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PLAQUE METABOLISM AND SUBSTRATE CONCENTRATION

by

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There is now an abundance of data to indicate that the bacterial production of organic acids in the dental plaque plays an important role in the aetiology of caries. It is quite natural that many attempts should have been made to inhibit the production of acid or to neutralize the acids produced. Enzyme inhibitors may be introduced into toothpastes or into the food. A substrate leading to alkali production in the plaque may be added to a toothpaste or perhaps to the diet. However, it is then necessary to know how the rate of acid or alkali production is dependent on substrate concentration and inhibitor concentration.

Production of alkali from nitrogen compounds such as amino acids and urea may play a part in the maintenance of the pH of the dental plaque — which is often found to be higher than that of the saliva in contact with the same plaque, (*Kleinberg,* 1961) and may contribute to the production of a milieu favourable for the existence of calculus. It is thus probable that differences in the concentrations of substrates leading to production of alkali and acids determine the pH of the dental plaque and influence the carious dissolution of the hard tissues, calculus formation and enamel remineralization.

In the present paper the anaerobic production of acids in suspensions of dental plaque material at different concentrations of glucose and sucrose is determined. Ammonia production of dental plaque material at different urea concentrations is also

studied and it is shown that acid production from the sugars studied and alkali production from urea are dependent in differing manners on substrate concentration.

EXPERIMENTAL PROCEDURE

Production of ammonia from urea by dental plaque material at different urea concentrations

Patients were allowed to accumulate dental plaque material for two days and appeared at 9.00 a.m. in the laboratory without having eaten or brushed their teeth. Plaque material was taken with a scaler and was transferred to a small spoon made of stainless steel. Saliva was removed by means of blotting paper. The spoons were kept in a moist chamber in order to avoid drying, (*Frostell & Larje,* in print). The spoons were weighed to the nearest tenth of a milligram on a Sartorius Selecta balance, and the wet weight was determined. The material was then transferred to 0.5 ml of Brain-Heart-Infusion (Difco) and was suspended in 9-12 ml of a buffered Ringer solution (NaCl, 8.0; KCl, 0.2; $CaCl_2$, 0.1; $Na_2HPO_4 \cdot 2\ H_2O$, 0.89; KH_2PO_4; 0.68 g/l).

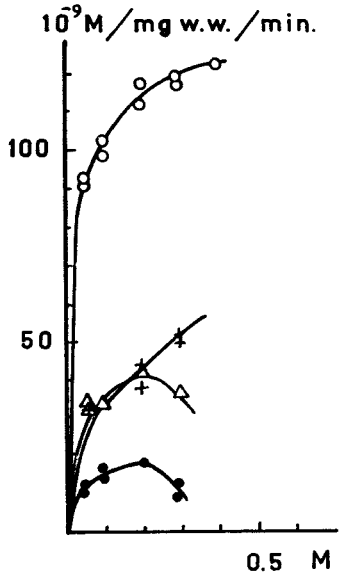


Fig. 1. Ammonia production from urea by oral staphylococci at different concentrations of urea.

Experiments were performed in the following way: In a water bath (37.0° ± 0.1) was placed a series of tubes containing 2.0 ml of a 0.2 M phosphate buffer (pH 6.9), 1.0 ml of plaque suspension, and 3.0 ml of distilled water. The experiment was started by adding 1.0 ml of a urea solution to each tube except one. The final urea concentrations were: 0.048 M, 0.095 M, 0.19 M and 0.29 M. In one experiment a tube containing 0.38 M was included. Urea decomposition was allowed to go on for 90 minutes, except in one experiment in which this period was 115 minutes.

Ammonia production from urea was determined as previously described (*Frostell*, 1960). Two controls were run with each concentration of urea in order to determine the decomposition of urea in the Parnas-Wagner apparatus.

In all, five experiments were performed. Representative results are given in Fig. 1. Two types of curves were found, one with an optimum at approximately 0.2 M and one which perhaps had an optimum at a higher concentration.

Urease activity of staphylococci at different urea concentrations

The author has previously found that oral staphylococci are often urease-active, and the activity of some selected strains has been quantitatively determined (*Frostell*, 1959). However, it was shown that in plaque material staphylococci capable of colony formation on Difco's Staphylococcus Medium 110 were too few in number, as determined by the methods used by the author, to account for the urease activity of such material. The author believes that other organisms may be responsible for the urease activity exerted by plaque material, but efforts to isolate these organisms have so far failed.

The urease activity of staphylococci at different urea concentrations has been studied previously (*Frostell*, 1959). It may be observed that none of the curves obtained with plaque material resembles that found earlier with staphylococci. This fact provided a reason for re-investigating the influence of urea concentration on the urease activity of staphylococci.

Urease activity of staphylococci has been determined from the amount of ammonia produced per unit time per unit weight

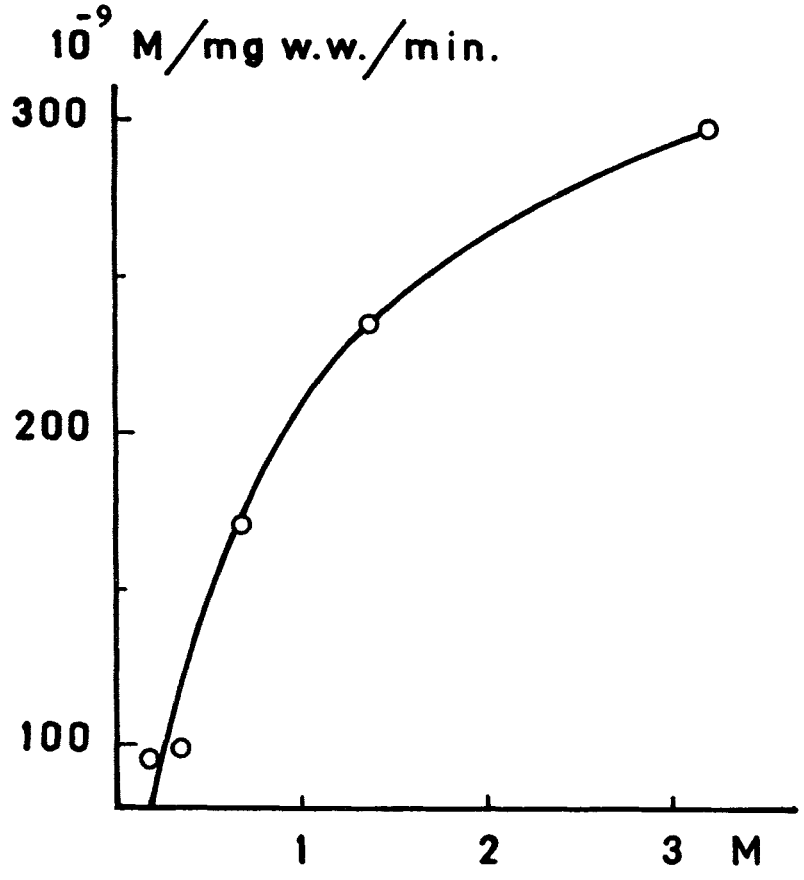


Fig. 2. Ammonia production from urea in suspensions of dental plaque material at different concentrations of urea.

during experiments with the titration method as previously described and by the method outlined above. These last-mentioned experiments were performed with a suspension of staphylococci produced as previously described (*Frostell*, 1959); owing to the high activity of the cells, the experimental period was only 15 minutes. The results were somewhat conflicting, partly owing to the fact that a great deal of ammonia may be produced by decomposition of urea during the ammonia determination. A representative curve is given in Fig. 2.

Urease activity increased very quickly up to a urea concentration of about 0.1 M. In contrast to the results previously obtained it was found that urease activity of staphylococci increased further with increasing urea concentration up to about 2—4 M. At still higher concentration of urea the urease activity again diminished.

Acid production by dental plaque material at different glucose or sucrose concentrations

Plaque material was gathered as previously described. The wet weight was determined. The material was homogenized in 3.0 ml of a Ringer solution in a glass tissue grinder, as will be described in greater detail in a future paper. The suspension was then made up to 10.0 ml with the same Ringer solution. A few millilitres of the suspension were dried for 24 hours at 105° C for the determination of the dry weight. Experiments were performed with the rest of this suspension as follows:

In a number of tubes containing 2.0 ml of a phosphate buffer (pH 6.90, 0.1 M), 3.0 ml of a sugar solution were pipetted. Nitrogen gas that had passed over red-hot copper and through distilled water was passed through the tubes, which were kept in motion in a water bath at 37° C. After gassing for 5 minutes, 1.0 ml of the plaque suspension was added, and the experiment was allowed to go on for 60 minutes. The pH of the tubes — which had been verified beforehand — was now between ½ and 1 unit lower than at the start. A blank was run containing only buffer, plaque suspension and distilled water. The tubes were titrated back to initial pH with 0.01N NaOH. Between 0.5 and 1.5 ml were usually required, except in cases where the activity was inhibited by high concentrations of sugar. A correction was made for the blank.

Three experiments were performed with each sugar. The percentage concentrations of sugar were 0.05, 1.0, 5.0, 10, 15, and 20 (Fig. 3 a) except in one experiment with glucose when 5.0 per cent was omitted and 40 per cent was used instead. Molar concentrations of glucose were 0.003, 0.06, 0.28, 0.55, 0.83, and 1.11. Two separate experiments were made with a glucose concentration of 0.0015 M. Molar concentrations of sucrose were 0.0015,

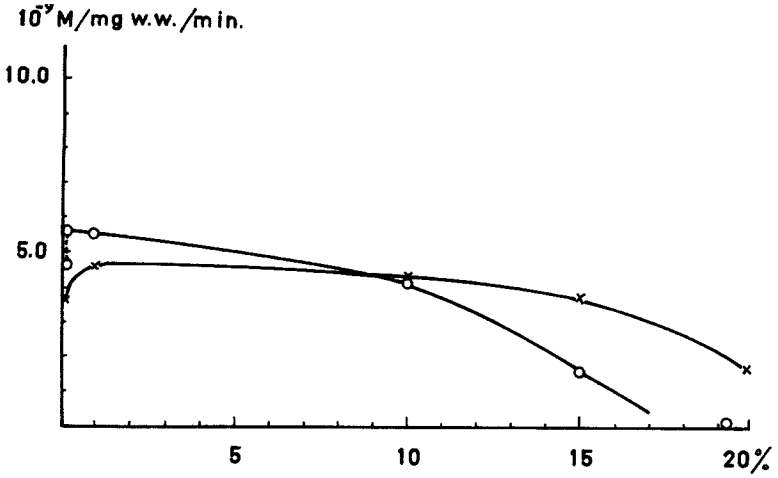


Fig. 3 a. Acid production from glucose and sucrose in suspensions of dental plaque material at different concentrations of sugar. Sugar concentrations expressed in per cent.

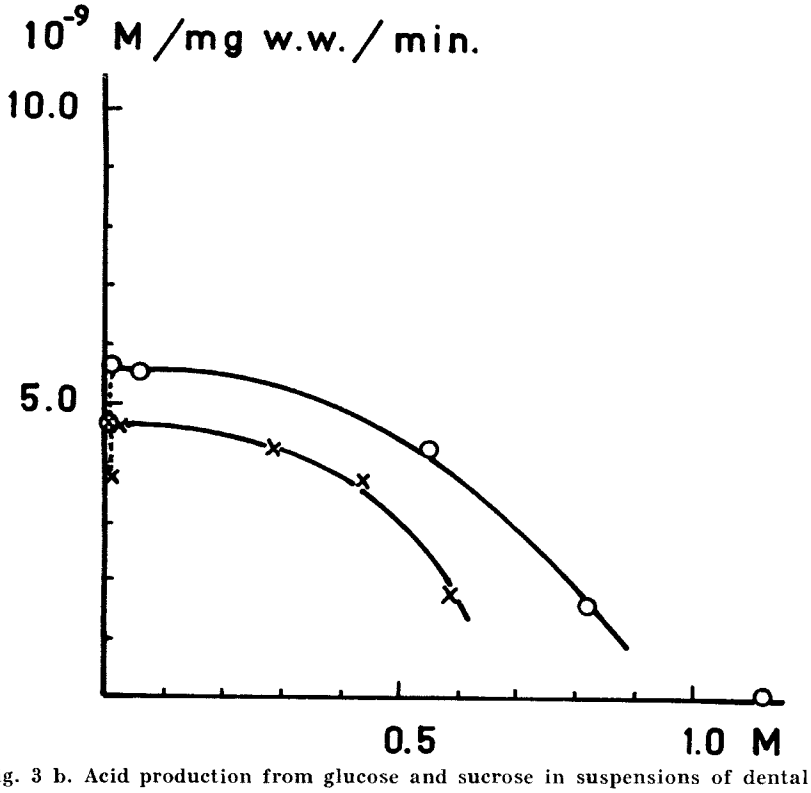


Fig. 3 b. Acid production from glucose and sucrose in suspensions of dental plaque material at different sugar concentrations. Sugar concentrations expressed in moles per liter.

o———o glucose

×·····× sucrose

0.03, 0.15, 0.29, 0.44, and 0.59. The curves presented represent the means of three experiments. It may be noted that when the concentration of the sugar is expressed in moles the two curves resemble each other closely (Fig. 3 b). Some points have been omitted from the curves if one of the values were lacking.

Acid production from glucose reached its optimum under the experimental conditions at very low sugar concentrations (0.003 M—0.06 M). Glucose concentrations over 0.6 M were inhibitory. A glucose or a sucrose concentration of 0.003 M gave optimum activity but 0.0015 M did not give optimum activity. The mean acid production activity from glucose at 0.0015 M was 86 per cent of the activity at 0.1 M.

DISCUSSION

As may be observed in Fig. 1, alkali production from urea by dental plaque material could be determined with some accuracy by the method used. Type II resembles the curve obtained with staphylococci, but Type I is different. It is believed that at least two different urease enzymes are present in the oral cavity.

The observation that the urease activity of staphylococci and of dental plaque material varies with the urea concentration as now described makes it very important to realize that the urease activities earlier observed (*Frostell,* 1959, 1960) must be referred to the urea concentration as well as to several other factors prevailing in those experiments. As stated before, the results do not refer to conditions in the oral cavity or in saliva where the urea concentration is very low, viz. about 0.002 M, (according to *Hench & Aldrich,* 1922, *Albrechtsen & Thaysen,* 1955). It is obvious that the ureolytic activity of dental plaque material as well as that of staphylococci will vary within wide limits, depending on the urea concentrations, but that the staphylococci will be about 10 times as active as plaque material which in turn is about 10 times as active as dental calculus (*Frostell,* in print) at a certain urea concentration. However, at concentrations over 0.3 M plaque ureolytic activity is often inhibited.

It is also obvious that when the titration method is used for the determination of urease activity (*Frostell,* 1959, 1960) it is important to avoid great changes of the volume of the bacterial

suspension during the experiments, since that will cause a change in the concentration of urea unless urea is added with the titrator solution. This has been pointed out before (*Frostell*, 1960).

When the acid production from glucose and sucrose in suspensions of dental plaque material is determined according to the method here described, it is important to explain that the activity observed must be referred to the experimental conditions and not to conditions prevailing in the oral cavity. For example, it has been observed that saliva contains some factor or factors which stimulate the glycolysis and the cell respiration (*Hartels & Wasdell*, 1955; *Frostell & Blomberg*, 1957; *Wasdell*, 1962). It is probable that the acid production activities presented here are somewhat lower than those which would be found if the determinations were performed in the presence of saliva. In 1950 *Strålfors*, however, determined the time required for the pH of dental plaque material to sink from neutrality to about 4.5. He also determined the buffering capacity of dental plaque material. From his figures, obtained in experiments with 10 persons, the acid production rate may be estimated at approximately 6×10^{-9} M per mg wet weight per minute between pH 6.5 and 5.5. The present results agree fairly well with this figure. In a series of experiments with several different sugars it was found that the mean activity under the present experimental conditions was about 8×10^{-9}. If the experimental period is kept short (30 minutes), even higher activity values will be obtained as the activity gradually decreases during the experiment. The author proposes to return to these problems in a future publication.

It is now interesting to compare the alkali production activity of dental plaque material with the acid production activity from glucose or sucrose. Fig. 4 gives an approximate idea of the relation between the acid production from sucrose and the ammonia production from urea in suspensions of dental plaque material at different substrate concentrations. Fig. 4 does not refer to experiments with the same plaque material, but in the author's experience these two curves may be representative as though they were obtained with material taken from one patient. It must be kept in mind, however, that when urea is hydrolysed, carbonic acid is also produced in such a quantity that the total alkali production is about 30 per cent lower than is indicated by

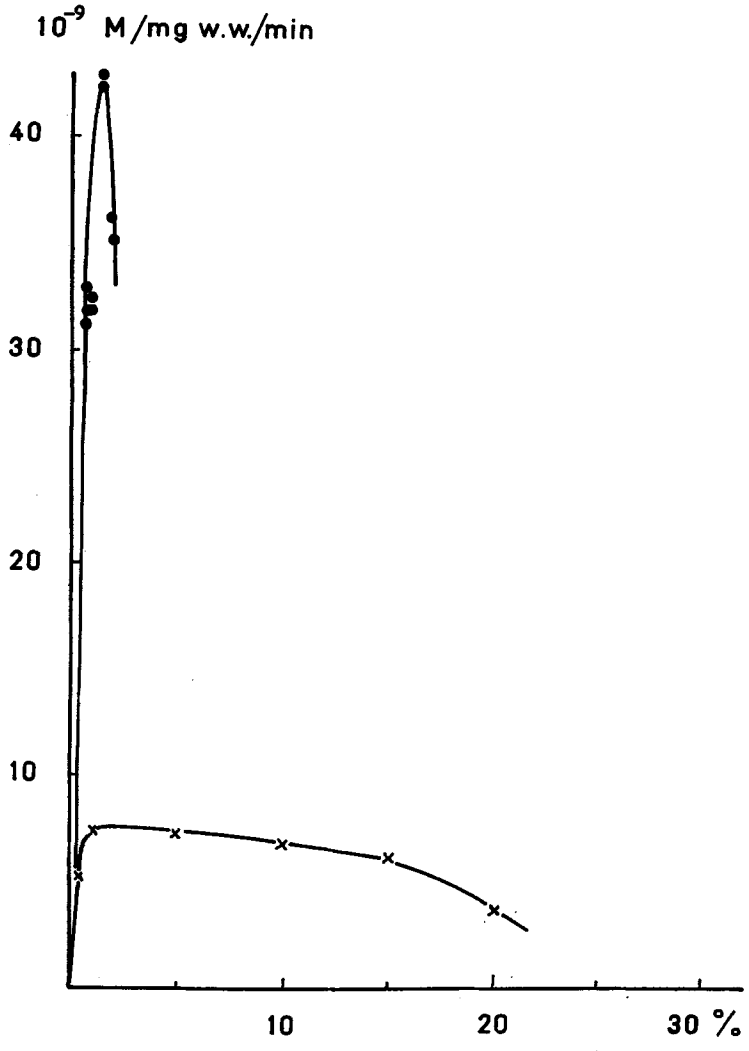


Fig. 4. Ammonia production from urea and acid production from sucrose at different concentrations of the substrates. A comparison of representative curves.

●——● ammonia production

×——× acid production

the curve. Anyhow, it may be seen that within a certain area of a comparatively high urea concentration the acids formed from sucrose will be completely neutralized by alkali formed from the

urea if the two substances are introduced into the suspension simultaneously. The figure gives some indication of the possibilities and also of the difficulties involved in efforts to use urea for the prevention of dental caries.

If, for example, urea or a urea substitute were to be mixed into sucrose powder, the urea concentration must be kept quite high since sugar is often used in diluted solutions. It would, for practical reasons, be extremely difficult to maintain, in all the different situations when sucrose is used, such a urea concentration that it would completely neutralize the acid production from sucrose within the wide sucrose concentration area which allows an appreciable acid production. If, however, urea could be coupled with a substance which adhered to the teeth and slowly gave off urea, it is probable that small amounts would be sufficient to cause an alkali production which neutralized or partly neutralized the acid produced from sugar to such a degree that the dental hard tissues would be protected against the acid attack. Thus, urea must be present in the oral cavity for many hours during the day and it is not to be expected that the introduction of urea — which is easily eliminated from the oral cavity — for example into a toothpaste used twice a day (see *Hawes & Bibby*, 1953), would have any appreciable effect as a prophylactic against dental caries.

Urea has been mixed into the food of experimental animals (*Chernausek & Mitchell*, 1950; *Mitchell & al.*, 1952) but has had little if any effect against dental caries. However, dental caries in laboratory animals (hamsters, rats) appears in a higher pH-range (about pH 7) than does human dental caries (pH 4.5—5.5). It may thus be expected that a prevention of the pH-fall after sugar intake by urea ingestion would be more easily obtained in man than in these laboratory animals.

The activity-concentration curves for glucose and sucrose are interesting to compare. Sucrose may be utilized for acid production within a wide concentration range as compared with glucose, when the concentration is expressed as percentage weight per volume. The difference, however, seems to be only apparent: when the concentration of the sugars is expressed in moles the curves closely resemble each other, i.e. it appears probable that the microorganisms are capable of fermenting the same number

of monosaccaride molecules per unit time at the different substrate concentrations irrespective of whether glucose or sucrose is the substrate.

If an enzyme inhibitor were to be mixed into the sugar used for the sweetening of foods in order to prevent the production of acids in the oral cavity from such foods it is obvious that the inhibitor concentration would have to be kept high enough to prevent acid production at very low sugar concentrations. This would mean that a great part of the sugar-inhibitor mixture used as sugar would consist of inhibitor even if the most effective inhibitors known were to be used. Unless the inhibitor is completely non-toxic and tasteless and causes no harmful effects whatsoever, it is thus clear that the inhibitor concentration must be made independent of the sugar intake or individually standardized for all the different types of foods. This would be rather difficult to achieve.

In this discussion no regard has been paid to all the different problems that arise when the diffusion of sugars, urea and enzyme inhibitors into the dental plaques is taken into consideration.

SUMMARY

Alkali production from urea and acid production from sugars in suspensions of dental plaque material varies with the concentration of the substrate. The dependence of plaque metabolism of substrate concentration was determined for urea, glucose and sucrose respectively. Some experiments were also performed with oral staphylococci.

It was found that the concentration-dependence of the urease activity of dental plaque material was different from that of the glycolytic activity of such material. Some practical consequences of these results are discussed.

RÉSUMÉ

LE MÉTABOLISME DE LA PLAQUE DENTAIRE ET LA CONCENTRATION DE SUBSTRATE

La production alcaline d'urée et la production acide de glucose entre des suspensions de micro-organismes variées avec la concentration de la substrate. On a déterminé le dépendance du

métabolisme de la plaque dentaire quant à la concentration de substrate dans respectivement urée, glucose et sucrose. Quelques expériences ont aussi été faites avec des staphylococques oraux.

Il a été démontré que la dépendance de l'activité uréolytique des matériaux de plaque dentaire était différente de celle de l'activité glucolytique. Quelques conséquences de ces résultats sont discutées.

ZUSAMMENFASSUNG

PLAQUEMETABOLISMUS UND SUBSTRATKONZENTRATION

Die Alkaliproduktion aus Harnstoff und die Säureproduktion aus Zucker in Suspensionen von dentalem Plaquematerial variiert mit der Konzentration des entsprechenden Substrates. Die Abhängigkeit des Plaquemetabolismusses von der Substratkonzentration wurde für Harnstoff, Glucose und Saccharose bestimmt. Einige Versuche wurden auch mit oralen Staphylokokken ausgeführt.

Es zeigte sich, dass die Konzentrationsabhängigkeit der Ureaseaktivität des dentalen Plaquematerials sich von der Konzentrationsabhängigkeit der glücolytischen Aktivität des gleichen Materials unterschied. Die praktische Bedeutung der Ergebnisse wurde diskutiert.

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