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REVIEW ARTICLE

The Stephan Curve revisited

William H. Bowen

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Abstract The Stephan Curve has played a dominant role in caries research over the past several decades. What is so remarkable about the Stephan Curve is the plethora of interactions it illustrates and yet acid production remains the dominant focus. Using sophisticated technology, it is possible to measure pH changes in plaque; however, these observations may carry a false sense of accuracy. Recent observations have shown that there may be multiple pH values within the plaque matrix, thus emphasizing the importance of the milieu within which acid is formed. Although acid production is indeed the immediate proximate cause of tooth dissolution, the influence of alkali production within plaque has received relative scant attention. Excessive reliance on Stephan Curve leads to describing foods as "safe" if they do not lower the pH below the so-called "critical pH" at which point it is postulated enamel dissolves. Acid production is just one of many biological processes that occur within plaque when exposed to sugar. Exploration of methods to enhance alkali production could produce rich research dividends.

Keywords Stephan Curve · Acid tolerance · Alkali generation · Polysaccharide · Plaque matrix

Introduction

For more than a century, oral microbiologists have focused on acid production by the oral flora as the primary etiological cause of dental caries. This line of research developed from the chemo-parasitic theory for the pathogenesis of dental caries proposed by Miller [1] and has been the core of much research into the etiology and pathogenesis of dental caries since.

Stephan [2, 3] became a leading proponent of the importance of acid production in the pathogenesis of dental caries. His early work investigated acid production by plaque removed from the tooth surfaces. These early observations, although an advance at the time, contributed little to the understanding of the processes occurring on the tooth surfaces and to the dynamics of acid production and other processes occurring at the plaque tooth interfaces. It is noteworthy that Stephan observed, "Nevertheless, the findings do not exclude of other factors other than pH which might produce or modify the decalcification of teeth in caries." Following the development of microelectrodes, it was possible to measure pH in dental plaques in situ, from multiple sites over time (for review see [4]). Data gleaned from such investigations lead to the construction of the Stephan Curve. The original published curve is shown in Fig. 1 (reproduced from J. dent Res with permission from Sage Publishing).

It is important to note that the Stephan Curve is a composite resulting from an average of numerous readings collected from many teeth.

Measurement of pH in plaque

A myriad of different approaches have been developed to determine pH values in dental plaque in situ. This review will examine just a few and illustrate the difficulties some of them present.

The most commonly used electrodes in early investigations were constructed from antimony or glass, iridium

W. H. Bowen (✉)
Center for Oral Biology, University of Rochester,
601 Elmwood Avenue, Box 611, Rochester, NY 14642, USA
e-mail: William_Bowen@urmc.rochester.edu

or field effect transistors [5]. Antimony electrodes were honed from a solid piece of antimony [6] or they could be fabricated by electro-plating antimony onto fine tinned copper wire or platinum thereby allowing for the manufacture of purposed shaped and extremely small electrodes [7]. They gave accurate readings but were sensitive to elevated levels of lactate. These electrodes were used to determine pH in plaque from rodents, primates, and humans [7, 8].

The introduction of small glass electrodes with fine tips certainly enhanced accuracy; unfortunately, they were delicate and fractured readily [9].

Using touch electrodes offered a major disadvantage, i.e., it was necessary to penetrate the plaque to obtain measurements and thereby disrupt its integrity. Furthermore, this approach provided data on the average pH within plaque, and not necessarily the pH values at the plaque tooth interfaces [10] and investigators soon recognized the shortcoming of this approach [11].

Constructing a prosthesis harboring a glass electrode

By constructing a prosthesis harboring a glass electrode, plaque could accumulate in vivo at the interface of the electrode and tooth surface [12, 13]. The indwelling electrodes had the disadvantage that they were difficult to construct; this certainly limited the number of subjects that could be included, and the subjects were restricted to adults with an appropriate available site. However, they did record what was occurring on average in the plaque electrode interface, in vivo. Various test solutions could be applied and the pH determined by running a lead ex oris to a pH meter. Clearly this represented a major technical advance, nevertheless, opinions differ on whether the data collected were superior to those collected using the touch electrodes [4, 14]. The technique also allowed for multiple measurements in sequence from different sites in the mouth.

The next advances in the area followed the introduction of radio telemetry [15]. The construction of the prosthesis was similar to that of the indwelling electrode but in addition included a small radio transmitter. This approach allowed for pH measurements to be recorded without the need for the patient to have wires coming from the mouth and permitted collection of data under physiological conditions [16, 17]. It is nevertheless important to point out that there is no ideal method of measuring pH of plaque, although recent evidence suggests that it is possible to determine pH within plaque by means of pH sensitive dyes. These data reveal that there may be multiple pH values heterogeneously distributed throughout dental plaque and also across the surface of biofilm attachment. Clearly, an electrode at least gives an average pH value [18].

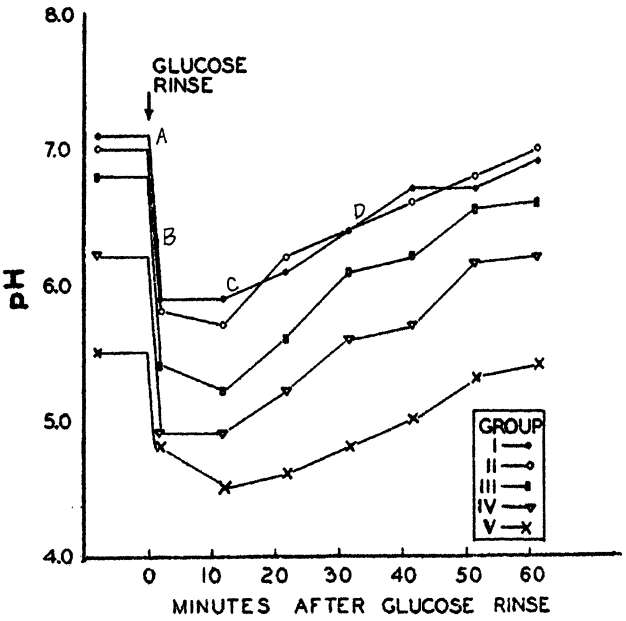

Fig. 1 pH Curves of plaques on labial surfaces of *upper* anterior teeth in different caries activity groups. Groups *I* caries-free, *II* marked caries-activity, *III* slight caries activity, *IV* marked caries activity, *V* extreme caries-activity [3]

The introduction of techniques utilizing indwelling electrodes led to widespread investigations of plaque pH values and perhaps this elevated concern about acid production in plaque may have been to the detriment of other aspects of the pathogenesis of dental caries, which will be discussed later.

Stephan Curve

The Stephan Curve can, for descriptive purposes, be divided into several phases; each one may represent physiological responses within the plaque, and offer possible relevance in the pathogenesis of dental caries. The resting phase, pH value (A) that is plaque not exposed to sugars for 12 h or more; the initial decline (B) in pH values following exposure to sugar; the time when pH is below the "critical pH" (C) (see later) and finally (D) the phase of recovery (Fig. 1).

Stephan [3] and others have noted that the fasting pH value of plaque (plaque that has not been exposed to food for 12 h or more) has lower values (more acid) in patients who were caries-active compared with those teeth who were cavity-free. Stephan apparently restricted many of his observations to carious teeth that it is important to note, however, Dong et al. [19] and Fejerskov et al. [20] confirmed these observations (see later). Like Stephan they noted that plaque in the upper jaw was significantly more acidogenic than plaque in the lower jaw.

Clearly, these observations illustrate that there is metabolic activity occurring in plaque in the absence of extraneous sources of carbohydrates. There are several sources of carbohydrates within plaque; when sugars are ingested several species of organisms found in plaque synthesize extracellular polysaccharides (PS), both soluble and insoluble [21]. The soluble PS found in plaque are usually α 1–6 linked glucan (dextran) and fructan β 2–6 linked fructose [22–24]. Both may be broken down to sugar by intrinsic enzymes (dextranase and fructanase) [25]. The sugar is then metabolized to acid via Emden Meyerhoff pathway.

In addition to extracellular polysaccharide, many organisms found in dental plaque have the capacity to synthesize intracellular polysaccharide, of the glycogen type [26]. This too may be metabolized to acid thereby maintaining low pH values within plaque [27–29]. Indeed the population of microorganisms storing intracellular polysaccharide correlates with the prevalence of dental caries. Thus, there appears to be a sound basis for the relationship between how pH values in fasting dental plaque on carious tooth surfaces compared with that observed on sound surfaces.

It is important to note that in some instances, particularly on cavity free teeth, the fasting values may be higher than the surrounding saliva, an observation that suggests strongly that alkali is being generated within plaque (see later) [30].

Following the application of sugar, e.g., glucose or sucrose to plaque, acid is generated within plaque rapidly as reflected in the fall of pH values [31]. A range of acids is produced rapidly: lactic and acetic are by far the most dominant [32, 33]. The pH value remains depressed as long as sugar is available and acid is produced within dental plaque; values as low as 3.9 may be recorded.

Critical pH

The concept of "critical pH" was also introduced by Stephan [3]. He suggested that the most significant way to relate pH changes to caries activity of different groups was to classify the values according to a "hypothetical critical decalcifying pH level." Interestingly, Stephan suggested a value close to pH 5.0. The so-called "critical pH" evolved from the concept that enamel does not begin to dissolve rapidly until pH 5.5 is reached in plaque; it is perhaps unfortunate that the "critical pH" concept has been enshrined as dogma. The critical pH of 5.5 was derived from the theoretical solubility of enamel in saliva and not plaque fluid. However, Dawes [34] points out many other factors determine whether enamel dissolves. The level of calcium, phosphorus, and F in plaque fluid are major determinants [35]. Clearly, as pointed out by Stephan, it may vary from person to person and from tooth to tooth. The "critical" pH varies over a wide margin and may be as low as 5.1 in plaque fluid. The "critical" pH for dentine and cementum, because of their lower mineral content may be much higher than that suggested for enamel.

Kind to teeth tests

As a direct result of identifying a "critical" pH, significant interest developed in Europe to a lesser extent in USA on identifying snacks and candies that would not lower the pH of plaque below 5.5–5.7 [13, 36, 37]. Manufacturers could submit their products for testing and earn the right to place a label on their product "Kind to Teeth" implying that the product was non-cariogenic. More detailed research into the physiology of dental plaque revealed several difficulties with this approach, not the least of which is that each product is of course just part of an overall dietary intake. This avenue also carried the implication that acid production was the sole determinant of cariogenesis.

Several authors have pointed out the limited value of a single pH measurement [38]. Exposure of plaque to sugar may lead to enhancement of physiological activity that can hinder or enhance the virulence of plaque without lowering the pH values below the "critical pH." These would include, for example, but not be limited to, extracellular PS production, intracellular polysaccharide formation, and stimulation of alkali production.

Area under the curve

Different patterns of the Stephan Curve are frequently observed even if the overall shapes are basically similar. Several investigations have paid particular attention to the "area under the curve" as opposed to simply the lowest pH values recorded. This refers to that part of the curve where the pH remains below the "critical" pH. The longer the pH remains below the value (calculated from the area) the more damage it allegedly does to enamel. Unfortunately, this assumption has little evidence to support it [38]. In a clinical study involving caries-active children and cavity-free children, caries status of the individual was unrelated to plaque pH in comparable non-carious sites. Plaque on cavity-free teeth returned to resting pH values much quicker than did plaque on teeth with carious lesions [20].

Persistent low pH values in plaque in addition to possibly damaging enamel may result in having the effect of enhancing the selection of aciduric and acidogenic microorganisms through up regulation of genes responsible for acid tolerance [39] (for review, see Lemos et al. [40] and

Kajfasz et al. [41]). The enhanced tolerance leads to selection of highly acid tolerant flora, which in turn enhances the potential virulence of plaque. Thus, it is clear that more frequently sugar is ingested, the more virulent the plaque may become.

Plaque returning to physiological pH values

Stephan [3] observed that the pH value of plaque following exposure to sugar returned to "regular" values over time.

Several mechanisms may affect this process not the least of which is the presence of saliva. In the absence of saliva, pH may remain depressed over considerable time, an observation that explains the presence of rampant caries in subjects who have lost or reduced salivary flow [42].

The intrinsic buffering capacity of plaque also aids in the elevation of pH of plaque [35]. The volume of saliva in the mouth and rate of flow of the salivary film affects the buffering effect of saliva and the diffusion of acids from plaque [43]. Data from this study revealed that the salivary film moves at different rates in various areas of the mouth, which explains in part, the uneven distribution of carious lesions in the mouth.

It is also apparent that plaque has the capacity to generate alkali and dispose of acid through a variety of mechanisms. This concept was illustrated by Stephan [3] who observed that the inclusion of urea in test solutions of sugar could prevent the fall in pH values.

Urea may also be a major source of ammonia in the mouth [44]. *Streptococcus salivarius* is a primary source of urease in the oral cavity; in addition, *Actinomyces naeslundii* and *A. viscsous* also are sources of urease. Significant levels of urea are found in saliva of normal subjects (3.7 ± 3.7 ug/dl) [45], which is readily broken down to ammonia and carbon dioxide [46]. It is noteworthy that plaque from patients on renal dialysis and hence have elevated values of urea in saliva (73.6 ± 26.4 ug/dl) did not lower pH values of sugar solutions. Urea was also detected in plaque from dialysis patients (6.75 ± 16.8 ug/mg). Urea was not detected in plaque from normal subjects [47].

In a clinical study of caries free, caries inexperienced, and caries-active subjects, Gordan et al. [48] found significantly higher levels of urease actively in plaque from caries-free subjects compared with controls.

Ammonia may also be generated by plaque organisms in an additional distinct pathway. The arginine deiminase system is inducible at low pH values such as those found in plaque after exposure to sugar. It is found, for example, in *S. sanguinis*, *S. gordonii* and some mutans streptococci and *A. naeslundii*. The arginine deiminase system results in the catalysis of arginine (to ammonia and citrulline). Citrulline in turn is broken down to ornithine and carbamyl phosphate. Carbamyl phosphate can be furthermore degraded to ammonia and carbon dioxide [49–51].

More recently, a related system, agmatine deiminase system (AgDS), has been described in *S. mutans* and other oral streptococci [52]. However, the authors note, "thus while possession of AgDS would benefit *S. mutans* it is unlikely to have a positive effect in the persistence of acid sensitive species in oral cavity."

Attention has been drawn to the importance of malolactic fermentation as a major source of alkali production by oral streptococci [53]. It was noted that the capacity of *S. mutans* to produce alkali in vitro was greater that it capacity acid from sugar at low pH values. Furthermore, this reaction may act additively with the arginine deiminase system of *S. sanguinis*. It does not act with urease from *S. salivarius*. This reaction also serves to enhance the survival of an acidogenic organism in a highly acid milieu.

It is noteworthy that Gordan et al. [48] also observed that plaque and saliva from caries-free subjects produced significantly higher levels of ammonia from arginine than those from caries-active subjects.

Ammonia may also be produced from additional pathways. For example, Curtis et al. [54] demonstrated the presence of elevated amounts of δ amino valeric acid in plaque and suggested that plaque microorganisms could carry out the Stickland reaction [55], which could generate ammonia and carbon dioxide.

Several species of microorganisms in dental plaque have the capacity to metabolize lactate. For example, *Veillonella parvula* ferments lactate to proprionate, acetate, carbon dioxide, and hydrogen [56, 57].

Truly caries-free subjects in the absence of fluoride are extraordinarily difficult to identify. However, those who have been identified appear to have elevated levels of low molecular weight peptides in their saliva. The peptides may be rapidly metabolized resulting in formation of ammonia [58, 59].

Clinical implications

The earliest studies on pH in dental plaque were limited largely to investigating acid production in carious lesions and comparing results with those found in cavity-free surfaces. Most of the studies included adults only. It has been widely assumed that the pH changes observed in plaque, on sound surfaces, following exposure to sugar would differ in caries-active subjects compared with those observed in cavity-free persons. In an extensive study, however, Fejerskov et al. [20] using a palladium-touch microelectrode showed that the carious sites of individual children were unrelated to plaque pH in comparable

non-carious sites. This observation is consistent with that of Rankine et al. [60] who also noted the absence of differences in pH values of plaque fluid from children with low caries activity compared with that from children with high caries incidence. Thus, a single observation on the response of plaque pH to sugar exposure is of little value. It is remarkable, despite the overwhelming interest in acid production by plaque how little the physiology of plaque biofilms has been explored following exposure to sugar. For example, the references in the literature to plaque acid production outnumber their reference to alkali by more that 50:1! Furthermore, synthesis of EPS by plaque in situ following exposure to sucrose has received scant attention. It is clear that distinct reactions are occurring at various time points in the Stephan Curve and it is assumed that mineral is being dissolved at various stages; however, direct experimental evidence is lacking.

In an elegant study, Dong et al. [19] explored the association between plaque acidogenicity, and both the prevalence and incidence of dental caries in 12-year-old children. They noted that cavity-free subjects had a higher maximum pH in their plaque as a much faster return to high pH values after 30 min. They also observed plaque from cavity-free subjects recorded less time below pH 7 than did plaque caries-active children.

It is important to note that none of the other classic parameters for example "minimum pH" in plaque after sugar area of curve below critical pH of 5.5 and time below critical pH were statistically significant.

These observations once again illustrate the danger of focusing on just one parameter, i.e. acid production in dental plaque. The concept of the importance of alkali production in modifying the virulence of dental plaque is not novel, but certainly has been neglected.

Any discussion of acid and dental caries is incomplete if the milieu within which the acid is formed is excluded [61]. For example, by labeling a food product that contains sucrose kind to teeth because it does not lower pH to 5.5–5.7 can nevertheless lead to enhanced plaque production through formation of elevated amounts of glucan and fructan. Glucan has been shown to be an essential property for the expression of virulence by *S. mutans*. Thus, although the pH may not fall to "critical" values, the potential virulence of plaque may be enhanced through enhanced glucan and fructan formation [62].

It has been proposed "that a deficiency in base formation especially that from arginine, may be as great or a greater risk for the development of dental caries as excessive formation of acid from fermentable carbohydrate" [63–65]. This observation is supported by data from a clinical study [48] who further ask, "Could alkali production be considered an approach for caries control?" as noted above, they reported significantly elevated levels of arginine deiminase activity in saliva from caries-free persons compared with caries-active persons. Further support for the concept resides in the observation that lower urease activity was found in saliva and plaque in children who had elevated levels of dental caries [66].

A novel approach to enhance alkali production in vivo in a rodent model was explored by Clancy et al. [67] who showed that animals infected with a recombinant ureolytic *S. mutans* experienced reduced incidence of dental caries that was associated with ureolytic capacity of dental plaque. Thus, in principle, it has been established that alkali production in situ can prevent or reduce the incidence of dental caries, an observation that leads strong support to the suggestion of Dong et al. [19]: "Our only significant finding was a stronger pH rise at the end of the Stephan Curve in our caries-free subjects and the role of base production in this feature might be studied with profit in the future."

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