フッ化物摂取の目安量の基準

齲蝕の疫学調査によりう蝕(むし歯)罹患率を有意に減少させる摂取量

0.02 ～ 0.05 mg/kg

最大値 0.05 mg/kg

文 献:
McClure F.J., 1943. ; Ophaug R.H. et al., 1980
Nohno K.et al.,2005(Japan); Tomori T.et al.,2002 (Japan)
Ophaug R.H., 1980.; Dabeka R.W., 1982.
Featherstone J.D.B., 1988.

年齢群別のフッ化物の食事摂取基準(目安量)

0.05mg/kg に日本人の標準体重を乗じて年齢群別のフッ化物摂取基準を計算した。

日本人のフッ化物摂取基準案

注1)年齢層の区分は日本人の食事摂取基準(2005年版)に依拠している
注2)母乳栄養児は母乳中フッ化物濃度が0.01ppm(中央値)であり、摂取量1000mlとして算出した

フッ化物の上限摂取基準の設定

上限量(UL)の基準は、LOAEL値を参照

Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine : Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride, pp.288- 313, National Academy Press, Washington, D.C.,1997.

フッ化物の歯の審美的副作用である「歯のフッ素症」の症度MO(Deanの分類の modelate)の発現頻度が飲料水中フッ化物濃度 2 ppm未満の場合では5%未満であるという疫学的事実に基づいている。

Dean H.D.: The investigation of physiological effects by the epidemiological method, Fluorine and dental health, pp.23- 31, American Association for the Advancement of Science, Washington, D.C., 1942.

A: 最大一日フッ化物摂取量

1)飲料水中フッ化物濃度の最大値を2 ppmとし、一日飲水量を1.5Lとする。

①飲料水からのフッ化物量
2 mg/L×1.5 L=3 mg/day

②食事からのフッ化物摂取量
0.25～0.3 mg/day

③フッ化物飲料水で調理した食事中フッ化物摂取量:
0.3×2=0.6 mg/day

①+③最大一日フッ化物摂取量=3+0.6=3.6mg/day

B: 最小一日フッ化物摂取量

1)飲料水中フッ化物濃度の最大値を2 ppmとし、一日飲水量を1.0Lとする。

①飲料水からのフッ化物量:
2 mg/L×1.0 L=2 mg/day

②食事からのフッ化物摂取量:
0.25～0.3 mg/day

③フッ化物飲料水で調理した食事中フッ化物摂取量:
0.25×2=0.5 mg/day

①+③最小一日フッ化物摂取量:
2+0.5=2.5mg/day

フッ化物摂取上限量の計算

- 最小値：2.5/30=0.083 mg/kg/day
- 最大値：3.6/30=0.12 mg/kg/day

$$\text{上限量} = \frac{0.083+0.12}{2} = 0.10 \text{ mg/kg}$$

8歳児がなぜ重要なのか

- 永久歯の発達成長期は、8歳までで決まることが、病理学的に明らかにされている。

Fejerskov O., Thylstrup A., Larsen M.J.: Clinical and structural features and possible pathogenic mechanisms of dental fluorosis. Scand J Dent Res 85; 579- 587, 1977.

- フッ化物の全身的応用としての永久歯へのう蝕予防効果の臨界年齢は、8歳までの摂取量によって決まる。
- 歯の審美的副作用である「歯のフッ素症」の発現も、この年齢までに摂取されたフッ化物摂取量によって決定する。

日本人のフッ化物摂取基準案

注1）年齢層の区分は日本人の食事摂取基準（2005年版）に依拠している
注2）母乳栄養児は母乳中フッ化物濃度が0.01ppm（中央値）であり、摂取量1000mlとして算出した

10歳以上の上限量はどのように決めるか

- 成人の体重を60kgとする。
- 体重あたりの上限摂取量=0.1 mg/kg

10歳以上の上限量＝0.1 mg/kg×60kg
＝6 mg

- 男女の区別はしない

妊婦と授乳婦のフッ化物摂取量

妊婦/授乳婦	目安量(mg)	上限量(mg)
妊婦	2.5	6.0
授乳婦	2.5	6.0

母乳にはフッ化物は移行しない事実

- Ekstrand J., Boreus L.O., de Chateau P.: No evidence of transfer of fluoride from plasma to breast milk, Br Med J 283;761- 762, 1981.
- Ekstrand J., Spak C.J., Falch J., Afseth J., Ulvestad H.: Distribution of fluoride to human breast milk following intake of high doses of fluoride, Caries Res 18;93- 95, 1984.

胎児への移行も制限されるという事実

- Gupta S., Seth A.K., Gupta A, Gavane A.G.: Transplacental passage of fluorides, J Pediatr 123;139- 141, 1993.
- Leverett D.H., Adair S.M., Vaugham B.W., Proskin H.M., Moss M.E.: Randomized clinical trial of the effect of prenatal fluoride supplements in preventing dental caries, Caries Res 31;174- 179,1997.

15－29歳の目安量と上限量と同じ値に設定

ライフステージにおけるフッ化物摂取基準案

妊婦／授乳婦のフッ化物摂取基準

妊婦/授乳婦	目安量(mg)	上限量(mg)
妊婦	2.5	6.0
授乳婦	2.5	6.0

フッ化物食事摂取基準案経緯の現況

- 。フッ化物食事摂取基準案の口腔衛生学会での承認支援（平成19年5月1日）
- フッ化物食事摂取基準案の日本歯科医学会の推奨（平成19年4月8日）
- 平成20年11月18日厚生労働省生活習慣病対策室第8次食事摂取基準策定のヒアリング（平成20年11月18日）
- 平成20年度厚生労働科学研究班の研究成果発表会（平成21年2月10日）

今後の研究課題

- 現在における食品中フッ化物濃度の継続的分析
- 上限量の意味するところの検討
- 妊婦におけるフッ化物胎盤透過性の薬理動態学的検証を最新の方法で再検討する。
- 歯のフッ素症の分子生物学的手法によるメカニズムの研究

日本人のフッ化物摂取基準が設定された場合の健康政策への波及効果

- 国民全体のう蝕罹患リスクは低減し、う蝕罹患率は減少する。
- う蝕が原因による抜歯の数が減少するだろう。
- 年齢に応じた現在歯数は増加傾向を示すだろう。
- 20歳から60歳までの現在歯の健康維持のための口腔ケア（疾病予防）のニーズが増加していくことが予想される。

日本人のフッ化物摂取基準に関わった研究者

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高江洲義矩（東京歯科大学）

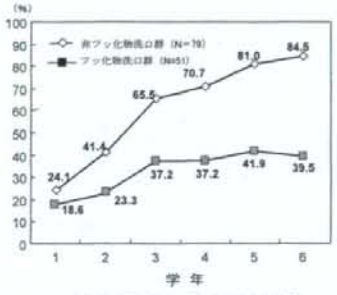
フッ化物洗口事業の普及率と歯科医療費

古賀　寛、眞木吉信

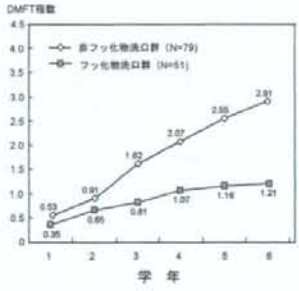
東京歯科大学衛生学講座

地方自治体のフッ化物洗口事業実施

- 地域：千葉県房総地区、人口約7000人
- 対象：保育園児、幼稚園児および学童
- フッ化物洗口剤：週1回法（900ppmF）
- 実施期間：1997年～現在まで



フッ化物洗口群と非洗口群における永久歯齲蝕有病者率の経年的推移



フッ化物洗口群と非洗口群におけるDMFT指数の経年的推移

フッ化物洗口事業による歯科医療費への効果 歯科医療費分析方法

対象者：国民健康保険加入者（0－15歳、6－11歳）
調査票：社会保険庁（天津小湊町）の歯科レセプト
　　歯科レセプトの期間：1997年～2001年度
調査方法：レセプトの総点数(1点10円）を記載
記載項目：年度別、年齢別、月別、
　　歯科受診保険点数の総計、受診回数
歯科受診：齲蝕（乳歯齲蝕、永久歯齲蝕）、他の疾患

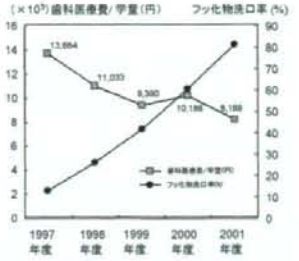
学童の国民健康保険加入率

年度	6～11歳児の人数	国保加入者数	国保加入率
1997年	525	167	31.8
1998年	502	161	32.1
1999年	454	140	30.8
2000年	393	133	33.8
2001年	368	117	31.8
2002年	348	105	30.2

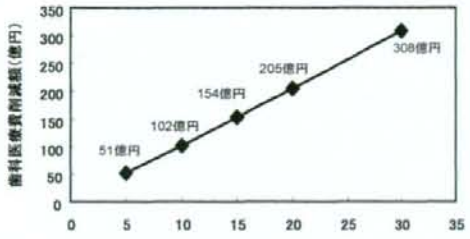
資料：天津小湊町役場

国保加入者(学童)の歯科医療費

項目・年度(平成)	1997年度	1998年度	1999年度	2000年度	2001年度
6～11歳国保加入者	167	161	140	133	117
総歯科医療費	2,285,250	1,776,340	1,314,580	1,354,680	958,070
歯科医療費/1人	13,684	11,033	9,390	10,186	8,189
6～11歳歯科受診延回数	274	205	181	193	113
1受診当たりの歯科医療費	8340	8665	72629	7019	8478



国民健康保険加入学童1人あたりの
歯科医療費とフッ化物洗口率の経年推移



全国学童のフッ化物洗口実施率と
歯科医療費の削減額推定値
(対照:フッ化物洗口率0%、1061億円の学童の歯科医療費(推定値))

ま と め

- 全国で学童(1-6年生)フッ化物洗口を実施しなかった場合の歯科医療費は1061億円と推定された。
- フッ化物洗口普及率からみた場合、5%では51億円、10%では102億円、15%では154億円、20%で205億円、30%では308億円の歯科医療費が削減できると推定された。
- フッ化物洗口事業の普及率の目標値と歯科医療費の削減額が、粗い推算で提示された。
- さらなる、詳細な推算が必要と考える。

平成 20 年度研究成果一覧

厚生労働科学研究「フッ化物応用による歯科疾患予防プログラムの構築と社会経済的評価に関する総合的研究」（H20－循環器等（歯科）－一般－001）平成 20 年度研究成果一覧

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学術論文等

口腔衛生会誌　J Dent Hlth 58：548-551, 2008

委員会報告

う蝕予防のための日本人におけるフッ化物摂取基準（案）の作成

眞木　吉信[1,2]，荒川　浩久[1]，磯崎　篤則[1]，小林　清吾[1,2]
飯島　洋一[1,2]，田浦　勝彦[1]，古賀　　寛[2]，西牟田　守[2]

口腔衛生会誌 58：548-551, 2008

日本人におけるフッ化物摂取基準案作成にいたる経緯

日本歯科医学会医療問題検討委員会フッ化物検討部会は日本歯科医学会 斎藤　毅会長の要請を受け，平成10年1月22日，第一回の委員会を開催した．以来，平成11年10月8日までに9回の会議を開催し，「フッ化物応用についての総合的な見解」をまとめるために検討を重ねてきた．その結果，平成11年11月1日に答申を行い，国民の口腔保健向上のために，う蝕予防を目的としたフッ化物の応用を推奨するとともに，わが国におけるフッ素の適正摂取量を確定するための研究の推進を奨励することとなった．この答申に基づき，平成12年4月に厚生省（現厚生労働省）は「歯科疾患の予防技術・治療評価に関するフッ化物応用の総合的研究」(略称：フッ化物応用の総合的研究，H 12-医療-003，主任研究者　高江洲義矩，東京歯科大学教授)を発足させることとなった．

厚生労働科学研究「フッ化物応用の総合的研究」班によるフッ化物摂取基準の検討

厚生科学研究「歯科疾患の予防技術・治療評価に関するフッ化物応用の総合的研究」班（主任研究者　高江洲義矩）は，日本歯科医学会の「フッ化物応用についての総合的な見解」(平成11年）の報告を受けて発足し，3年後に，厚生労働科学研究「フッ化物応用による歯科疾患の予防技術評価に関する総合的研究」班（主任研究者高江洲義矩，眞木吉信）に引き継がれた．さらに，3年間の研究として，「フッ化物応用による歯科疾患予防プログラムの構築と社会経済的評価に関する総合的研究」班(主任研究者　眞木吉信）において継続されている．

日本歯科医学会の「フッ化物応用についての総合的な見解」において，フッ化物応用の基礎となるフッ化物摂取基準を確定するための研究を推進することが提唱され，研究班における「フッ化物の栄養所要量と健康」グループに課せられたテーマは，わが国において健康増進のためのフッ化物応用を推奨していくために，乳児から成人，老人にいたる生涯を通したう蝕予防のためのフッ化物摂取の目安量（Adequate Intake；AI）および上限量（Tolerable Upper Limited Intake Level；UL）を策定することにあった．

フッ化物の栄養学的評価は，近年の微量元素の摂取基準がアメリカから発信された栄養摂取概念をもとに展開されており，日本においても第6次栄養摂取基準改定から援用されている．初期の厚生労働科学研究においては，各年齢群別におけるフッ化物摂取量に関する知見を収集するとともに，乳児，幼児および児童のフッ化物摂取に関する調査と実験研究を行った．また，母乳および日本において市販されている主な調製粉乳のフッ化物濃度の分析を通して乳児のフッ化物摂取量を推定した．その後，幼児（3～5歳児）のフッ化物摂取量を陰膳食法による食事調査から求め，さらに浄水場の平均フッ化物濃度も考慮して0.16 ppmF未満の低フッ化物濃度飲料水地区でのフッ化物摂取量を実際に求めた．その結果，平均値0.28 mg/day（1～6歳児），および0.29 mg/day（3～5歳児），フッ化物配合歯磨剤を含めた総フッ化物摂取量でも0.35 mg/day，最大値でアメリカの上限値(UL)を超えることなく，目安量（AI）の2分の1程度であった．

また，わが国におけるフロリデーション（水道水フッ化物添加）を考慮した幼児（3～5歳）のフッ化物摂取量を試算すると，食事からのフッ化物摂取量がアメリカの

[1]日本口腔衛生学会フッ化物応用委員会
[2]厚生労働科学研究「フッ化物応用による歯科疾患予防プログラムの構築と社会経済的評価に関する総合的研究」(H 18-医療-一般-019)

設定の目安量（AI）を満たし，上限量（UL）を超えない摂取量となり，0.8 ppmFの飲料水において平均値0.73 mgと推定され，最大値でも上限量(UL：1.7 mg/day)を超えることなく，3歳児の目安量(AI)程度と評価された．

次に，飲料水フッ化物濃度が異なる2つの地域の小児における食事からのフッ化物摂取量を陰膳食法で検討したところ，飲料水フッ化物濃度0.6 ppm地域の中学生は低濃度（0.1 ppmF以下）地域の生徒と比べう蝕経験歯数が有意に少なく，歯のフッ素症も審美的に問題となるレベルの発現はないことが示された．

食品中フッ化物分析については，まず，普遍的なフッ化物分析法である微量拡散—フッ化物イオン電極による食品中フッ化物分析法の信頼性と妥当性を検証した．この方法では，無機のフッ化物添加回収実験で91％以上の回収率が得られ，数種類の食品を複数の研究機関で比較しても有意な差は認められないので，本法は食品のフッ化物分析法として適切であることが示された．

食品中フッ化物分析値においては，海産物を中心として，魚類32品目(可食部)のフッ化物濃度は，0.02～9.07 μg/g，変動係数0.7～39.4％の範囲であった．そのなかでフッ化物濃度1.0 μg/g以上のものが，9品目あった．さらに，マーケットバスケット方式によって国民栄養調査成績表(平成11年度)の分類に準じた，66品目を分析したフッ化物濃度では，米0.14 μg/g，小麦粉0.03 μg/gと低値を示した．麺類0.14 μg/g，砂糖0.07 μg/g，乳製品0.05 μg/g，魚介類（魚の可食部）0.44 μg/g，で魚介類が最も高い値を示した．肉や豆腐，野菜，果物，ジャガイモはおおむね0.1 μg/g以下の低値を示した．

これまでの日本における飲食物からのフッ化物摂取量の文献をレビューしたところ，飲食物からの1日あたりの総フッ化物摂取量は，成人では0.89～5.4 mg/dayと文献間のバラツキが大きいが，1990年以降の報告では，0.90～1.28 mg/dayであった．また，乳児ではドライミルクと乳児用食品を摂取した場合0.09～0.27 mg/day，幼児では0.23～0.38 mg/dayであった．乳幼児における総摂取量はアメリカの設定基準（Dietary Reference Intakes；DRI）が示したAI（目安量）の約2分の1であった．

以上の研究知見をまとめた結果が表1，2に示した「日本人におけるフッ化物摂取基準（案）」である．

日本口腔衛生学会における「日本人におけるフッ化物摂取基準案」の検討と承認支援

日本口腔衛生学会では平成19年3月1日，厚生労働科学研究「フッ化物応用による歯科疾患予防プログラムの構築と社会経済的評価に関する総合的研究」(H 18-医療-一般-019)，主任研究者　眞木吉信，東京歯科大学教授より，上記案の承認支援の依頼を受けて，フッ化物応用委員会において検討を重ね，その結果を全理事へ諮ったところ，この提案を支援するにいたった．

日本歯科医学会における「日本人におけるフッ化物摂取基準案」の推奨

アメリカやカナダに代表される北米やヨーロッパの先進諸国では，フッ化物が健康の保持，増進のための栄養素として認められ，摂取基準量が策定されている．一方，日本においても，前述したように日本歯科医学会の見解に基づき，フッ化物の摂取基準に関する研究が平成12年から厚生労働科学研究として実施されてきた．その研究成果として表1，2の「日本におけるフッ化物摂取基準(案)」が作成された．この基準案は日本口腔衛生学会においても承認と支援を受けて学術的にも問題のない数字が提示されたと考えられる．日本歯科医学会としても，健康の推進と疾病のリスク低減の観点で，この摂取基準(案)を推奨する立場から，厚生労働省の策定する「2010年版日本人の食事摂取基準」に上記のフッ化物の摂取基準（案）の収載を依頼している．

日本人におけるフッ化物摂取基準（案）

生涯にわたる健康を維持・増進するうえで，フッ化物応用によるう蝕予防は基本的かつ不可欠であり，多くの疫学調査から実証されている[12]．このようなフッ化物の摂取基準は，アメリカでは推定平均必要量（EAR：estimated average requirement）の推定が困難なことから，各年齢層別の一日あたりのフッ化物の目安量（AI：adequate intake）と上限量（UL：tolerable upper intake level)が提示されている[3]．しかしながら，日本人の食事摂取基準では2005年版（2005年～2009年使用）現在においてもフッ化物の摂取基準は，いまだ設定されるにいたっていない[4]．フッ化物はあらゆる食品に含有されているため，その摂取基準の設定が困難であり，日本ではその基礎資料も示されていなかった．日本人の基準値を策定するには，フッ化物摂取のう蝕予防効果と過剰摂取

表1　ライフステージにおけるフッ化物摂取基準

年齢	フッ化物（mgF/日）					
	男			女		
	目安量（mg）	上限量（mg）	基準体重（kg）	目安量（mg）	上限量（mg）	基準体重（kg）
0-5（月）	母乳栄養児 0.01	0.66	6.6	母乳栄養児 0.01	0.61	6.1
0-5（月）	人工栄養児 0.33	0.66	6.6	人工栄養児 0.31	0.61	6.1
6-11（月）	0.44	0.88	8.8	0.41	0.82	8.2
1-2（歳）	0.60	1.19	11.9	0.55	1.10	11.0
3-5（歳）	0.84	1.67	16.7	0.80	1.60	16.0
6-7（歳）	1.15	2.30	23.0	1.08	2.16	21.6
8-9（歳）	1.40	2.80	28.0	1.36	2.72	27.2
10-11（歳）	1.78	6.0	35.5	1.79	6.0	35.7
12-14（歳）	2.50	6.0	50.0	2.28	6.0	45.6
15-17（歳）	2.92	6.0	58.3	2.50	6.0	50.0
18-29（歳）	3.18	6.0	63.5	2.50	6.0	50.0
30歳以上	3.40	6.0	68.0	2.64	6.0	52.7

注1）年齢層の区分は日本人の食事摂取基準（2005年版）に依拠している．
注2）母乳栄養児は母乳中フッ化物濃度が0.01ppm（中央値）であり，摂取量1000mlとして算出した．

表2　妊婦・授乳婦のフッ化物摂取基準（mgF/日）

妊婦/授乳婦	目安量（mg）	上限量（mg）
妊婦	2.5	6.0
授乳婦	2.5	6.0

による危険性，すなわち，日本の小児における歯の審美的副作用（adverse cosmetic effect）である「歯のフッ素症（enamel fluorosis）」の発現とその基準値設定の基礎資料が必要となる．また，食品に嗜好飲料水や居住地域の水道水を含めた食事からのフッ化物摂取量と歯磨剤からの飲み込み量を合わせた総フッ化物摂取量の把握が必要である[5-7]．

2000年4月に発足した厚生科学研究（現厚生労働科学研究）は「歯科疾患の予防技術・治療評価に関するフッ化物応用の総合的研究」（主任　高江洲義矩）から始まり，2003年度には「フッ化物応用による歯科疾患の予防技術評価に関する総合的研究」，2006年度には「フッ化物応用による歯科疾患予防プログラムの構築と社会経済的評価に関する総合的研究」（H 18-医療一般-019）（主任　眞木吉信）に改組され，口腔保健に関するフッ化物応用の総合的研究を実施している．フッ化物摂取基準の策定は歯科保健を推進するうえで必須であり，ライフステージごとに飲食物からのフッ化物摂取量と歯磨剤の口腔内残留量も加味して，目安量（AI）と摂取上限量（UL）を設定した．

フッ化物摂取の目安量の基準は，疫学的調査からう蝕罹患率を有意に減少させる体重1kgあたり0.02から0.05 mg/kgである事実[8,9-12]に基づいて，その高い値である0.05 mg/kgとした．また上限量（UL）の基準は，LOAEL値を参照した[3]．すなわち，MO（Deanの分類のmodelate）の発現頻度が飲料水中フッ化物濃度2 ppm未満の場合では5%未満であるという疫学的事実[13]に基づいている．上限量の明確な計算過程は文献には示されていないが，推考すると次のような計算過程で求められていると考えられる．

1）飲料水中フッ化物濃度の最大値を2 ppmとし，一日飲水量を1.5 *l*とする．

①飲料水からのフッ化物量：2 mg/*l*×1.5 *l*=3 mg/day

②食事からのフッ化物摂取量：0.25-0.3 mg/day

③フッ化物飲料水で調理した食事中フッ化物摂取量：0.3×2=0.6 mg/day

①+③最大一日フッ化物摂取量=3+0.6=3.6 mg/day

2）飲料水中フッ化物濃度の最大値を2 ppmとし，一日飲水量を1.0 *l*とする．

①飲料水からのフッ化物量：2 mg/*l*×1.0 *l*=2 mg/day

②食事からのフッ化物摂取量：0.25–0.3 mg/day
③フッ化物飲料水で調理した食事中フッ化物摂取量：0.25×2＝0.5 mg/day
①＋③最小一日フッ化物摂取量＝0.5＋2.0＝2.5 mg/day

8～9歳児の体重を約30 kg[4]と仮定すると，2)より，最小2.5/30＝0.083 mg/kg/day，1)より，最大3.6/30＝0.12 mg/kg/dayと計算される．すなわち，上限量の範囲は，0.083–0.12 mg/kg/dayとなる．そして，その平均値をとると0.1 mg/kg/dayとなる．どうして8～9歳児を基準としたかは，永久歯の発生学的解釈から成熟期と密接に関連[13]しているからである．したがって，上限量は0.1 mg/kg/dayと設定した．この上限量はフッ化物摂取による健康障害の発現ではなく歯の審美的副作用である[3]．この体重あたりの目安量と上限量に各年齢層の日本人の基準体重[4]を乗じて男女別に8～9歳までの摂取基準値を設定した（表1）．さらに「歯のフッ素症」のmoderateが進行する臨界副作用（critical adverse effect）の感受性年齢（susceptible age groups）は病理学的には8歳までである[14]．したがって，日本人の食事摂取基準の年齢区分における10歳以上の上限量は，成人の体重を約60 kg[4]と仮定して，0.1 mg/kg×60 kg＝6 mg/dayと推定し，男女ともに6 mg/dayに統一した（表1）．

また，妊婦と授乳婦における目安量と上限量の範囲では，母乳にはフッ化物は移行しない事実[15,16]，胎児への移行も制限されるという事実[17,18]から15～29歳の目安量と上限量と同じ値に設定した（表2）．表1，2の目安量と上限量は，食品，飲料水，栄養補助食品およびフッ化物配合歯磨剤からの摂取量である．

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Current Topic

Caries Research

Caries Res 2008;42:409–418
DOI: 10.1159/000159604

Received: October 23, 2007
Accepted after revision: August 20, 2008
Published online: October 3, 2008

Caries Ecology Revisited: Microbial Dynamics and the Caries Process

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Key Words

Actinomyces · Dental biofilm · Dental caries · Dental plaque · Ecology · Mutans streptococci · Non-mutans bacteria · Non-mutans streptococci

Abstract

In this essay we propose an extension of the caries ecological hypothesis to explain the relation between dynamic changes in the phenotypic/genotypic properties of plaque bacteria and the demineralization/remineralization balance of the caries process. Dental plaque represents a microbial ecosystem in which non-mutans bacteria (mainly non-mutans streptococci and *Actinomyces*) are the key microorganisms responsible for maintaining dynamic stability on the tooth surface (dynamic stability stage). Microbial acid adaptation and subsequent acid selection of 'low-pH' non-mutans bacteria play a critical role for destabilizing the homeostasis of the plaque by facilitating a shift of the demineralization/remineralization balance from 'net mineral gain' to 'net mineral loss' (acidogenic stage). Once the acidic environment has been established, mutans streptococci and other aciduric bacteria may increase and promote lesion development by sustaining an environment characterized by 'net mineral loss' (aciduric stage). Hence, high proportions of mutans streptococci and/or other aciduric bacteria may be considered biomarkers of sites of particularly rapid caries progression. This cascade of events may change the surface texture of caries lesions from smooth to rough (enamel) or hard to soft (dentin). These clinical surface features can be reversed at any stage of lesion development provided that the acidogenic/aciduric properties of the biofilm are resolved. From an ecological point of view it is therefore not only important to describe which bacteria are involved in caries, but also to know what the bacteria are doing.

The dental biofilm supports a 'micro-ecosystem' of bacteria that exhibit a variety of physiological characteristics. In particular, the production of acid resulting from sugar metabolism by these bacteria and the subsequent decrease in environmental pH is responsible for demineralization of the tooth surface and formation of dental caries [for review, see Marsh and Nyvad, 2008].

Much research has suggested that mutans streptococci (MS) are the major pathogens of human dental caries. This is because, first, MS are frequently isolated from cavitated caries lesions; second, MS induce caries formation in animals when fed a sucrose-rich diet; third, MS are highly acidogenic and aciduric [Hamada and Slade, 1980; Loesche, 1986], and fourth, MS are able to produce water-insoluble glucan, which promotes bacterial adhesion to the tooth surface and to other bacteria [Hamada and Slade, 1980]. A systematic literature review by Tanzer et al. [2001] confirms a central role of the MS in the initiation of dental caries on enamel and root surfaces.

However, some recent studies indicate that the relationship between MS and caries is not absolute: high proportions of MS may persist on tooth surfaces without le-

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sion development, and caries can develop in the absence of these species [Nyvad, 1993; Bowden, 1997; Aas et al., 2008]. Under such circumstances, it is suggested that acidogenic and aciduric bacteria other than MS, including 'low-pH' non-MS and *Actinomyces* [van Houte et al., 1994, 1996; Sansone et al., 1993] are responsible for the initiation of caries. Recent molecular analyses have strengthened this concept by showing that the microflora associated with white spot lesions is more diverse than hitherto appreciated and that novel phylotypes and species including *A. gernesceriae, A. naeslundii,* and *A. israelii* as well as a broad range of non-MS and *Veillonela* spp. may also play a role [Becker et al., 2002; Aas et al., 2008]. Since all the bacteria that have been associated with caries belong to the normal microflora of the oral cavity, dental caries has been described an endogenous infection [Fejerskov and Nyvad, 2003]. Endogenous infections may occur when members of the resident flora obtain a selective advantage over other species whereby the homeostatic balance of the biofilm is disturbed [Marsh and Martin, 1999]. Therefore, an ecological hypothesis is attractive [Marsh, 1994].

Concurrently with these changes in the interpretation of the microbial etiology of caries, novel concepts have evolved around the caries process itself. Thus, there is a growing awareness that caries lesions can be managed by non-operative interventions [Fejerskov, 1997; Fejerskov et al., 2008]. Moreover, it has been demonstrated that the effect of such interventions is reflected in the clinical appearance and activity of the lesions [Nyvad and Fejerskov, 1997; Nyvad et al., 2003, 2005; Thylstrup et al., 1994].

So far, these clinical and microbiological advances have not been integrated into a comprehensive concept that may broaden our understanding of caries. Given these circumstances, the aim of this paper is to revisit the 'ecological plaque hypothesis' pioneered by Carlsson [1986] and Marsh [1994, 2003] and to clarify the relationship between the ecological succession of bacteria in dental plaque and the caries process.

Recent Concepts of the Caries Process

Dental caries has been described as a chronic disease that progresses slowly in most individuals. The disease is seldom self-limiting and, in the absence of treatment, caries progresses until the tooth is destroyed. The localized destruction of the hard tissues, often referred to as the lesion, is the sign or symptom of the disease [Fejerskov et al., 2008]. Lesion progression is often depicted on a linear scale ranging from initial loss of mineral at the ultrastructural level to total destruction of the tooth. In reality, however, caries lesion development is a highly dynamic series of processes with alternating periods of progression and arrest/regression [Backer-Dirks, 1966; Nyvad et al., 2003]. Lesion progression may be arrested at any stage of lesion development, even at the stage of frank cavitation [Lo et al., 1998], provided the local environmental conditions, e.g. biofilm control and topical fluoride exposure, are favorable [Nyvad and Fejerskov, 1997]. Hence, the clinical stages of caries represent nothing but historical signs of past caries experience. What may be perceived clinically as an 'incipient' or 'early' lesion may in reality turn out to be an 'aged' established lesion that has been present in the oral cavity for months or years. Likewise, carious cavities may have experienced major differences in their history in the oral cavity.

Changes in the progression rate of caries are associated with alterations of the surface features of the lesions, active non-cavitated enamel lesions being dull and rough and inactive non-cavitated enamel lesions being shiny and smooth [for review, see Thylstrup et al., 1994]. These clinical distinctions have been shown to provide a reliable and valid classification of caries lesion activity [Nyvad et al., 1999, 2003]. Furthermore, such classification has offered novel information about caries lesion transition patterns [Baelum et al., 2003; Lima et al., 2008] and served as a useful basis of selecting high-risk patients [Hausen et al., 2007] in randomized clinical trials. Therefore, when trying to understand the clinical dynamics of caries, assessment of the surface texture of lesions may be a more sensitive parameter than merely assessing the stage of severity of a lesion as revealed by the presence or absence of a cavity.

From a biochemical point of view, the caries process is much more complex. Metabolic processes are constantly taking place in the dental plaque as a result of microbial activity, and this is reflected by continuous, rapid fluctuations in plaque pH, both when the plaque is starved and fed [Newman et al., 1979]. Hence, any clinically sound or carious tooth surface that is covered by an undisturbed plaque may experience minute mineral losses and mineral gains depending on the metabolic status of the microflora. The key point is that only when the cumulative result of the de- and remineralization processes produces a net mineral loss over time may a caries lesion develop or progress [Manji et al., 1991]. Such situations are likely to occur when there is a drift of pH in the biofilm, e.g. as a consequence of increased carbohydrate availability or reduced salivary clearance. By contrast,

when the integrated de- and remineralization processes result in a net mineral gain over time, this may lead to deposition of minerals in the tooth surface and arrest of lesion development. This explains why the caries process has been regarded as a ubiquitous and natural phenomenon [Manji et al., 1991]. Because of the constantly metabolically active biofilm, these processes cannot be prevented, but they can be controlled to the extent that caries does not appear clinically [Fejerskov, 1997; Kidd and Fejerskov, 2004]. This new microdynamic concept of caries suggests that an updated explanation of the microbial ecology of caries must take into consideration that the caries activity may change over time in response to pH drifts in the biofilm.

Microbial Characteristics and the Caries Process

Distribution of MS and Non-Mutans Bacteria in Supragingival Dental Biofilm at Clinically Healthy Sites and in Carious Lesions

In situ studies have shown that the initial colonizers of newly cleaned tooth surfaces constitute a highly selected part of the oral microflora, mainly *S. sanguinis*, *S. oralis* and *S. mitis* 1 [Nyvad and Kilian, 1987]. Together, these three streptococcal species may account for 95% of the streptococci and 56% of the total initial microflora [Li et al., 2004; Nyvad and Kilian, 1987]. Surprisingly, MS comprise only 2% or less of the initial streptococcal population, irrespective of the caries activity of the individual [Nyvad and Kilian, 1990a]. These observations imply that the vast majority of the early colonizers on teeth belong to the 'mitis group'. These bacteria as well as other viridans group streptococci, except for the MS, are often referred to as the non-MS, which are genetically distinguished from the MS that belong to the 'mutans group' [Kawamura et al., 1995]. As the microflora ages it shifts from *Streptococcus*-dominant to *Actinomyces*-dominant [Syed and Loesche, 1978; van Palenstein Helderman, 1981]. The predominant species in mature smooth surface plaque belong to *Actinomyces* and *Streptococcus*, most of which are non-MS [Ximénez-Fyvie et al., 2000]. MS are found in very low numbers [Bowden et al., 1975].

The proportion of MS in plaque covering white spot lesions in enamel is often higher than at clinically healthy sites, although still rather low, ranging between 0.001 and 10% [van Houte et al., 1991b]. Meanwhile, non-MS and *Actinomyces* still remain major bacterial groups in enamel lesions. In fact, it has been shown that in the absence of MS and lactobacilli, the initial dissolution of enamel can be induced by members of the early microflora, exclusively [Boyar et al., 1989].

In cavitated lesions in dentine, including rampant caries, MS constitute about 30% of the total flora [Boue et al., 1987; Loesche et al., 1984; Milnes and Bowden, 1985], indicating that these species are associated with progressive stages of caries. By contrast, MS are encountered less frequently at the advancing front of dentin caries where lactobacilli, prevotellae and *Bifidobacterium* are more prevalent [Aas et al., 2008; Becker et al., 2002; Chhour et al., 2005; Edwardsson, 1974; Munson et al., 2004].

Non-MS as Generalists and MS as Specialists: How Non-MS Can Become Dominant in Supragingival Plaque

Most non-MS have adhesins [Gibbons, 1989; Kolenbrander, 2000] which adhere to proteins and sugar chains of acquired pellicles coating the tooth surface. This seems to be one of the reasons for the dominance of non-MS at the initial stage of plaque formation. In addition, most oral streptococci produce extracellular polysaccharides such as glucans and fructans [Banas and Vickerman, 2003; Whiley and Beighton, 1998]. Polysaccharides can fill the gaps between bacteria and form the matrix of plaque, and accelerate plaque formation.

On the other hand, MS do not attach efficiently to the acquired pellicle [Nyvad and Kilian, 1990b], although they have adhesins such as the antigen I/II. Instead, these bacteria have been emphasized to produce water-insoluble glucans, which are adhesive and capable of accelerating bacterial accumulation. However, it should be noted that glucans only act as additional factors in plaque formation, and that not only MS but also non-MS can produce glucans [Banas and Vickerman, 2003; Vocca-Smith et al., 2000].

Both non-MS and MS metabolize various sugars and produce acids. When sugar is supplied in excess, streptococci can store the extra sugars as intracellular polysaccharides (IPS) [Hamilton, 1976; Takahashi et al., 1991; van Houte et al., 1970], and they can utilize the IPS as an energy source to produce acids when sugar is limited as occurs between meals. The final pH values of non-MS when grown with sugars are heterogeneous, ranging from 4 to 5.2, whereas those of MS are more homogeneous, being around 4 [Hardie, 1986]. In general, on the basis of final pH values, MS are more acidogenic and aciduric than non-MS. It should be realized, however, that the final pH values of non-MS can be much lower than pH 5.5 [Hardie, 1986], the 'critical' pH for the demineralization of enamel.

Non-MS have a variety of extracellular glycosidases [Whiley and Beighton, 1998] that can liberate sugars and amino-sugars from glycoproteins such as the mucin contained in saliva. Furthermore, all non-MS grow on amino-sugars [Byers et al., 1996; Whiley and Beighton, 1998]. This is an advantage for non-MS in the oral cavity, where salivary glycoproteins are always available.

In addition, most non-MS can utilize arginine or arginine-containing peptides available in saliva through the arginine deiminase system, which degrades the arginine molecule to ammonia and carbon dioxide with production of ATP. Overall, this metabolic pathway produces alkali and neutralizes the intracellular and the environmental pH [Burne and Marquis, 2000]. Arginine deiminase system is helpful for non-MS not only to utilize arginine as an energy source but also to survive under the acidic conditions in the oral cavity. However, most MS do not have these metabolic features.

In summary, non-MS have diverse physiological activities, suggesting that they are generalists, versatile enough to adapt to various conditions in supragingival biofilm, and this could be the reason why they are the dominant streptococci in supragingival biofilm. On the other hand, MS are aciduric specialists in sugar metabolism and acid production, which make them less competitive in clinically sound supragingival environments.

Acidogenicity and Acidurance of Non-MS: Key Factors in the Caries Process

It is clear that an ability both to produce acid (acidogenicity) and to tolerate a low-pH environment (aciduranc) is a crucial feature for microorganisms responsible for caries. Sansone et al. [1993] compared the microbial composition of dental plaque at clinically healthy sites and white spot lesions and found that non-MS were dominant at both sites while MS were present at low and similar levels at both sites. However, the ability of plaque to reduce pH in vitro was significantly greater at white spot lesions (pH 4.13) than at clinically healthy sites (pH 4.29). These results suggest that MS are neither a unique causative agent for white spot lesions, nor a main determinant of the acidogenicity of plaque.

In order to evaluate the acidogenicity of the non-MS, Sansone et al. [1993] further grew these bacteria in liquid culture media supplemented with 1% glucose and measured the final pH of the culture media. In agreement with Svensäter et al. [2003], they found that non-MS are heterogeneous for acidogenicity: some strains lowered the culture pH to below 4.4, a pH comparable to that produced by MS, whereas for other strains the pH-lowering capacity was less pronounced. In addition, the proportion of acidogenic non-MS was higher at white spot lesions than at clinically healthy sites. The acidogenic non-MS, identified as *S. gordonii*, *S. oralis*, *S. mitis* and *S. anginosus*, were subsequently designated as 'low-pH' non-MS [van Houte, 1994], and it was suggested that the pH-lowering capacity of plaque may be related to the proportion of 'low-pH' non-MS [van Houte et al., 1991a, 1991b]. Later observations by van Ruyven et al. [2000] have supported this notion.

The question still remains: Which of the non-MS are to be considered 'low-pH' non-MS? Alam et al. [2000] obtained two groups of *S. oralis* – one comprised the total *S. oralis* population in dental plaque, whereas the other comprised aciduric strains that were able to grow at pH 5.2. They then differentiated these strains into 15 genotypes on the basis of genetic similarity. The distributions of genotypes were different between the total bacterial group and the aciduric group; only some genotypes of *S. oralis* seemed to be aciduric and to form an aciduric subpopulation. These results are in line with another study showing that strains of non-MS differ distinctly by their rate of acid production at decreasing pH; in particular some strains within *S. mitis* 1, *S. oralis* and *S. gordonii* are capable of producing acids as rapidly as many *S. mutans* strains at pH 5.0 and 5.5 [de Soet et al., 2000]. Collectively, it is suggested that the group of 'low-pH' non-MS comprise a mosaic of acidogenic subpopulations of each species of non-MS.

Involvement of Actinomyces

Most of our knowledge about the role of *Actinomyces* in caries stems from studies of root surface caries. However, there is no evidence that *Actinomyces* spp. have a specific role in root caries. In fact, a review of the literature has concluded that the basic patterns of microbial colonization are identical on enamel and root surfaces, structurally as well as microbiologically [Nyvad, 1993].

As with enamel caries, MS comprise only a small proportion of the microflora of root surface caries lesions. van Houte et al. [1996] reported that non-MS and *Actinomyces* spp. were dominant in dental plaque covering root surface caries and that the isolated *Actinomyces* strains were heterogenous with respect to acidogenicity: strains isolated from root surface caries were more acidogenic than those from clinically healthy root surfaces. Meanwhile, Brailsford et al. [2001] observed that, in subjects with root surface caries, aciduric bacteria able to grow at pH 4.8 comprised 21.6% of the total microflora in root surface caries lesions (lactobacilli and *Actinomyces* were

dominant), whereas aciduric bacteria comprised 10.7% in clinically sound root surfaces (*Actinomyces* dominant). However, in subjects without root surface caries, aciduric bacteria comprised only 1.4% of total microflora in clinically sound root surfaces. These findings indicate an association between acidogenic/aciduric *Actinomyces*, i.e. 'low-pH' *Actinomyces* and root surface caries.

Actinomyces are as versatile to adapt to the dental biofilm environment as are the non-MS; they have adhesin-mediated adhesion to tooth surfaces, produce acids from various sugars, and synthesize intracellular and extracellular polysaccharides. In addition, *Actinomyces* have a unique glycolytic system [Takahashi et al., 1995] in which they utilize high-energy polyphosphate and pyrophosphate compounds for synthesis of hexokinase and phosphofructokinase, respectively, acting as phosphoryl donors instead of ATP. This means that *Actinomyces* are able to exploit a surplus ATP to synthesize polyphosphate as an energy reservoir, and salvage energy from pyrophosphate, a high-energy-phosphoryl-bond-containing byproduct from the metabolism of polymers such as nucleic acids and glycogens. In addition, *Actinomyces* are often ureolytic [Kleinberg, 2002; Yaling et al., 2006] and can utilize lactate as a carbon source for growth [Takahashi et al., 1996]. These diverse physiological characteristics of *Actinomyces* seem to be advantageous to survive and dominate in supragingival plaque [Takahashi and Yamada, 1999b].

Acid Adaptation and Acid Selection: Adaptive Changes in Acidurance and Acidogenicity and the Consequent Selection of 'Low-pH' Non-MS

Non-MS are not only genotypically heterogenous, but they are also able to change their physiological characteristics adaptively. Takahashi and Yamada [1999a] have shown that when these bacteria were exposed to an acidic environment, they increased their acidogenicity. These bacteria were grown first at pH 7.0 and afterwards at pH 5.5 for a short time: 30, 60 and 90 min. The bacteria were then harvested, washed and incubated with glucose, and the final pH values were measured as a marker of acidogenicity. Their acidogenicity or final pH values varied (pH 4.04–4.33), but after incubation at pH 5.5 for 60 min, all the bacteria increased their acidogenicity (pH 3.93–4.12).

Non-MS were also able to increase their acidurance adaptively [Takahashi and Yamada, 1999a]. Bacteria initially grown at pH 7.0 were killed by acid stress in a strain-dependent manner following exposure to pH 4.0 for 60 min (survival rate: 0.0009–71%), but after pre-acidification at pH 5.5 for 60 min, all the bacteria increased their acidurance (survival rate: 0.4–81%).

The biochemical mechanisms underlying the acid adaptation are considered to involve the following mechanism [Quivey et al., 2000]: (1) an increase in proton impermeability of the cell membrane; (2) induction of proton-translocating ATPase (H^+-ATPase) activity that expels proton from cells; (3) induction of the arginine deiminase system that produces alkali from arginine or arginine-containing peptides, and (4) induction of stress proteins that protect enzymes and nucleic acids from acid denaturation. In non-MS, the increase in activities of H^+-ATPase and arginine deiminase and expression of stress proteins (homologues of heat shock protein, Hsp60 and Hsp70) were observed following incubation at pH 5.5 [Takahashi and Yamada, 1999a].

In the oral cavity, acidification of the biofilm due to frequent sugar intake or poor salivary secretion can be a driving force to enhance the acidogenicity and acidurance of the non-MS, resulting in establishment of a more acidic environment. Even if acid adaptation occurs, non-MS are still so heterogeneous with respect to acidurance that the population of more aciduric strains, i.e. 'low-pH' non-MS will increase selectively in this environment. This will cause a shift in the composition and acidogenic potential of the biofilm, which, provided the demineralization/remineralization balance is disturbed over an extended period of time, leads to dental caries. Similar microbial acid adaptation and acid selection processes may occur in *Actinomyces*.

Competition between Non-MS and MS

Transient Acidification. Although 'low-pH' non-MS can adaptively increase their acidurance and acidogenicity, and take over the position in supragingival plaque, MS are more competitive under severely acidic conditions. Following a rapid exposure to pH 4.0 for 60 min as often observed in dental plaque after a sugar exposure, *S. sanguinis* ATCC 10556, a strain of 'low-pH' non-MS, was able to survive. However, this bacterium temporarily lost the ability to grow, along with the inactivation of glycolytic enzymes, and did not start growing again until 90 min after the pH had returned to neutral [Takahashi et al., 1997]. By contrast, the growth of *S. mutans* NCTC 10449 at pH 4.0 was not influenced at all. In view of this observation, it is expected that the population of non-MS decreases gradually during frequent acidification, whereas the proportion of MS would increase.

Prolonged Acidification. Experiments using in vitro cultures of mixtures of oral bacterial species have clearly

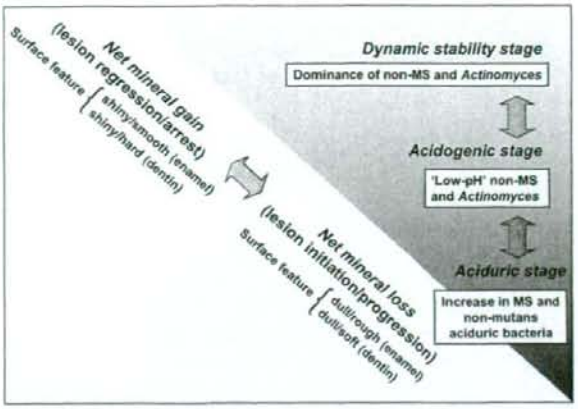

Fig. 1. An extended caries ecological hypothesis explaining the relationship between acidogenic and aciduric shifts in the composition of the dental biofilm and changes in the mineral balance of the dental hard tissues. Note that the cascade of ecological events in the biofilm is reversible and is reflected in the surface features of the dental hard tissues at any stage of lesion formation. MS = Mutans streptococci. For detailed explanation, see text.

shown that prolonged acidification is the driving force behind the emergence of MS in dental plaque. Bowden and Hamilton [1987] demonstrated that *S. sanguinis* (formerly *S. sanguis*) was dominant when the pH was kept at 7.0–6.0 in a mixed continuous culture, whereas when pH was shifted to 5.5, *S. mutans* overcame *S. sanguinis*, although *S. sanguinis* survived in the culture at pH 4.5. A similar phenomenon was observed by Bradshaw and Marsh [1998]. They established a continuous culture with 9 oral bacterial species and demonstrated that non-MS *(S. oralis* and *S. gordonii)* were dominant when the pH was kept at pH 7.0 during daily pulses of glucose for 10 days, whereas when the pH was allowed to fall to a preset value of 5.0, MS as well as lactobacilli became dominant; non-MS were excluded from the consortium when pH was allowed to fall without control (final pH = 3.83). Similarly, Takahashi et al. [1997] showed that a strain of 'low-pH' non-MS (*S. sanguinis* ATCC 10556) was not able to grow at pH $\leq$ 4.2 in a complex liquid medium under anaerobic conditions, while *S. mutans* was still able to grow at pH 4.2. Given these observations, it is suggested that prolonged acidic conditions around pH 5.5 may cause the emergence of MS in the microbial flora and that more severe acidic conditions around pH 4 may exclude the non-MS. In the oral cavity, prolonged acidic conditions (pH $\leq$ 5.5) can occur in carious cavities [Dirksen et al., 1962; Hojo et al., 1994], where clearance of acids is hampered. This may be the reason why MS and particularly lactobacilli are frequently isolated from established carious cavities.

An Extended Caries Ecological Hypothesis

In the light of the foregoing we suggest an extended caries ecological hypothesis that explains the relationship between the composition of the dental plaque and the caries process (fig. 1). In this hypothesis, dental plaque is a dynamic microbial ecosystem in which non-mutans bacteria such as non-MS and *Actinomyces* are the key players for maintaining dynamic stability. These bacteria can produce acids from sugary foods and the resulting acids can demineralize the enamel. However, the temporary decreases in pH are easily returned to neutral level by homeostatic mechanisms in the plaque [Marsh and Martin, 1999]. This is a natural pH cycle, which occurs numerous times daily in supragingival plaque (dynamic stability stage).

However, when sugar is supplied frequently or salivary secretion is too scarce to neutralize the acids produced, the pH decreases in the plaque may enhance the acidogenicity and acidurance of the non-mutans bacteria adaptively. Under such conditions the population of the 'low-pH' non-MS and *Actinomyces* then increases via acid selection, leading to a microbial shift to a more acidogenic microflora. These changes in the phenotype and genotype of the microflora may shift the demineralization/remineralization balance from 'net mineral gain' to 'net mineral loss' and initiate lesion development (acidogenic stage). At this stage, lesion development could also be arrested with de-adaptation of the microflora, provided that the mineral balance is restored to a 'net mineral gain' by reduced environmental acidification.

If prolonged acidic environments prevail, lesion development ('net mineral loss') is likely to progress. In these environments, more aciduric bacteria such as MS and lactobacilli may replace the 'low-pH' non-mutans bacteria and further accelerate the caries process (aciduric stage). However, even at this highly aciduric stage, the mineral balance and composition of the microflora could possibly be reversed by modification of the acidic environment, e.g. as a result of sugar restriction [de Stoppelaar et al., 1970].

In this scenario, the microbial acid adaptation and the subsequent acid selection of 'low-pH' non-mutans bacteria play a crucial role in destabilizing the homeostasis of

the biofilm and facilitating lesion development. Moreover, once the acidic environment has been established, the proportion of aciduric bacteria such as MS and lactobacilli may increase and act as promoters of lesion progression by sustaining an environment characterized by 'net mineral loss'. Hence, high proportions of MS and/or other aciduric bacteria may be considered biomarkers of sites that undergo particularly rapid caries development [Bowden et al., 1976; Chhour et al., 2005; Macpherson et al., 1990; Nyvad and Kilian, 1990b]. We suggest that this cascade of events is associated with changes in the surface texture of the dental hard tissues from smooth to rough (enamel) or from hard to soft (dentin) [Nyvad et al., 1999, 2003].

Two decades ago, Carlsson [1986] presented a caries microbiological hypothesis by which he speculated that ecological changes in the oral flora were determined by competition for nutrients. Carlsson proposed that at low levels of sugars, the oral microflora would be dominated by bacteria with a high affinity for sugars (the 'gleaners'), whereas at consistently higher concentrations of sugars, bacteria with lower affinity for sugars, but with high growth rates, would be favored (the 'exploiters'). Under the latter condition the metabolic end products established acidic environments favoring an outgrowth of aciduric bacteria, the so-called 'pH-strategists' [Carlsson, 1986]. This concept was further developed as the ecological plaque hypothesis by Marsh [1994, 2003], who focused on the dynamic and reversible processes of de- and remineralization in the plaque by linking between sugar supply, pH change and microflora shift. We suggest that the ecological concept of caries should be extended and strengthened by including clinical manifestations of caries lesion processes, and by detailing the microbial acid adaptation and acid selection processes.

Clinical and Scientific Perspectives

The extended caries ecological hypothesis supports the 'mixed-bacteria ecological approach' proposed by Kleinberg [2002] that the proportion of acid- and base-producing bacteria is the core of caries activity. Clearly, the extended hypothesis undermines the view that dental caries is a classical infectious disease, and therefore that prevention and control of this condition by elimination of a specific group of microorganisms, such as the MS, through vaccination, gene therapy or antimicrobial treatment, is unwise. Rather environmental control of the microflora should be achieved by stimulating the non-mutans bacteria such as non-MS and *Actinomyces* by avoiding acidification of the dental biofilm.

Practical solutions to this strategy may include mechanical plaque control, reduction/substitution of the intake of sugary foods and/or application of pH-neutralizing techniques such as saliva stimulation. Even if the effect of such interventions on the composition of the microflora is sparsely documented, it has been shown that dietary modification may facilitate such changes. Hence, de Stoppelaar et al. [1970] observed a clear reduction in the proportion of MS at carious and filled sites at the expense of *S. sanguis* following a 3-week period of sucrose restriction. These changes were reversed when individuals resumed a normal diet containing sucrose. Conventional culture studies of young dental plaque in caries-inactive individuals have failed to reveal a consistent microbial response pattern to sucrose-regulated diets [Staat et al., 1975; Scheie et al., 1984]. In these studies, sucrose-related modulation of the microflora was found to depend on prior oral colonization by mutans streptococci, and these species were not entirely eliminated on a low-sucrose diet. It is interesting to speculate that differences in the propagation of MS might reflect differences in acid tolerance between clones of these species [Welin-Neilands and Svensäter, 2007]. Future studies describing the site-specific microbial shifts in response to sucrose should therefore focus on both the MS and the non-mutans bacteria, e.g. by applying molecular identification methods.

An important consequence of the extended hypothesis is that knowledge about the acidogenic and aciduric properties of bacteria, i.e. the phenotypic characteristics, and their regulatory mechanism may be a more relevant parameter than knowledge about their taxonomy. The phenotypes of most bacteria have already been well described in textbooks such as the Bergey's Manual of Systematic Bacteriology [Holt, 1984]. Nevertheless, such descriptions are not particularly helpful to explain the in vivo behaviors since bacterial phenotypic characteristics may change depending on the local environmental conditions. Therefore, from an ecological point of view it is not only important to describe which bacteria are involved in caries but also to know what the bacteria are doing [Takahashi, 2005].

Recently, van Ruyven et al. [2000] have detected non-mutans aciduric bacteria other than non-MS and *Actinomyces* from dental biofilms covering white spot lesions. They found that these bacteria consisted of various species including lactobacilli and *Bifidobacterium*. Interestingly, the samples differed with respect to dominance of

particular bacterial species, suggesting that any bacterial species can participate in the development of caries as long as they are aciduric and dominant [Bowden, 1984]. In this essay we have focused on the non-MS and the *Actinomyces* as the major non-mutans aciduric bacteria because detailed studies have been conducted for these bacteria. However, it would not be surprising if other non-mutans aciduric bacteria were found to be associated with dental caries. As stated above, it is not the genotype per se, but the phenotype in a certain environment, i.e. the acidogenic and aciduric potential of the bacteria, that is conducive to a microbial shift leading to caries.

According to the extended hypothesis, there is a firm association between the de- and remineralization balance of caries lesions and the overall composition of the microflora. In the in situ study of Nyvad and Kilian [1990b], root surface caries lesions experiencing the highest mineral loss, as assessed by quantitative microradiography, were dominated by uniform *Actinomyces* spp., or a combination of MS and *Lactobacillus* spp., whereas lesions experiencing a smaller mineral loss were associated with a more diverse microbiota including non-MS, MS, *Actinomyces*, lactobacilli, *Bifidobacterium* as well as lactate-metabolizing species (*Veillonella* spp.). Such differences in the pattern of the microflora in response to different lesion progression rates not only lend support to the suggested acidogenic and aciduric stages of bacterial succession in caries, but also conform with the concept that microbial diversity may exert a protective effect on the dynamic stability of the biofilm community, recently referred to as the 'insurance hypothesis' [Yachi and Loreau, 1999; Boles et al., 2004]. Therefore, in the future, if we truly wish to advance the ecological understanding of caries, it is important to describe the total microbiota of caries lesions by studying lesions with a known age and history in the oral cavity or, alternatively, employ clinical caries diagnostic methods that reflect the activity state of lesions [Nyvad et al., 1999, 2003].

Acknowledgements

This essay was developed on the basis of joint discussions following our presentations at the ORCA Symposium Japan (November 13–14, 2006, Nagoya, Japan).

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