point.

Addition of base to this solution of isoelectric gelatin will cause a slow rise of pH, the protein forming an anion and behaving as an acid:

$$\text{H-(E)-OH} + \text{NaOH} \longrightarrow (\text{EOH})' + \text{Na}\cdot + \text{H}_2\text{O} \quad \text{(I)}$$

The resulting proteinate will be able to effect a DONNAN *distribution, its solution becoming acid and the other compartment alkaline as in fig. 2.* When all negative valencies have been saturated with $Na\cdot$, the base-binding capacity of the gelatin is exhausted, the pH having risen to about 13.

Addition of acid to the solution of isoelectric gelatin will slowly lower its pH, the gelatin again forming a salt but now behaving as a base:

$$\text{H-(E)-OH} + \text{HCl} \longrightarrow (\text{HE})\cdot + \text{Cl}' + \text{H}_2\text{O} \quad \text{(II)}$$

The $H\cdot$-ion from the acid will disappear as such, the pH only falling because of hydrolysis, until all positive valencies have been saturated with anions. With most proteins this is the case at a pH of about 1.5. *The chloride formed will also be able to cause a* DONNAN *distribution of $H\cdot$ and OH'-ions, now however as shown in fig. 3, because the ionized protein has a positive charge.*

Addition of still more acid will cause a pH drop below 1.5. As now the acid-binding capacity is exhausted, the solution will contain gelatin chloride *and free acid.*

The behaviour of gelatin, in short, is dependent on the pH of its solution:

1. Upwards from its isoelectric point (I.P.) of 4.7 the gelatin is negatively ionized.
2. Between pH 4.7 and 1.5 it is positively ionized, but no free acid is present.
3. Below pH 1.5, the saturation point (S.P.), any excess of acid is no longer buffered.

On the strength of the foregoing considerations the influence on enamel of gelatin solutions at different pH values can be predicted. In pH region

1. it will be protected by an alkalifying DONNAN effect and suffer no ill effects at all. In region 2. it will become decalcified as a result of an acidifying DONNAN effect, a „white spot" developing without loss of surface continuity or histologic structure. In the third region this indirect decalcification will be at its maximum, but in addition a direct decalcification of the enamel will occur, caused by the presence of free acid.

To verify these speculations freshly extracted as well as formalin-fixed teeth were submerged for different lengths of time in gelatin solutions at different pH values after having been prepared as in the experiment described above. The concentration of the gelatin varied between 2% and 20%. A few drops of phenol. liq. were added to prevent moulding. pH values of 5 and over were obtained by addition of NaOH, while pH values below I.P. were obtained by adding hydrochloric or lactic acid. All values were determined electrometrically and controlled weekly. As expected, pH levels around I.P. (between 4.5 and 5) caused hardly any or no opacity. At higher pH levels all teeth without exception remained unaffected. From I.P. downwards the decalcifying process was accelerated as demonstrated by the depth of the lesions. At pH levels under S.P. the opacity was accompanied by etching of the surface, a depression developing in the centre of the white spot. All white spots obtained resembled initial caries closely in every respect. S.P. was 2 with HCl, and 3 with lactic acid (fig. D).

Preliminary conclusions

Saliva normally contains about 2% mucin. This will mainly be present in the form of its Na·-salt, the pH being practically neutral. In contact with a tooth the anion will cause an excess of OH′-ions to accumulate in the enamel, and it will continue to do so as long as these large anions are present. This protective action of saliva has been known for a long time, but in our opinion it is not due to some protecting substance but to the fact that its mucin content is charged negatively.

When a little lactic acid is formed, e.g. as a result of microbial carbohydrate decomposition, the pH will drop slowly because of the buffering action which is a property of all proteins:

$$(EOH)'\,Na\cdot + HM \longrightarrow H\text{-}(E)\text{-}OH + Na\cdot M' \quad \text{(III)}$$

Of course this equation is incorrect in so far as each protein molecule is able to bind a considerable number of Na·-ions. When these have all been replaced by H·-ions the protein is uncharged and the pH conforms to its isoelectric point. As mucin possesses acid properties like gelatin, its

isoelectric point however being situated at pH 3, it will be clear that even when the pH drops from 7 to 3, the saliva protein will keep its anion character, the alkalifying DONNAN effect exerting its protective influence on the enamel.

When acid production is increased to such a degree that all mucin is neutralized, its viscosity will have become minimal and the film of saliva on the tooth surface watery thin, being washed away easily and replaced by fresh saliva. In stagnant areas however, where replacement by buffer solution does not readily occur and where, owing to the presence of other proteins, the critical isoelectric „point" is probably not as low as 3, a further drop of pH below this critical level may cause the neutralized protein mixture to become for the greater part kationic, behaving now as a base, and forming lactate:

$$\text{H-(E)-OH} + \text{HM} \longrightarrow \text{(HE)}\cdot + \text{M}' + \text{H}_2\text{O} \quad \text{(IV)}$$

Please note that now a different buffering mechanism is present and functioning, all positive valencies becoming available as soon as the pH starts to drop below the critical level. If enough lactic acid is formed, all these valencies are saturated according to (IV), before free acid can be present. *The protein however, being positively charged, will now exert an indirect decalcifying action on the enamel.* Anions will enter the enamel in exchange for OH′-ions, which will at least assist in neutralizing the acid that is being formed. A white spot will develop at the site of greatest intensity of the DONNAN effect. This need not always coincide with the area of maximal saliva stagnation, or plaque formation, or food retention, neither of these factors alone determining the location of initial decalcification.

When lactic acid continues to be produced in sufficiently large amounts, the second critical level will be reached, i.e. the saturation point (S.P.) of the protein solution, and free acid will accumulate for the first time during the process. It will exercise a direct decalcifying action, usually, as a matter of course, in or near the centre of the white spot, where cavitation as a rule starts.

As long as no free acid is present the low pH is the result of hydrolysis, the „salt" (HE)·M′ being derived from an extremely weak base (protein) and a rather strong acid (lactic acid). A stationary pH indicates that the acid production is balanced by the renewal of buffering compounds and the flow of OH′-ions from the enamel. The reader will have noted that the positively charged protein is not used up in any way, which is typical of membrane phenomena of this kind.

Fig. A

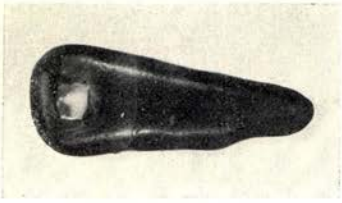

Fig. B

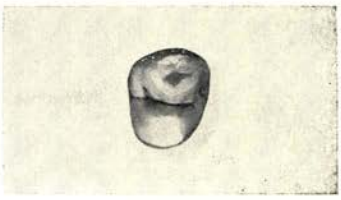

Fig. C

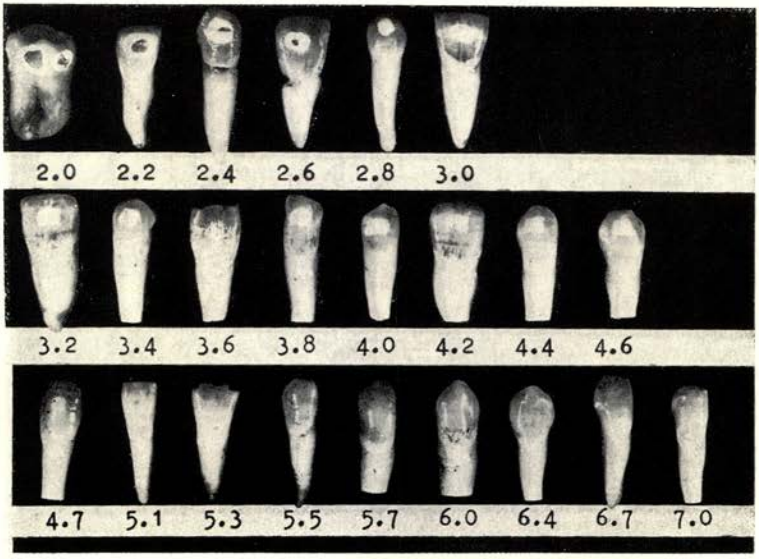

Fig. D

Fig. A. Freshly extracted human molar, part of its surface having been subjected to the action of 3% lactic acid (pH 3.5) for three days. The enamel has been etched away without previous formation of a white spot

Fig. B. Human incisor with paraffin coating, showing the window which limits the action of a solution to the exposed part of the enamel surface

Fig. C. Occlusal view of human premolar, the lingual surface of which has been decalcified with 3% quinine hydrochloric

Fig. D. A series of human teeth after three weeks immersion in 10% gelatin solutions as described in the text. The numbers indicate the pH levels which were obtained by adding either 30% lactic acid (pH 2 — 4.7) or 1% NaOH (pH 5.1 — 7)

The whole process may be summarized in the following scheme:

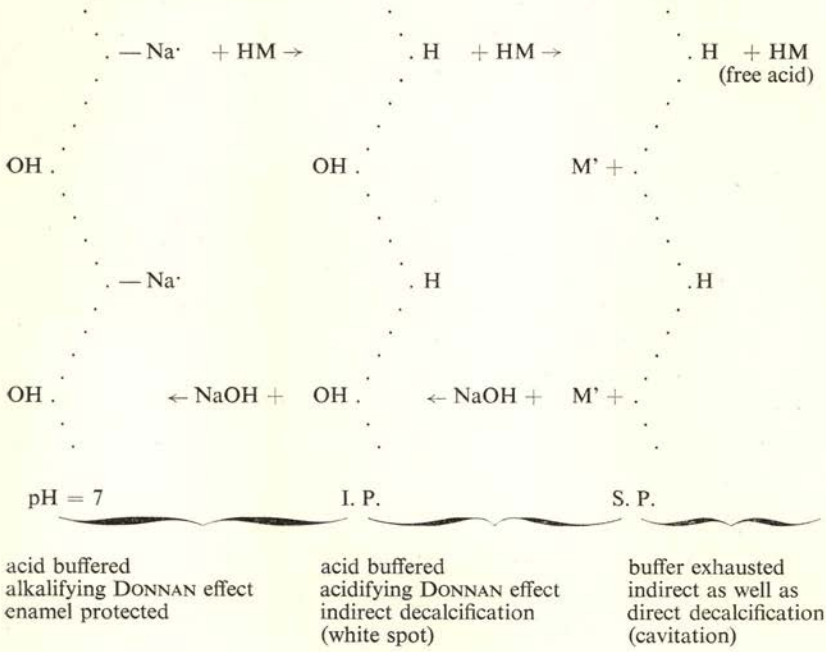

fig. 5

The only factors that are able to effect a rise of the pH are the removal of the now useless and noxious protein, and the flow of OH′-ions from the enamel. The last mentioned factor is always very small, enamel containing very little water. If the protein remains in situ, having assumed a viscous, perhaps even tacky consistency again, practically no pH raising factors are immediately available. A minimal acid production therefore is sufficient to maintain the pH at the low level which probably is most favourable for the micro-organisms. Moreover, all protein lactate would have to be converted into uncharged protein again, before the acidifying DONNAN effect would stop, allowing the pH to rise above I.P.

It should be emphasized that all symbols used in this article have been chosen for the purpose of making our point clear. The symbol E stands for all protein components, the symbols Cl′, M′ for all anions present, etc., the only important factors in DONNAN equilibria being the degree of dissociation and the size and charge of the ions. By I.P. is meant the average degree of acidity at which the protein solution behaves isoelectrically,

rather than an exact pH reading. S.P. also does not indicate a definite pH level. It signifies the presence of free acid.

Summarizing our findings and speculations we are convinced that pH as measured on the tooth surface bears no direct relation to the amount of acid actually present at the moment. Initial caries of enamel, the so-called white spot, is explained as the result of ionic distribution of the DONNAN-type, dependent on the presence and concentration on the tooth surface of positively charged ionized protein; not on the presence of free acid. Important factors are acid production rate, average isoelectric „point" and concentration of the protein or proteins present, and the rate of drainage of all compounds concerned. The same DONNAN theory explains the protecting action of saliva on enamel, its ionized protein being negatively charged at pH values above its isoelectric point.

We feel justified in stating tentatively that in caries-resistant individuals the pH on the enamel surfaces will as a rule vary between 7 and the average I.P. of proteins present. It will not drop below the last mentioned value because of:

Low acid production rate,
Low average isoelectric point of the proteins present,
High concentration of proteins (ample buffering capacity and strong alkalifying DONNAN effect),
High rate of drainage.

In caries-susceptible subjects pH will drop relatively often below the critical level, because of:

High acid production rate,
High average isoelectric point of proteins involved,
High concentration of proteins (strong acidifying DONNAN effect),
Low rate of drainage.

A comprehensive article containing a survey of the literature, which will deal with the principles involved in initial enamel caries and which will include the results of experiments now under way, is in preparation.

Samenvatting

De carieuze aantasting van het glazuur begint als een witte verkleuring, zonder dat de kenmerkende structuren aan de oppervlakte en in het inwendige van het glazuur verloren gaan. Macroscopisch doet het aangetaste gebied zich voor als een opake massa, krijtachtig wit in tegenstelling tot het overige doorschijnende weefsel. Het is met een sonde gemakkelijk te krassen. Microscopisch is het beeld slechts in zoverre veranderd

dat in het algemeen de structuur van het glazuur duidelijker is geworden; prismagrenzen, lijnen van RETZIUS en banden van SCHREGER komen beter uit dan in transparant onaangetast glazuur. Voorts is het opake weefsel bij doorvallend licht geelachtig getint. Het bevat minder kalkzouten, hetgeen beschouwd wordt als een gevolg van een langzame ontkalking, bewerkt door de zure stofwisselingsprodukten van bepaalde mondbacteriën. Deze bevinden zich voornamelijk in karakteristieke retentieplaatsen op het tandoppervlak, waar zij een vast hechtend laagje vormen bekend als de „bacterial plaque". De afbraak van koolhydraten ten behoeve van hun energievoorziening gaat gepaard met de vorming van organische zuren, in hoofdzaak melkzuur, en dus met een daling van de pH op het betrokken glazuuroppervlak. Tot voor kort waren geen andere stoffen bekend of aangetoond, die in staat waren de kalkzouten op te lossen, zodat de chemisch-parasitaire theorie van MILLER zich tot de huidige dag heeft gehandhaafd. Een zwak punt in die theorie wordt gevormd door de voorstelling dat de gevormde zuren het glazuur zonder meer zouden aantasten zodra de pH tot een zekere waarde zou zijn gedaald.

In de eerste plaats is het in vitro niet gelukt, een directe aantasting van het glazuur te bewerkstelligen door verdunde zuren, zonder dat het weefsel werd weggeëtst. Met andere woorden, de proeven in vitro leverden steeds een „caviteit" op zonder voorafgaande vorming van een witte ontkalking.

In de tweede plaats bestaat er in het algemeen geen duidelijke correlatie tussen de gemeten pH van speeksel of bacterial plaque en de cariësvatbaarheid, hoewel anderzijds de laagste pH-waarden steeds worden gevonden in monden die de meeste cariës vertonen. Ook bestaat er een correlatie ten opzichte van het waargenomen aantal lactobacillen in de mond, en bij experimentele beïnvloeding van dat aantal. Deze feiten duiden er op, dat de rol van de zuren gecompliceerder is dan een eenvoudige oplossing van het glazuur.

In dit verband wordt er door de schrijver op gewezen, dat een lage pH niet betekent dat er veel zuur aanwezig is, of dat er per tijdseenheid veel zuur geproduceerd wordt. De pH van een milieu geeft de actuele zuurgraad ervan aan, ongeacht de sterkte, de aard, of de concentratie van eventueel aanwezig zuur. Een oplossing van een zout van een sterk zuur en een zwakke base, b.v. NH_4Cl, vertoont een lage pH tengevolge van hydrolyse. Het is echter onmogelijk, met een dergelijke oplossing glazuur te ontkalken, omdat de hoeveelheid werkelijk aanwezig zuur, de potentiële zuurgraad, practisch nihil is. Een bepaalde pH gemeten op een tandoppervlak geeft uitsluitend een *toestand* aan, namelijk dat op het ogenblik van de meting een bepaalde verhouding bestaat tussen factoren die de pH verhogen en factoren die hem verlagen. Wanneer eerstgenoemde minimaal werkzaam zijn, behoeven de laatstgenoemde ook slechts zeer gering te zijn om de pH constant te houden. Deze feiten zijn van groot belang bij het beoordelen van biologische processen en worden veel te vaak over het hoofd gezien. In het onderhavige onderzoek wezen zij op een proces waarbij ontkalking plaats heeft tengevolge van een pH-verlaging, die echter niet rechtstreeks doch indirect door zuren wordt veroorzaakt. Een dergelijk proces is door DONNAN en zijn medewerkers onderzocht en beschreven.

Een kort begrip van de principes ontwikkeld door DONNAN werd ontleend aan TENDELOO. Wanneer twee vloeistoffen gescheiden worden door een semi-permeabele membraan, en één der vloeistoffen bevat een ion dat het membraan niet kan passeren, terwijl het tegengesteld geladen ion dat wel kan, treden merkwaardige verschijnselen op. Fig. 2 geeft aan, dat in het geval van een negatief geladen groot ion E′ er een uitwisseling zal plaatsvinden van Na· en H·-ionen, respectievelijk uit het linker en het

rechter compartiment, met het gevolg dat rechts een overmaat OH′ ionen ontstaat en de pH daar stijgt. Het omgekeerde geval wordt in beeld gebracht door fig. 3. Hier veroorzaakt de aanwezigheid van een positief geladen groot ion links, dat het membraan niet passeren kan, een overmaat H·-ionen, dus een zure reactie, in het rechter vak. Wanneer bovendien in een der compartimenten een chemische reactie zorgt voor geregelde wegname van een of meer der betrokken ionen, dan kan zich geen evenwicht instellen en ontstaat een geregelde ionenstroom door het membraan in de richting van de pijltjes.

Daar het glazuur kan worden opgevat als een waterhoudend gel van zeer dichte structuur, kunnen wij het als een semi-permeabele membraan beschouwen; tevens kan het de rol van één der „compartimenten" vervullen. Het andere „compartiment" wordt gevormd door het vloeistoflaagje dat zich onmiddellijk op het glazuur bevindt; dit kan uit speeksel ofwel uit de vloeibare plaque-inhoud bestaan. *Bevat deze vloeistof een groot ion, dat het glazuur niet kan binnendringen, dan zijn de voorwaarden voor een* DONNAN-*effect aanwezig*. Is dit ion positief geladen, dan heeft een pH-daling in het glazuur plaats, in principiële tegenstelling dus tot de toestand waarbij een tand in verdund zuur zou worden gedompeld. Fig. 4 geeft dit zeer schematisch weer. Wèlke anionen het glazuur binnendringen is niet van belang. Het lag voor de hand, in deze uiteraard zeer eenvoudig weergegeven verschijnselen de verklaring te zien voor de typische vorm van ontkalking die zich bij beginnende cariës voordoet. Wij vonden dit bevestigd in experimenten waarbij chinine hydrochloride de rol van de hypothetische stof E·Cl′ vervulde. Deze betrekkelijk groot-moleculaire stof werd gekozen omdat de pH van een 3% oplossing ervan in water 6,7 bleek te zijn, ver boven de critische waarde van ± 5 dus, die voor de carieuze ontkalking van glazuur noodzakelijk wordt geacht. Na enkele weken aan de invloed van deze oplossing te zijn blootgesteld geweest, vertoonde het glazuur van menselijke gebitselementen typische witte vlekken aan de oppervlakte, identiek aan die der initiële cariës.

In het mondmilieu zijn geen andere grootmoleculaire en bovendien in oplossing geïoniseerde stoffen aanwezig dan de eiwitten uit het speeksel en afkomstig van het voedsel. Op de tandkronen is een laagje speeksel aanwezig dat ± 2% mucine bevat. In de plaques, fissuren, pits en andere retentieplaatsen zal zich bovendien een mengsel van allerlei eiwitten en hun afbraakprodukten bevinden. Alle eiwitten vertonen de eigenschap, dat zij zich nu eens als zuren, dan weer als basen kunnen gedragen, afhankelijk van de pH van de oplossing. Bij een voor elk eiwit specifieke pH, het iso-elektrische punt (I.P.) van dat eiwit, is het ongeïoniseerd. Bij een pH hoger dan I.P. gedraagt het zich als een zuur en vormt zouten van het type Na·E′. Bij een pH lager dan het I.P. is het eiwit wederom geïoniseerd, maar nu draagt het een positieve lading en vormt in zijn zouten het kation, b.v. E· Cl′. Bij een negatieve lading der mucine-ionen in het speeksel zal het glazuur H·-ionen verliezen en een zekere basiditeit verkrijgen ook al ligt de pH van het milieu tussen het I.P. en 7, en is dit dus in absolute zin zuur. Dit stabiliserende DONNAN-effect verklaart de beschermende werking die algemeen aan het speeksel wordt toegekend. Het I.P. van mucinen ligt namelijk ongeveer bij pH 3, een voor eiwitten bijzonder lage waarde. Eerst bij een pH beneden deze waarde kan een ontkalkend effect door het nu positief geladen mucine worden veroorzaakt in de vorm van een witte vlek; daarboven heeft het een beschermende werking. Andere eiwitten echter kunnen bij een veel hogere pH een ontkalkend DONNAN-effect veroorzaken. Experimenten met gelatine-oplossingen van verschillende concentraties en geleidelijk afnemende pH bevestigden de juistheid van bovenvermelde beschouwingen. Geheel volgens verwachting bleek het mogelijk drie gebieden te onderscheiden in de pH-schaal van 0 tot 7:

1. het gebied tussen pH 7 en het I.P. van gelatine, dat 4,7 bedraagt. Hier werden de verschillende pH-waarden bereikt door toevoeging van NaOH-oplossing met behulp van een pH-meter. *In dit gebied, waar het eiwit negatief geladen is, bleven alle gebitselementen onbeschadigd.*

2. het gebied tussen het I.P. en het punt, waar alle eiwit geheel met anionen verzadigd was, het „saturation point" (S.P.). Voor eiwitten ligt dit in het algemeen bij pH 1 tot 2, wanneer van een sterk zuur gebruik wordt gemaakt zoals HCl; hoger wanneer het zuur zwakker geïoniseerd is. De verschillende pH-waarden werden in dit gebied bereikt door toevoeging van verdund zuur, waarvoor HCl en melkzuur werden gebruikt, met S.P. bij resp. pH 2 en 3. *Het gevormde gelatine-chloride of -lactaat veroorzaakte reeds na enkele dagen witte vlekken in het glazuur, in alle opzichten identiek met die van beginnende cariës.* De structuur zowel aan de oppervlakte als in het inwendige veranderde in die van opaak glazuur, en wel tot een diepte, die evenredig bleek met de pH-daling en de duur der inwerking. Etsing van de oppervlakte trad echter niet op.

3. het gebied waar de pH lager was dan S.P. Hier was naast het gelatine-chloride of -lactaat ook vrij zuur aanwezig. *Behalve een ontkalkend* DONNAN-*effect van maximale intensiteit trad nu ook een directe zuurwerking in, waarbij het wit verkleurde glazuur langzaam werd weggeëtst.*

Samenvattend kunnen wij uit het bovenstaande besluiten, dat het optreden van witte vlekken in het glazuur bij beginnende cariës te verklaren is als het gevolg van de aanwezigheid van positief geladen eiwit-ionen op de oppervlakte, en niet van vrije zuren. Een negatieve lading van de aanwezige eiwit-ionen daarentegen werkt stabiliserend op het glazuur, een toestand welke in het algemeen bestaat wanneer bespoeling van het glazuur en reiniging ervan voortdurend gewaarborgd zijn. Pas wanneer door grote zuurproduktie en stagnatie van het speeksel (plaquevorming) de buffercapaciteit van het aanwezige eiwit is uitgeput, zonder dat dit ververst wordt, en de aanwezigheid van vrij zuur dus mogelijk is, wordt het ontkalkte glazuur ook weggeëtst en begint zich een caviteit te vormen, in het algemeen in het centrum van de witte vlek. De mening wordt uitgesproken en gemotiveerd, dat bij de vorming van „white spots" behalve de bekende factoren als zuurproduktie, bespoeling door speeksel en buffercapaciteit van het speeksel, een tot nu toe niet in aanmerking genomen factor van betekenis is, *namelijk het punt op de pH-schaal waar de op het glazuuroppervlak aanwezige eiwitten van lading veranderen. Bij aanwezigheid van verschillende eiwitten zal dit mengsel zich over een zekere pH-breedte isoelektrisch gedragen, zodat dan van een gemiddeld isoelektrisch punt gesproken moet worden.*

Een volledig artikel, waarin alle verrichte en nog in bewerking zijnde experimenten zullen worden beschreven, wordt in het vooruitzicht gesteld.

Literatuur

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