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MEMBRANE PHENOMENA IN CARIOUS DISSOLUTION OF THE TEETH

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Abstract—Experiments are described that have been conducted to ascertain the role of DONNAN membrane effects in carious dissolution of the mineral components of the tooth structures. When a solution of an ionized macromolecular substance is separated from another solution by a semipermeable membrane, which arrests the macromolecules but allows free passage to small ions from both sides, the latter are distributed over both solutions in a peculiar way. Under certain circumstances, depending on the net particle charge, a drop of the pH on one side of the membrane can be effected, accompanied by a rise of the pH on the other side. We applied this principle to the problem of carious tooth decalcification. It was possible to predict and obtain results *in vitro* that closely resemble actual caries, much more so than could be achieved until now with the use of buffered or unbuffered acids. Further experiments, partly still under way, strongly suggest that the beneficial effect of saliva on the tooth substance, mostly described as a mineralizing or remineralizing activity, may also be ascribed to a DONNAN membrane effect, the macromolecular agent being salivary mucin.

Zusammenfassung—Der Haupteinwand, der gegen MILLERS chemoparasitäre Theorie erhoben werden kann, ist die Tatsache, daß es sich als unmöglich erwiesen hat, die typischen kariösen Läsionen *in vitro* durch direkte Säureentkalkung nachzuahmen.

Diese Tatsache hat zur Suche nach biochemischen Theorien zur Erklärung des Mechanismus der kariösen Entkalkung geführt. Diese Theorien waren jedoch genau an demselben Punkt nicht erfolgreich; sie gewannen über ihre theoretischen Aspekte hinaus keine weitere Bedeutung, weil sich sogar noch weniger experimentelle Beweise zu ihrer Stützung erbringen ließen. Der Autor kam zu der Schlußfolgerung, daß es nicht richtig ist, die Anwesenheit von freier Säure in den bakteriellen Plaques anzunehmen; sobald Säure gebildet wird, reagiert sie mit den Bestandteilen der Plaques, und es entstehen Verbindungen, die eine Entkalkung des Zahnschmelzes mittels des Membranphänomens, wie von DONNAN beschrieben, bewirken können. *In vitro* Experimente konnten bei Berücksichtigung aller in Frage kommenden Faktoren den Beweis einer Entkalkung von Zahnschmelz und Dentin erbringen, die morphologisch von der natürlich vorkommenden Karies nicht zu unterscheiden war. Diese Ergebnisse scheinen endgültig bewiesen zu haben, daß die Theorie von MILLER im wesentlichen korrekt ist.

INTRODUCTION

IN THE literature on dental caries indirect evidence of the role of fermentation acids as aetiologic agents is abundant. On the other hand direct proof of their action is entirely lacking, as all *in vitro* experiments, using acids as decalcifying agents, result in etching and cavitation of the tooth surface in a way that is basically different from the clinical aspect and course of dental caries. This study was undertaken to investigate the possible role of membrane phenomena of the DONNAN type, resulting in an acid reaction within the tooth substance and thus causing a decalcification that might resemble dental caries more closely.

According to the views of MILLER a number of organic acids are formed by micro-organisms that constitute and infest the bacterial plaque and the adhering food rests at the well-known sites of carious attack. These acids are the product of carbohydrate fermentation and when present in sufficient concentration may attack the enamel surface, causing the chalky white appearance of the area involved and all other histologic changes that are too familiar to be enumerated here *in extenso*. The main characteristic of these changes is the loss of calcium salts. When the carious attack, *in casu* the opaque area of the enamel, reaches the dentino-enamel junction, the dentine in turn is subject to decalcification which extends in all directions more or less equally, thereby undermining the overlying sound enamel. The dentinal tubules are in most cases found to be infected with micro-organisms, and it is commonly believed that these are responsible for the decalcification of the dentine, as they too are supposed to produce acids from fermentable carbohydrates that allegedly reach them from the oral cavity.

All clinical evidence obtained by MILLER and numerous other workers point to the correctness of these views, insofar as the forming and accumulation of acids is undoubtedly a causative factor in the initiation and the progress of dental caries. At the same time the exact nature of the decalcification mechanism is a much disputed issue, mostly because of the following facts:

1. Time and again it has proved impossible to imitate the carious lesion *in vitro* by the action of acids on sound enamel. Whether weak or strong, organic or mineral, diluted or concentrated, buffered or unbuffered, acids do not cause the same changes as are seen in dental caries. In cases of initial caries the enamel shows a partial dissolution of the apatite underneath a superficial layer of more or less intact enamel, while the histologic structure of the opaque area remains intact to a certain extent (Fig. 1). In experiments with acids however it is commonly found that the hard superficial layer of enamel dissolves first, followed by a progressive softening and total destruction of the enamel, the remains of which are easily washed away by a jet of water (Fig. 2).

2. Even in more advanced cases of carious decalcification, the so called white spot may still show an intact enamel surface. Nevertheless a longitudinal section through this area often discloses extensive decalcification and discoloration of the

underlying dentine with the formation of a dead tract and infection of the tubules (Fig. 3). In these cases, the carious enamel, though opaque and crumbly to the explorer, has retained its anatomical outline, its surface gloss and a considerable

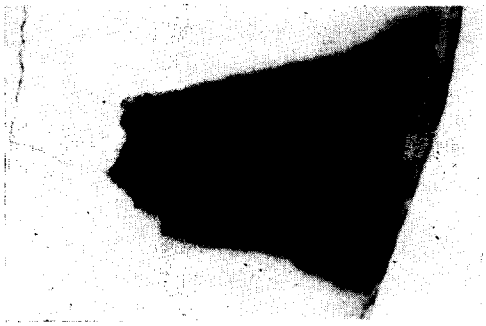

FIG. 1. Ground section of human premolar. Initial enamel caries. Transmitted light. × 60.

density in regard to its permeability. To many workers it has therefore been difficult to accept the idea of soluble carbohydrate passing first the bacterial plaque and then diffusing through the opaque enamel in sufficient quantities to enable the micro-organisms in the tubules to decalcify the dentine.

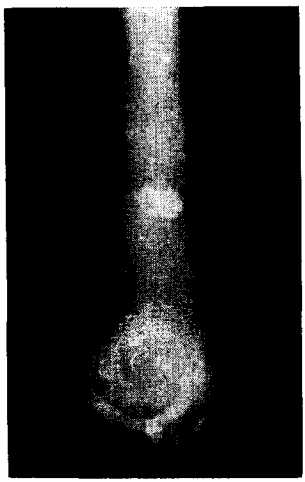

FIG. 2. Human upper lateral. Enamel etched with lactic acid on the labial surface. Incident light, × 8.

3. Experiments *in vitro* with the aim of sustaining the bacteria in the tubules by a diffusion of carbohydrate through opaque enamel have never succeeded.

4. A penetration of free acid from the bacterial plaque is also very improbable, as it is hard to understand how the dentine could be decalcified completely by acids passing first through only partly decalcified enamel.

5. In sections of carious teeth, the decalcification zone in the dentine always shows a clearly defined curved border, the softened dentine occupying a more or less spherical "cavity". This regular cavity form and the distinct borderline, so

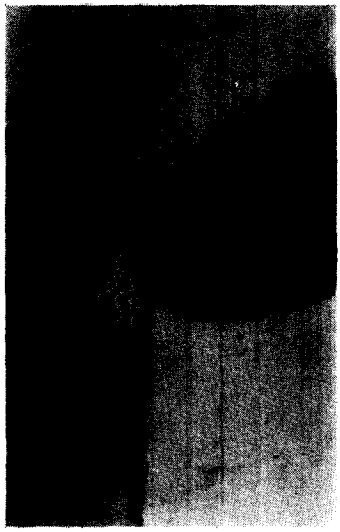

FIG. 3. Ground section of human premolar. Caries of enamel and dentine. Transmitted light, × 40.

FIG. 4. Paraffin section of human premolar. Gram stain. Dentine caries. Only a few tubules contain micro-organisms. The decalcified area is sharply defined and distinctly spherical. Transmitted light, × 60.

well known from röntgenograms, are explained very unsatisfactory in most text-books by correlating them with the progress of the micro-organisms in the tubules. Moreover, in many cases the decalcified dentine is only partially infected or even completely sterile, as was already discovered and described by MILLER (Fig. 4).

These facts and other considerations based on clinical experience have caused many research workers to drop MILLER's views altogether, and to search for another explanation of the carious process. The results were invariably of doubtful value, as the importance of acid production cannot be minimized without giving rise to new and still more puzzling problems. At present the literature on dental caries abounds with aetiologic factors, most of which are purely fantastic.

A thorough survey and analysis of all information has convinced the author that the acids formed in the plaque are an essential factor in dental caries but that they act indirectly, setting off some independent physico-chemical process that causes decalcification by lowering the pH *within* the tooth structures. A process of this kind has been described by DONNAN and coworkers in 1911. For a complete description of his work we must refer the reader to the literature. An outline of the principles involved is taken from TENDELOO (1952).

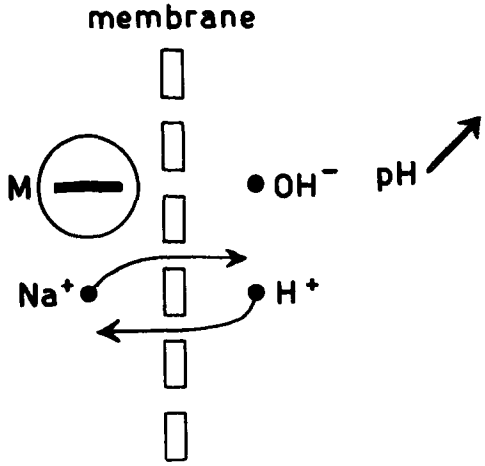

FIG. 5.

DONNAN studied the ionic concentrations in solutions separated by a semi-permeable membrane. When one of the solutions contains an ion large enough to be arrested by the membrane, interesting diffusion phenomena occur. For our purpose, the most important of these is the presence of large non-diffusing ions on one side of the membrane and their influence on the dissociation of pure water in the opposite compartment and on the distribution of its ions.

Let us consider a substance Na^+M^- dissolved and completely dissociated on the left side of the membrane. Under conditions as pictured in Fig. 5 the Na^+ ions can diffuse through the membrane to the right side, but they will only do so if the M^- ions go with them (which is impossible) or if an H^+ ion passes to the left in exchange for each Na^+ ion. DONNAN showed that this exchange actually takes place until a state of equilibrium is reached, which of course is influenced by the

concentration of Na^+ M^-. The right compartment then contains OH^- ions in excess of the remaining H^+ ions, in other words, its reaction has become 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 (Fig. 6), the substance E^+ Cl^- providing the non-diffusing ion E^+, an exchange 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 pH as low as that of gastric juice may be attained. It must be kept in mind, that the low pH reading on the right side does not necessarily indicate the presence of large quantities of HCl. When however any of the ions concerned are continually removed as a result of some secondary chemical reaction in either

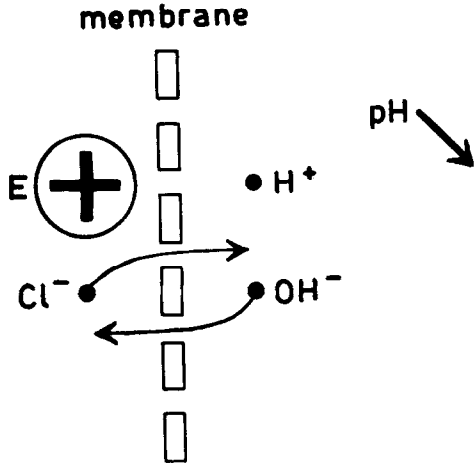

FIG. 6.

compartment, the transport of OH^- and Cl^- ions through the membrane will also be continuous. The possibilities outlined above will serve to show that under appropriate conditions ionic distribution causing a pronounced change of pH is possible and predictable as the result of membrane equilibria of the type described by DONNAN. In a number of experiments we have applied this theory to the problem of carious decalcification.

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 is quite impenetrable by macromolecules such as proteins. We may therefore consider the enamel surface as a membrane or barrier in the sense of

DONNAN's theory, and at the same time its mass as one of the "compartments", the other being the oral cavity. The fluid bathing the tooth surface may be a film of salivary mucin *or* the gelatinous contents of a bacterial plaque.

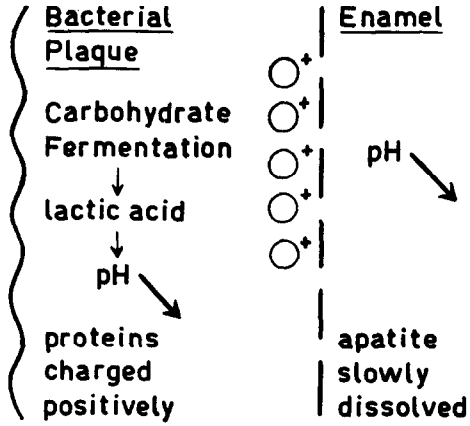

FIG. 7.

In the immediate proximity of the enamel surface we may assume the presence in solution of positively charged ions, too large to diffuse into the enamel (Fig. 7). A DONNAN ionic distribution will take place, resulting in a drop of the pH within

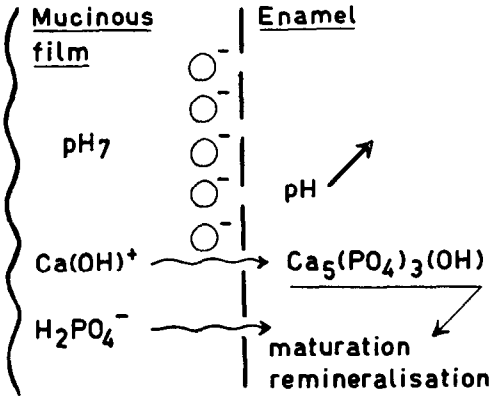

FIG. 8.

the enamel. The latter will "excrete" OH^- ions in exchange for any small anions present in the surrounding fluid. As an increasing excess of H^+ -ions develops within the enamel, we may suppose that the latter will be decalcified under conditions that are exactly opposite to decalcification by immersion of a tooth in an acid.

It has been shown that this particular kind of decalcification is actually responsible for the development *in vitro* of the characteristic "white spots" of initial enamel caries, and, in later stages, for the decalcification of the dentine as well. As the excess of H^+ ions will take part and be used in the ensuing process of decalcification the ion exchange will continue as long as positively charged ions are present on the enamel surface, or in other words, as long as predominantly positively charged macromolecules are present in true or colloidal solution.

The presence of negatively charged ions must give rise to the reverse situation, causing the pH in the enamel to rise slightly and thus exerting a stabilizing influence on the apatite structure (Fig. 8). Our experiments indicate that this situation actually occurs, the results pointing to salivary mucin as the active agent in enamel maturation and remineralization.

Perhaps the most important fact, that can be easily deduced from Fig. 5–8, is that the ionized macromolecules are not used up in any way. They need not even exist freely in a molecular solution, but can exert their influence as well or even better when forming a hydrated gel on the enamel surface (BOOY, 1956).

EXPERIMENTAL METHODS

All experiments were conducted at room temperature on extracted sound human teeth, except in those cases where carious teeth were selected for comparative purposes. In most cases the teeth used were upper and lower premolars that had been kept for some time, varying from a few days to several weeks, in 4% formol; the results did not differ in any way from those obtained with freshly extracted specimens. The teeth were cleaned with curettes, pumice and chalk, dried superficially and covered with paraffin by immersing them in a mixture of 90 parts paraffin of 60°C melting point and 10 parts beeswax, heated slightly above its melting point. The beeswax was added to render the paraffin less brittle. Windows were punched in the paraffin layer in order to expose one or more round areas of enamel to the influence of different solutions. After varying exposure times the teeth were cleaned and ground sections or paraffin sections were prepared through the centre of the former exposure window and through the tooth's axis. Three series of experiments were conducted.

In the first series the influence of acids was studied as to concentration and pH of different organic and mineral acids. In some cases the effect of different buffer mixtures was examined, because in the opinion of some authors the buffering action of the plaque constituents may be held responsible for the typical aspect of carious decalcification. The results of this series are compiled in Table 1.

All experiments with mineral acids (hydrochloric, sulphuric, and nitric acid) have been omitted, because these acids cause a rapid destruction of the ename that does not bear the slightest resemblance to caries. Buffer formulae were taken from LEHMANN (1928).

In a second series of experiments the effect of solutions of positively charged ionized macromolecules was studied. 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

TABLE 1. SITUATION AFTER 30 DAYS

	pH Values								
	7	6·5	6	5·5	5	4·5	4	3·5	3
Aq. dist. + lactic acid	—	—	—	Ag	Ag	Ag	Bd	Cd	Cd
Buffer solution "Soloid" ph 7 + lactic acid	—	—	Ag	Ag	Bd	Cd	Cd	E	E
Buffer solution "Soloid" pH 4 + NaOH or lactic acid	—	—	Cg	Cd	Cd	Cd	Cd–D	E	E
Buffer solution Prim. -Sec. phosphate ("SÖRENSEN")	—	—	—	—	Ag				
SÖRENSEN buffer solution pH 7 + lactic acid	—	—	—	—	Bg	Bg	D	E	E
Buffer solution Sec. phosphatecitric acid (LEHMANN, p. 55)	—	—	—	Cd–D	D	D	D	E	E
Buffer solution K. monocitrate-borax (LEHMANN, p. 52)	—	Bg	Bd-D	Bd–D	D–E	E	E	Cd	
Buffer solution K. monocitrate-borax (LEHMANN, p. 52) + lactic acid	Bg	Bd	D	D	E	E	E	E	E

Index to symbols: A = very slight white spot, g = glossy surface, d = dull surface,
B = slight white spot,
C = white spot,
D = etched-out surface,
E = surface etched away, enamel changed into soft pappy mass,
F = surface layer intact, underlying enamel soft and pappy.

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. After 2 weeks the enamel 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.

Further experiments were conducted with gelatin and gliadin solutions. Because of their typical protein structure they possess both basic and acid groups in watery solution, (mainly $—NH_3(OH)$ and —COOH). The H^+ 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. Addition of acid to the solution of isoelectric gelatin will slowly lower its pH, the gelatin forming a salt while behaving as a base (Fig. 9). The H^+ ions from the acid will

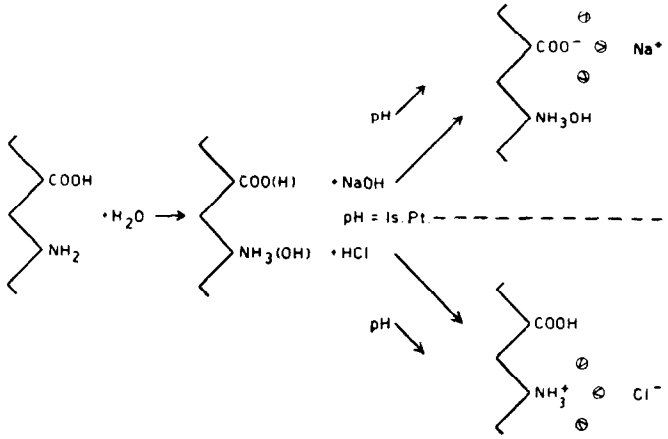

FIG. 9.

disappear as such, the pH falling only because of hydrolysis, until all positive valencies have been saturated with anions. The salt formed will be able to cause a DONNAN distribution of H^+ and OH^- ions as shown in Fig. 7, because the ionized protein has a positive charge. After addition of still more acid the solution will contain gelatin chloride and free acid.

Addition of base to the solution of isoelectric gelatin will cause a slow rise of pH, the protein now forming an anion and behaving as an acid. The same speculations apply to gliadin, a vegetable protein that has its isoelectric point at pH 6·8. Addition of lactic acid caused the formation of a precipitate; the sticky mass obtained after decanting the clear fluid was used in the same way as the acidified gelatin. A series of sterile containers were filled with solutions with different pH values, and a number of teeth, prepared as previously described, were immersed in each container so that the uncovered parts of the tooth surface were in direct contact with the solution. For all solutions distilled water was used with a little thymol added to prevent the growth of molds. A PYE pH meter was used for determining and controlling all pH values. After a certain length of time the teeth were cleaned and ground sections of about 100 μ prepared. Tables 2–4 represent some of the most important experiments.

TABLE 2

	pH Values										
	7	6·5	6	5·5	5	4·7	4·5	4	3·5	3	2
Gelatin 10%, NaOH or lactic acid 30%, 8 days	—	—	—	—	—	—	Bg	Cg	Cg	Cg	—
Gelatin 10%, NaOH or lactic acid 30%, 14 days	—	—	—	—	—	—	Ag	Bg	Cg	E	—
Gelatin 10%, lactic acid 30%, 21 days	—	—	—	—	—	Ag	Cg	Cg	Cg	F	E

For explanation of symbols see Table 1.

TABLE 3

	pH Values						
	7	6·8	6·5	6	5·5	5	4·5
Gliadin 10% NaOH or lactic acid 30% 30 days	—	—	—	Ag	Ag	Cd	Cd

For explanation of symbols see Table 1.

TABLE 4

No. days	Mean depth of white spot (mm)	No. days	Mean depth of white spot (mm)
5	0·08	75	0·47
10	0·12	80	0·51
15	0·15	85	0·54
20	0·18	90	0·60
25	0·21	95	0·67
30	0·26	100	0·74
35	0·28	105	0·76
40	0·33	110	0·81 DE
45	0·39	115	0·83
50	0·40	120	0·87 DE
55	0·41	125	0·99 DE
60	0·42	130	1·10 DE
65	0·42	135	1·01 DE
70	0·44	140	1·14 DE

The markings DE denote that in one or more of the specimens concerned the dentine-enamel junction was reached.

Gelatin 20% brought to pH=4 with 30% lactic.

In a third series of experiments the DONNAN phenomena caused by salivary mucin were studied. At the oral pH of about 7, mucin is negatively charged and probably present as its K^+ salt (RADSMA, 1954). As mucin has little or no basic groups, addition of only a little acetic acid to saliva will repress the dissociation of the carboxyl groups, the neutralized substance falling out from solution as a viscous whitish mass, which is readily soluble again in very diluted alkali. This procedure enabled us to obtain pure solutions of mucin of known concentration. Attempts to acidify these solutions so as to obtain positively charged mucin particles were of course unsuccessful. The mucin precipitated immediately, redissolving only after addition of an excess of mineral acid. The resulting solutions had the same effect on enamel as if they had contained acid only. From these results it may be concluded that strictly speaking mucin is not amphoteric like most other proteins. At its normal pH it is strongly negative, but a slight drop of the pH readily converts it to a neutral, unionized and therefore insoluble precipitate, that has no further part in any accompanying reaction. These findings agree with the work of OLDFELDT (1936) who found strongly acid properties in the case of salivary mucin (submaxillaris) with an isoelectric point as low as 2·5, which is in good agreement with the value of about 2·7 found by other workers. The isoionic point was somewhere at pH 3·4–3·45. A very characteristic finding for mucin was its very slight acid-binding capacity as compared to its capacity to bind base (1 : 5·3).

RESULTS

All teeth from the first series of experiments (Table 1), in which acids were used to attack the exposed tooth surface, showed a more or less pronounced etching and dissolution of the enamel after a varying length of time. Only in cases of very slight and superficial attack did the surface remain glossy during the first few weeks, but soon it became as dull as was the case with the stronger solutions. In all series sooner or later the enamel lesions passed through the same stages as described at the foot of the table. In the first experiment the unbuffered solutions of course required only very small additions of acid to obtain and maintain pH values of 6·5–4·5. Therefore only the solutions from pH 4 downward had sufficient strength to attack the enamel surface with visible results. The buffers chosen were all composed of substances of low molecular weight, and presumably unable to cause a DONNAN effect. In the buffered solutions the attack started immediately from pH 6 downward. Otherwise there was no difference from the unbuffered solutions. This proves that the buffering in itself has no effect on the course of acid decalcification, insofar that no better resemblance to carious decalcification could be obtained than with acids alone. A striking exception was found with the SÖRENSEN phosphate buffers, where no lesion occurred at pH 5·5 and over. It would seem that the presence of phosphate ions lowers the critical pH where attack may start. As for the remaining

pH values, once attacked the decalcifications showed the same characteristics as in the other specimens.

In the second series the results of the experiments with gelatin and gliadin confirmed our speculations regarding the posibility of a decalcification of the tooth substance by way of DONNAN membrane phenomena, as could already be expected from the aforementioned preliminary set-up with quinine hydrochloric.

Results are presented in Tables 2–4. Fig. 9 shows the general principles of these experiments. Part of a protein chain bearing an acid and a basic group is represented diagrammatically by the angular line. Depending on the presence and relative number of positive and negative groups per molecule a given protein has a specific isoelectric point. The watery solution of this protein therefore has a pH that corresponds with the specific isoelectric point of the protein. Addition of a base (e.g. NaOH) will result in the formation of the proteinate, the Na^+ ions remaining in solution, and the protein molecules bearing a number of negative charges at appropriate sites of the side groups. The free base disappears as such and is said to be buffered; it should be kept in mind that the process is in fact a salt being formed, and that it is not buffering in the strict sense. The pH rises because of hydrolysis, and the ionized protein obtains a water mantle, causing the solubility to increase and the solution to become more viscous. Addition of acid has the opposite effect as to the charge obtained, the protein now being charged positively at the sites of its aminogroups. The acid is "buffered", the pH slowly drops below the isoelectric point, and protein chloride, lactate, or another compound is formed according to the acid used. Again a water mantle forms around the ionized protein molecule, accompanied by increased solubility and viscosity.

When all negative groups have been saturated with base, or all positive groups with acid, the buffer capacity of the protein will be exhausted. If in the last mentioned case more acid is added, this will be present as free acid and cause the same lesions as in the first series of our experiments. In the case of salivary mucin however, which abounds with acid (—COOH) groups, basic groups lacking almost entirely, it is of course impossible to obtain a salt with an acid, except by a crude treatment with excess of mineral acid.

In the experiments now under consideration we used solutions of gelatin and gliadin. As has been explained above both substances can be given a positive or negative charge at will; the only difference is in their isoelectric points, which are at pH 4·7 and 6·8 respectively. The acid added was buffered, the important difference with the first series of experiments being that the buffering or salt-forming substance was non-diffusable. On the strength of this fact we could assume that a DONNAN effect would ensue. A positively charged gelatin or gliadin would tend to cause a drop of the pH within the enamel, while a negative charge will result in a basic reaction of the free water in the enamel structure.

Some experiments are summarized in Tables 2, 3, and 4, which only mention the macroscopic appearance of the enamel surface. Not included are the microscopic findings from the corresponding ground sections which will be dealt with separately. The most important conclusions that can already be drawn from these tables are the following. In conformity with our expectations, at all pH values above the isoelectric points (irrespective of their absolute numerical value) the teeth remained undamaged, even if these values were under pH 7. In our opinion this situation exists normally in the mouth, where the tooth surfaces are in contact with saliva, the mucinous film derived from this fluid being strongly negative at the normal oral pH.

Below the isoelectric point the macromolecules change their overall charge from predominantly negative to positive and the enamel is subjected to internal decalcification. As will be shown, this decalcification is identical to caries as far as the loss of calcium salts are concerned, both from the enamel and the dentine. As can be deduced from Tables 2 and 4 the best and quickest results are obtained when the gelatin concentration is high, and the pH is only slightly under its isoelectric point of 4·7. The drop of pH in the enamel depends on the gelatin concentration according to

$$[H^+] = \sqrt[3]{(K_w \cdot C_{\text{gelatin}})}$$

as computed by DONNAN, which explains the necessity of a high gelatin concentration. The acid-binding capacity of the concentrated gelatin gel should not be exceeded, solutions of pH 3·5 already showing marks of direct acid action. The most suitable pH at room temperature proved to be pH 4 or slightly over.

The histological aspects of the teeth decalcified under these optimal conditions are the following:

1. The exposed surfaces show a white discoloration which starts after a few days, sometimes even after a few hours.

2. The white spot keeps all surface characteristics of sound enamel, as could be affirmed by incident light microscopy and a replica technique described by PANTKE (1956).

3. In longitudinal and transverse ground sections the lesions show the same alternations as can be observed in dental caries, of course with the exception of infection, pigmentation and proteolysis. We studied these with incident and transmitted light, darkfield illumination, phase contrast and polarization microscopy (Fig. 10a, b, c).

4. When the lesion reaches the dentino-enamel junction, the dentine is decalcified in the form of a slowly widening spherical area, thereby progressively undermining the enamel (Fig. 11).

5. In most cases two transparent bands can be observed to extend from the decalcified zone to the pulp chamber, following the course of the dentinal tubules.

As in actual caries, these bands—in reality a tube of "sclerosed" dentine—enclose the "dead tract" described by FISH (Fig. 12). The cause of this transparency could not be ascertained with certainty, but it seemed to be part of the total picture, a "defence mechanism" of course being excluded absolutely.

FIG. 10 (a). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 360.

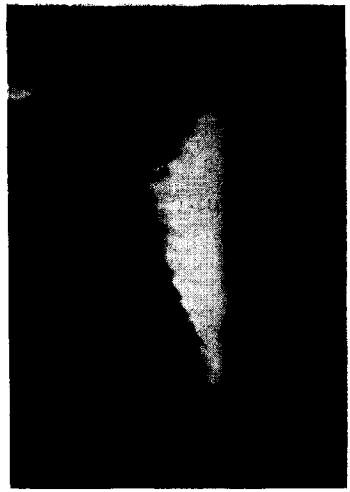

FIG. 10 (b). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Incident light, × 15.

6. In some cases the undermined enamel shows a retrograde decalcification (Fig. 13).

7. The border between totally decalcified and undecalcified dentine is rather abrupt and sharply defined (Fig. 11 and 13). When, however, calcium monophosphate is added from the start to the gelatin in excess, both areas blend evently into

FIG. 10 (c). Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Polarized light, total extinction, × 50.

FIG. 11. Ground section of human premolar. Artificial enamel caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 30.

one another (Fig. 14). As in the first series of experiments, addition of phosphates strongly retarded decalcification in those experiments where the time factor was varied, and it lowered the critical pH in those series, where a descending scale of

pH values was prepared. As yet we are unable to offer an explanation of this phenomenon.

At the present time our observations on the activity of the gliadin solutions are limited to those given in Table 3, the development of lesions with involvement of

FIG. 12. Ground surface of human premolar. Artificial caries. Gelatin 20% with lactic acid 30%, pH = 3·5. Decalcified dentine stained with bromthymol blue, added to the mixture from the start. Incident light, × 10.

the dentine as shown in Fig. 12 taking from 1 to 2 years. However, from the results obtained already with gelatin as outlined above, confirmed by comparative studies with different microscopic techniques mentioned before, we have come to the

FIG. 13. Ground section of human premolar. Artificial caries Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 100.

conclusion that the presence of positively charged ionized macromolecules in solution, or as a hydrated gel on the enamel surface, is sufficient but necessary to create a DONNAN membrane effect, thereby decalcifying the enamel and successively the dentine in a way that bears the closest resemblance to actual caries.

In a recent experiment the effect was studied of adding a neutral salt to a standard decalcifying mixture of 25% gelatin brought to pH 4 with lactic acid. It was assumed that this addition of NaCl, in itself a harmless substance, would intensify the decalcification, as it could be expected that the Cl^- ion would diffuse better into sound enamel, being smaller and having a greater mobility and activity than the lactic acid ion, apart from its probably greater dissociation. As expected, the teeth from mixtures containing 1 or 2% NaCl showed a nearly doubled decalcification intensity as measured in depth of the white spot, and as compared to the controls without NaCl.

FIG. 14. Ground section of human premolar. Artificial caries. Gelatin 20% with lactic acid 30%, pH = 4. Transmitted light, × 20.

With greater amounts of NaCl the effect disappeared again, probably because the dissociation of the gelatin was suppressed by the higher NaCl concentrations. Perhaps the same situation exists in the bacterial plaque in the human mouth, where a definite concentration of Cl^- ions may be an important factor in the very first stages of enamel attack rather than the actual presence of lactic acid ions, taking into consideration the very narrow "spaces" that are present in normal enamel, and seem to remain so in the superficial layer during a considerable time before surface etching and cavitation occur.

In the third series of experiments the influence of negatively charged macromolecules was tested. Neither gliadin nor gelatin solutions at pH values above their isoelectric points proved to cause ill effects on the enamel, even if the actual pH readings were under 7. In the opinion of the author this is a result of the pH shift caused by the presence on the tooth surface of negatively charged particles, as

already represented in Fig. 5. Any small ions present in the fluid, when diffusing into the enamel, will find an alkaline reaction there. The saliva being saturated with calcium monophosphate at normal oral temperature and pH, the salt will precipitate after diffusing into the enamel, and the precipitate must pass via secondary phosphate to tertiary phosphate in the form of hydroxyapatite. Probably this is what happens in the mouth when a tooth surface attacked previously by initial caries is exposed to the cleansing action of mastication and to salivary flow, as is so often the case when a neighbouring tooth is removed. Evidently a DONNAN membrane effect of this kind may also form the basis of normal tooth mineralization and enamel maturation (Fig. 8).

DISCUSSION

In the opinion of the author the results described, when applied to the problem of dental caries provide a clear insight in the mechanism of carious decalcification. Initial caries of enamel, the so-called white spot, is the result of a ionic distribution of the DONNAN type, dependent on the presence and concentration on the tooth surface of positively charged ionized macromolecules, namely the proteins and their derivatives in the bacterial plaque, rather than the presence of free acid. Important factors are acid production rate, average isoelectric point and concentration of the proteins present, and the rate of clearance of all compounds concerned. The lactic acid formed in the plaque lowers the pH on the enamel surface only slightly, at the same time reacting with the proteins present from food debris, etc. If the pH drops sufficiently to cause a predominantly positive ionization of the soluble proteins present, a white spot will form, followed by the well-known other phenomena of carious decalcification.

The same DONNAN membrane laws serve to explain the dentine decalcification. As was proved by our experiments the dentine can be decalcified completely by a DONNAN effect. This proves that in actual caries the presence of micro-organisms in the tubuli is only secondary, and their activity is directed mainly to a proteolytic destruction of the remaining dentine matrix and TOMES' fibres.

When the tooth surface is not covered by a bacterial plaque, the situation and the ensuing DONNAN effect are exactly the opposite of the foregoing. A thin film of mucin is always present on the tooth surfaces that are freely exposed to the saliva. This film adheres rather strongly to the tooth, and when removed is quickly formed again. At the normal salivary pH of about 7 this substance is very strongly ionized, bearing negative charges and thus exerting a favourable influence on the state of mineralization of the hard tissues.

Experiments to establish the degree of ionization at pH 7 of individual samples of human salivary mucin are under way. Obviously at this pH a given mucin may carry a greater number of negative charges per molecule than some other sample.

Comparative measurements of this relative particle charge may prove to show better correlation with caries activity than has been obtained until now by simple pH measurements of whole saliva.

REFERENCES

BARTHELD, F. VON 1958. Decalcification in initial dental caries, *Tijdschr. Tandheelk.* **65**, 76.
BOOY, H. L. 1956. *Inleiding tot de Physische Biochemie.* (ed. 1). Haarlem, Boon.
DONNAN, F. G. 1925. *Chem. Rev.* **1**, 73.
DONNAN, F. G. 1932. *Kolloid Z.* **61**, 160.
DONNAN, F. G. 1934. *Z. phys. Chem.* A **168**, 369.
DONNAN, F. G. 1939. *J. Chem. Soc., Lond.* (1939), 707.
LEHMANN, G. 1928. *Die Wasserstoffionenmessung.* (ed. 1). Leipzig, Barth.
PANTKE, H. 1956. Die Replica-Technik. *Stoma* **2**.
RADSMA, W. 1954. *Inleiding tot de Physiologische Chemie* (ed. 1). Groningen, Wolters.
TENDELOO, H. J. C. 1952. *Kolloidchemie* (ed. 3). Gorinchem, Noorduijn.