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The Buffer Value of Saliva and its Relation to Dental Caries.[1]

By

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Introduction.

In the research work on dental caries, the rôle of the saliva has been stressed by a great number of investigators. True dental caries can arise only when the tooth is in connection with the oral cavity and is surrounded and moistened by the oral fluids. It is therefore quite natural that the analysis of the external factors concerned with the origin of caries has included the study of the qualities of the saliva in relation to dental decay. Despite the vast amount of work done by able investigators, there are still many basic problems to be examined, some of which will be discussed in the following.

The acidity of saliva has played an important part in the theoretical discussion of caries. The carious disintegration of the tooth was by many early writers regarded as the effect of acids (viz. an acid saliva) upon the lime-salts of enamel and dentine. The more modern conception of the formation of acid as a localized phenomenon in the mucinous plaque or food débris cannot leave the acidity of the saliva without consideration, as the reaction of the saliva might have influence on the biological and chemical processes in the plaque. The rate of acid formation in the plaque can possibly be modified by the composition of the saliva and its buffering power, and on the other side, the chemical activity in the plaque may be reflected in diluted form in the saliva and,

[1] This article is part of the research studies of the group for caries investigations at the Dental Institute of Stockholm.

for instance, result in changes of the reaction. Any attempt to establish a definite quantitative or qualitative connection between the properties of the saliva and the activity of caries has however been of no avail. The results of different authors have not been consentient.

Regarding the relation between the actual acidity of saliva and caries, most investigators have noted a small difference between the *mean values* of caries susceptibles and caries resistants, the former having a lower pH average than the caries free (KARSHAN, KRASNOW & KREJCI 1931, KARSHAN 1399, HUBBELL 1933, USHIKUBO & IWAMOTO 1937). Women have a slightly more acid saliva during pregnancy (FRIESELL & VOGT 1926, ZISKIN & HOTELLING 1937, SCHMIDT-NIELSEN 1946 a).

KRASNOW 1938, HANKE 1939 and BELDING & BELDING 1939 find more significant differences in the pH of caries susceptible and caries resistant persons.

BRODSKY 1933, BUNTING 1936, HAWKINS 1939, and TURNER & CRANE 1944 could demonstrate no correlation between salivary pH and amount of caries.

The acid neutralizing power of saliva or the potential alkalinity (and acidity) of saliva has been studied in its relation to caries by many authors. The methods have been varying. *e. g.* colorimetric or potentiometric titrations against a known acid or base, and determination of CO_2 combining capacity. Most investigators find a difference in neutralizing ability between the saliva from susceptible individuals and that from caries resistant ones, but they ascribe more or less value to these findings. The problem is complicated in so far as the potential alkalinity of the saliva also is a function of stimulation, activated saliva having greater neutralizing power than has resting saliva. MARSHALL 1915 found that the buffer value of resting saliva was not a factor in the "causation or progress of caries", but that the activated saliva was a factor in "immunity". KARSHAN, KRASNOW & KREJCI 1931, HUBBELL 1933, FOSDICK & CAMPAIGNE 1939, MESSERLI 1946 have shown an existing relationship between the acid neutralizing ability of saliva and the amount of caries; the significance of which is of uncertain statistical value. DREIZEN, MANN, CLINE & SPIES 1946, however, stress the usefulness of a buffer determination as a test on caries activity. BUNTING 1936, BELDING & BELDING 1938, and SCRIVENER 1938 more or less disclaim the significance of such findings.

The reasons for these diverse results may be manifold. In the first place, perhaps, a real correlation does not exist between dental decay and the properties of saliva. Secondly, proper regard may not have been given to the complexity of the problem and to other connected facts. Furthermore there may be experimental errors in the evaluation of caries and in the assessment of alkalinity and acidity and in the chemical analyses. Since, in the following account the chief stress will be laid on the estimation of buffer values, the proper determination of pH is a salient point. It is probable, judging from the varying results of different investigators, that unsuitable methods have often been used, or that the writers have not been attentive to sources of error due to the inherent instability of saliva.

Theoretical Part.

The actual acidity of the saliva can be measured by several colorimetric and electrometric methods. Because of the protein constituents of the oral fluids the use of H-ion indicators that change colour at particular pH-levels will not give reliable results. For work on the salivary reaction, the electrometric measurement with a glass electrode seems to be the method of choice. The glass electrode is fit for use even in viscous, opalescent and opaque liquids, and it allows continued successive readings. Besides it covers a wide pH-range. A commercial apparatus with glass electrode (Radiometer PHM 21) was used for the potentiometric titrations of this investigation.

The actual acidity, the pH, of the saliva is dependent, among other things, on the amount of carbon dioxide dissolved in the liquid, and that amount is proportional to the partial pressure of CO_2. In the salivary glands the CO_2 tension can be approximately estimated to 40—42 mm Hg, but when the fluid is secreted into the oral cavity, where the partial pressure of CO_2 is changeable and indeterminate, the dissolved carbon dioxide may escape to a certain degree, thus decreasing the H-ion activity. The mixed saliva from a more or less open mouth, therefore, ought to be less acid than the secretion taken directly from the closed mouth or the glandular ducts, and a sample of saliva that has been standing in a beaker, even for a short time, will have a higher pH-value than it had in the mouth (B. SCHMIDT-NIELSEN 1946 b, and fig. 1, 2). To avoid loss of CO_2 when sampling saliva, various methods

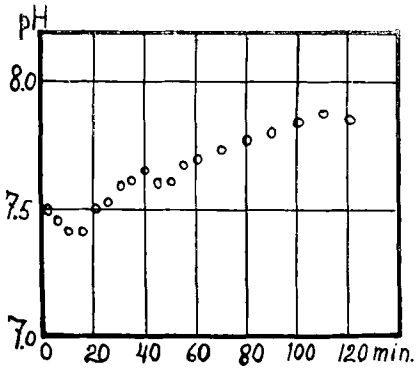

Fig. 1. pH variations of saliva during 2 hours in room temperature. (The increase of pH during the voiding of the saliva is not regarded.)

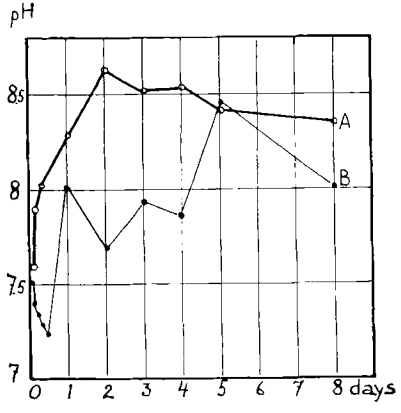

Fig. 2. pH variations of long standing saliva. Saliva, 10 ml. was divided in two parts. One part, B., was incubated directly, to the other, A., was added thymol as a preservative before incubating. pH-readings were made several times the first day and thereafter once a day.

have been tested. Collecting the saliva under a layer of paraffin oil is of limited value. It seems that a more practicable means of avoiding the above mentioned difficulties might be to saturate the collected saliva with a suitable gas-mixture of CO_2 or with alveolar air. For purely scientific work the saliva may be drawn from the mouth with some sort of a syringe and pH determined directly in the closed container.

Through the works of HENDERSON (1909) and HASSELBALCH (1916) we know that by determining the relation between the physically dissolved CO_2 and the bicarbonate we can calculate the H-ion activity of the fluid. The Henderson-Hasselbalch formula

$$pH = pk' + \log \frac{BHCO_3}{CO_2}$$

seems to hold true for body fluids such as blood, urine, and probably, saliva. For blood the constant $pk'_{38}{}^{\circ}$ has been determined to 6.1; for saliva, however, it is unknown. In the following the value 6.2 has been used as an average giving reasonable results.

The determination of bicarbonate and of free CO_2 in body fluids is usually carried out as an analysis of the total CO_2 content of the fluid, the amount of free CO_2 being calculated according to Henry's

law and subtracted from the total. The rest represents the bicarbonate, as the carbonate-ion is negligible at the pH-values in question. There are volumetric, manometric and titrimetric methods for the quantitative determination of CO_2. VAN SLYKE et al. have worked out an elaborate technique for biologic gas analyses with volumetric or manometric apparatus. (PETERS, J. P., VAN SLYKE D. D. Quantitative clinical chemistry II, London 1932.)

As the Van Slyke apparatus is rather expensive and not available everywhere, a titrimetric method suggested by WEST, CHRISTENSEN and RINEHART 1940, WENNESLAND 1941 and LEHMAN 1943 was also tried. The method of proceeding is essentially the following. In a Conway diffusion vessel the inner container is filled with a known $Ba(OH)_2$ solution (*e. g.* 1 ml; 0.1 N.) and in the outer annular compartment is dropped 1 ml of 2 N. H_2SO_4. The lid of the vessel is put on, well greased with vaseline at the margin in order to attain an airtight connection. Through a slit cut at the rim of the glass vessel, 1 ml saliva is delivered in the outer compartment by means of an exactly graduated syringe. The slit is then immediately filled with soft wax to avoid the escaping of carbon dioxide. The CO_2 liberated reacts with the $Ba(OH)_2$ and $BaCO_3$ is precipitated. After one hour the residual $Ba(OH)_2$ is titrated against HCl until thymol-blue changes to greyish green. Blanks without saliva are necessary to correct for CO_2 in the air and in the reagents. The difference between the amount of HCl required for the blank and the amount required for the saliva test is equivalent to the CO_2. Tests made as treble determinations of known bicarbonate solutions (5 and 10 mMols/litre) gave a mean error varying from 3 % to 14 %. There was no systematic difference between the theoretic value and the experimental CO_2 value. The method can possibly, when more worked out, be used for clinical saliva tests. For research work or when many determinations are necessary, the Van Slyke apparatus should be preferred.

In the following the manometric Van Slyke apparatus has been used for the CO_2 determination of small samples of saliva.

Saliva was allowed to accumulate without stimulation in the mouth for some minutes, while the mouth was held closed. Immediately after it was delivered in a test tube, 0.2 ml of saliva were drawn up in a pipette and the total CO_2 was determined in the Van Slyke apparatus. Table 1 column 2 shows the total amount of CO_2 in millimols per litre, the values being based on double determinations.

The saliva not used for the immediate analysis was kept in the open tube exposed to the atmospheric air for ten minutes and then tested for CO_2. As expected there is a fall in the total CO_2 and if the analysis of the same saliva is repeated one hour later

Table 1.

Total CO_2 of saliva (millimols/litre).

Specimen	Immediately	After 10 minutes	After 1 hour
1	5.00	2.63	—
2	5.23	3.50	1.17
3	6.20	—	1.73
4	4.01	2.88	0.79
5	5.23	2.37	1.21

Table 2.

Total CO_2 of saliva saturated with alveolar air at room temperature compared with the "immediate" value (millimols/litre).

Specimen	Immediate value CO_2 mM/litre	Saturation value CO_2 mM/litre
1	5.00	5.23
2	5.23	6.12
4	4.01	4.30
5	5.23	5.73

the CO_2 value has fallen still more to about 1/4 of the original value. It is apparent that the difference between the "immediate" value of total CO_2 and the one hour value must not only result from loss of the free CO_2 but also from loss of great amounts of bound CO_2. The pH, however, does not exceed the level of 8—8.5.

There is consequently no definite bicarbonate concentration in a particular saliva, the bicarbonate content having quantitative meaning only for a well defined CO_2 pressure and temperature.

Saturation with alveolar air in the manner suggested by Van Slyke and Cullen (1917) for blood plasma will readjust the lowered total CO_2 of saliva to a "normal" physiologic level. Salivas no. 1, 2, 4 and 5 were exposed to expired alveolar air for 15—30 minutes at room temperature. Table 2 shows a comparison between the "immediate" value and the "saturation" value.

The slight difference between the first and the second value can probably be ascribed mainly to the fact that no correction for temperature has been made, the absorption of CO_2 varying with temperature. For + 38° C the solubility of CO_2 in water can be expressed by the Bunsen absorption coefficient $\alpha = 0.545$ and for + 20° C $\alpha = 0.875$. For serum α 38° = 0.510. The exact

absorption coefficient of CO_2 in saliva is not known, but for preliminary clinical work the constants for solubility in water seem appropriate, especially since they have been determined for all temperatures that can here be questioned.

Alveolar air, or 5.5 % CO_2 air mixture, from a tank will give a partial CO_2 pressure of approximately 40 mm Hg and may be used with advantage for the saturation, if a thermostat is avail-

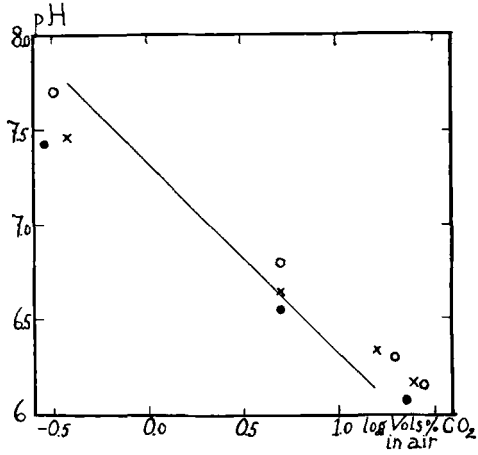

Fig. 3. The relation between pH and CO_2 contents in the atmosphere of three different salivas (○, ×, ●). The unbroken straight line shows the theoretical curve of a bicarbonate solution.

able to keep the liquid at body temperature. For the saturation a separatory funnel of at least 50 times the saliva is used and the gas mixture should first be drawn through a layer of moist glass beads in an Erlenmeyer flask. A few small crystals of thymol should be added to the saliva as a preservative. For small samples of saliva (*e. g.* 1—2 ml) and with "physiologic" CO_2-air mixtures it is not difficult to attain saturation, but difficulties of a practical sort arise, when there is need of a greater amount of saliva and of higher CO_2 tension.

The acidity, measured as H-ion activity, increases with growing CO_2 pressure, which is easy to demonstrate qualitatively as well as quantitatively. Blowing alveolar air through a sample of saliva that has been standing exposed to the air for a time will gradually lower the pH. If saliva is exposed to known gas mixtures of CO_2 in a separatory funnel, there will be a particular pH level

for each CO_2 mixture. Plotting these values against each other will give a curve of exponential character but not necessarily satisfying the Henderson-Hasselbalch equation (fig. 3). Probably the saturation of the liquid is not always complete. If the amount

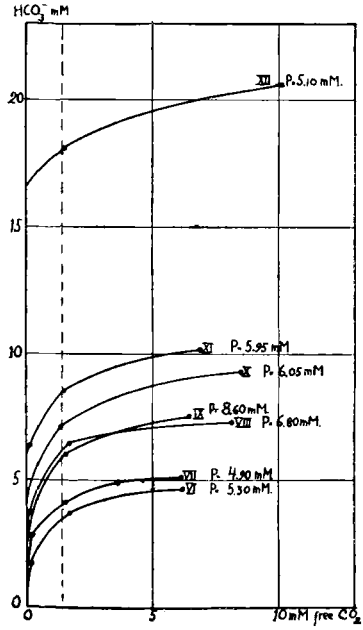

Fig. 4. CO_2 absorption curves of saliva. The calculated amount of physically dissolved carbon dioxide (proportional to the CO_2 tension) along the abscissa and the bicarbonate concentration along the ordinate. Adjacent to each curve the phosphate concentration of that particular saliva is denoted. Specimen 12 is stimulated, all the others are "resting" salivas.

of free CO_2 and that of chemically combined CO_2 at various pressures are plotted in a coordinate system, the following facts appear. For saliva there is a steep increase in the bicarbonate value up to a level, characteristic of the saliva in question. An additional increase in the CO_2 tension will mainly result in a higher absorption of dissolved carbon dioxide in the fluid. For an aqueous solution of bicarbonate of known strength, on the other hand, the curve is represented by a straight line on the known bicarbonate level (the conditions at extremely low CO_2 tension are neglected). For some of the "saturated" saliva specimens, the uncorrected curve showed a slight decrease in the chemically combined CO_2 by higher pressure, which can be explained as the graphic descrip-

tion of the above mentioned incomplete saturation. If, however, the values of the free CO_2 are corrected by applying the Henderson-Hasselbalch formula, the pH and the total CO_2 being known and the pk' estimated to approximately 6.2, we get the adjusted curves of fig. 4.

The CO_2 absorption curve of saliva will be similar to that of a solution of secondary phosphate, in as much as there is the initial ascent until an almost permanent level of bicarbonate is reached; for the phosphate solution approaching the molarity of the phosphate. As seen from the reaction

$$B_2HPO_4 + CO_2 + H_2O \rightleftarrows BH_2PO_4 + BHCO_3 \qquad (2)$$

the maximum amount of bicarbonate is equimolar with the phosphate. It is apparent, consequently, from the curves that, contrary to the conditions in blood plasma, *the phosphates are of great importance in the buffer systems of saliva.* The inorganic phosphate contents of the saliva specimens are denoted adjacent to the particular curve in the diagram (fig. 4). From the curves it can be inferred that part of the bicarbonate present is dependent on the amount of phosphate (and the CO_2 tension). Another part, however, is independent thereof. As the latter fraction can be estimated empirically as the difference between the total bicarbonate value at high CO_2 pressure and the phosphate concentration, it can be called the *residual bicarbonate* (b). The residual bicarbonate is obviously a very variable factor, probably a function of stimulation (specimen 12 is a paraffin stimulated saliva). Applied to an aqueous solution of $B_2HPO_4 + BHCO_3$, "the residual bicarbonate" is equal with the original amount of bicarbonate, and the "maximum amount of bicarbonate" is equal with the total phosphate-bicarbonate concentration of the solution. If the solution is exposed to carbon dioxide, the secondary phosphate will react according to formula (2) and bicarbonate will be formed to a certain degree. Mathematically the CO_2 absorption curves of any aqueous solution of phosphate-bicarbonate can, approximately and within reasonable limits, be deduced in the following way:

The equilibria are

$$pH = pk''_{(H_3PO_4)} + \log \frac{B_2HPO_4}{BH_2PO_4}$$

$$pH = pk'_{(H_2CO_3)} + \log \frac{BHCO_3}{CO_2}$$

Let the known total phosphate-concentration (cP) denote the unit and let all concentrations be given as fractional parts of cP.

$$x = \text{physically dissolved } CO_2$$
$$y = BH_2PO_4$$
$$b = \text{"residual bicarbonate"}$$
$$\log a = pk''_{(H_3PO_4)} - pk'_{(H_2CO_3)}$$

The equations are then

$$pH = pk''_{(H_3PO_4)} + \log \frac{1-y}{y} \tag{3}$$

$$pH = pk'_{(H_2CO_3)} + \log \frac{b+y}{x} \tag{4}$$

Subtraction gives

$$\log \frac{b+y}{x} \cdot \frac{y}{1-y} = \log a$$

$$y^2 + yb + yax = ax \tag{5}$$

(N. B. The result is given in fractional parts of cP.)

The theoretical curves of the diagram fig. 5 are calculated by means of this equation, the cP given the value of 5 mM/litre and b the values of 0 and 1 (*i. e.* 0 and 5 mM/l respectively). It is quite clear that the greater the original ratio bicarbonate/phosphate is, the flatter will the curve be. Looking at the experimental curves of the different salivas (fig. 4) we find that there is a rather good agreement with the theoretical curves.

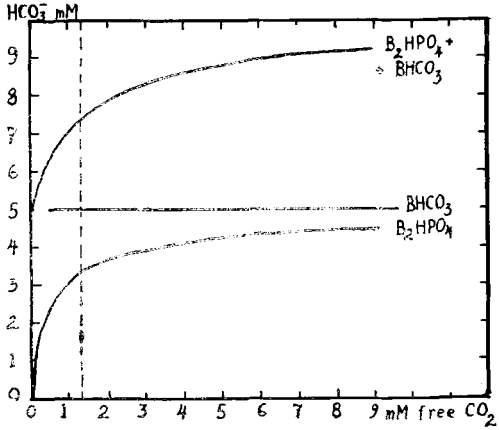

Fig. 5. Theoretically calculated curves for the CO_2 absorption of solutions of bicarbonate, phosphate and bicarbonate + phosphate. The curves are computed by means of the equation (5).

Table 3.

The salivary contents of phosphate (col. 2), maximum bicarbonate, calculations based on physiologic CO_2-tension (col. 3) and on a higher pressure (col. 4), and the "residual bicarbonate" (b) calculated on the two pressures (cols. 5 and 6).

1	2	3	4	5	6
Specimen	Phosphate mM/litre	Maximum bicarb. mM/l	Maximum bicarb. mM/l	"Res. bic." (b_1)	"Res. bic." (b_2)
6	5.30	5.45	5.30	0.154	0.00
7	4.90	5.78	5.55	0.88	0.65
8	6.80	9.40	8.25	2.60	1.45
9	8.60	9.93	9.11	1.33	0.51
10	6.05	10.22	9.93	4.17	3.88
11	5.95	11.72	11.50	5.77	5.55
12	5.10	21.70	—	16.60	—

Assuming then that the above deduction is valid also for saliva it may be possible to compute the "residual bicarbonate" *i. e.* that fraction of the total bicarbonate that is not dependent on the CO_2 pressure and the phosphate content. In table 3 the maximum bicarbonate and the residual bicarbonate are calculated by means of the above equation (5) and based on the values obtained from analyses of phosphate and bicarbonate content. (As seen from the table there is a small systematic difference between the value calculated on the physiologic CO_2 pressure and that based on a higher pressure, the nature of which difference has not been investigated.) By comparison of the table and the diagrams it is obvious that CO_2 combining protolytes or ampholytes other than the phosphate must play an unimportant rôle in the saliva.

By applying the equations (5) and (4) it is possible to compute the effect of phosphate on the slope of the bicarbonate curve of fig. 5. It can be shown that the phosphate contents can be held responsible only for a minor part of the discrepancies between the saliva specimens and the fixed bicarbonate curve of the diagram.

Previously it was suggested that the increased buffer value of stimulated saliva was mainly due to an increase of the "residual bicarbonate". As stimulation will not augment the phosphate concentration, the inference is that a growth of the total CO_2 of the saliva after stimulation means an increase of the "residual bicarbonate". Cf. SAND 1946.

Table 4.

Total CO_2 of resting and of paraffin activated saliva.

Specimen	CO_2 mM/l Resting saliva	CO_2 mM/l Activated saliva
13	5.82	10.20
14	3.79	7.40
15	5.30	10.70
16	4.29	7.40

The relation between stimulation and the amount of residual bicarbonate should be subjected to further studies on physiologic basis.

From the dissociation constants of carbonic and phosphoric acids it can be concluded that salts of these acids must play the most important buffering rôle at the physiologic pH levels of saliva. The capacity of a buffer system has its maximum at a pH level corresponding to the $pk_{(acid)}$ and in this specific case, the $pk^1_{(H_2CO_3)}$ is estimated to 6.2 and the $pk^2_{(H_3PO_4)}$ calculated to 6.9, the ionic strength taken into account. The inorganic buffer constituents of the saliva have consequently their greatest effect in the vicinity of the normal pH of the oral fluids. It must therefore be of considerable interest to perform an electrometric titration of salivas simultaneously analysed for the phosphate and carbonate contents.

Three specimens of table 3, viz. 10, 11 and 12, were titrated against 0.1 N.HCl (figs. 6, 7 and 8). As depicted on the figures, (the titration curve for a 10 mM mixed solution of phosphate and bicarbonate is also plotted for comparison in fig. 7) the salivas have buffering properties surpassing those of the phosphate-bicarbonate solution. Acid, equivalent to the maximum bicarbonate value of the particular saliva only presses down the pH to resp. 6.05, 5.10, 5.00 and does not alter the slope of the curve to any great extent.

It is evident that other buffer systems partly overlap the phosphate and bicarbonate and replace them down the pH scale. It does not seem probable that other inorganic substances form proteolytic systems that could offer sufficient resistance to the pH lowering acid. The proteins of saliva, though more diluted than in plasma, are on the other hand, certainly acting as effective buffers.

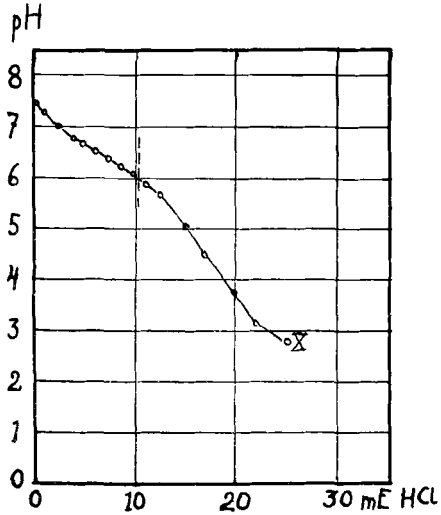

Fig. 6. Potentiometric titration curve of saliva specimen 10. Acid, equivalent to the "maximum bicarbonate", will press down the pH to 6.05.

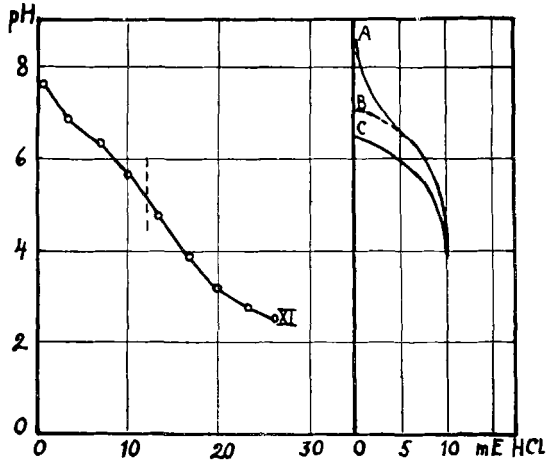

Fig. 7. Potentiometric titration curve of saliva 11. Acid equivalent to the "maximum bicarbonate" will press down the pH to 5.10. A, B and C are titration curves of a 10 mM solution of bicarbonate-phosphate, A directly titrated, B after saturation with 3.6 % CO_2 and C after blowing 25 % CO_2 through the solution. As seen, the curves A and B join above pH 6 and then coincide downwards.

However, the titration curves are not representative of what really occurs in the mouth when acid is formed and neutralized by the saliva; we must remember that under physiologic conditions the process takes place in an atmosphere containing CO_2.

Theoretically the titration ought to be performed after and during saturation *e. g.* with 3.6 % CO_2 at room temperature, but usually the titration seems to be carried out on salivas that have been standing for a time, which fact can be established for example by comparing the published curves of

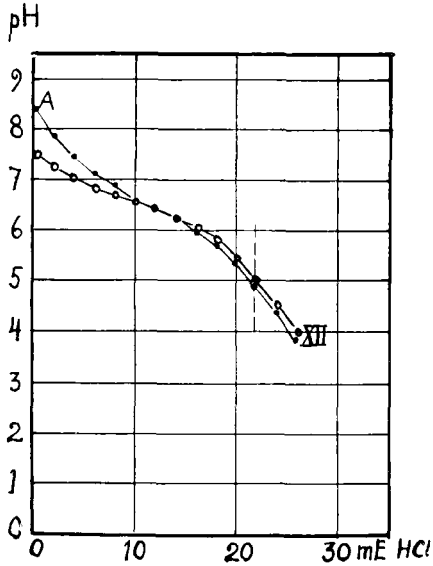

Fig. 8. Potentiometric titration curves of saliva 12. In the curve A, the saliva had been standing for an hour in an open Petri dish, the other curve represents a specimen of the same sample, that had been saturated with 3.6 % CO_2. The point of junction lies above pH 6.

DREIZEN et al. with fig. 8, where the difference between saturated saliva and a saliva that has been exposed to the air is obvious. It is then an important question whether this difference is of practical significance. The potentiometric titration curve of a particular saliva can be used to estimate the amount of acid necessary to lower the pH to a "dangerous" level, where disintegration of the tooth can take place. Conventionally this point can be set to pH 5. It is possible that the resistance to the lowering of the pH can be an indicator of the liability to caries of an individual (DREIZEN et al.). If the CO_2 saturation should have influence on the quantity of acid necessary, it is apparent that the curves should be of little value, proper regard not being paid to this fact. Looking at figs. 7 and 8 we find however that the curves of saturated and not saturated saliva (or a test solution) have a

point of junction or intersection just above pH 6 and then they practically coincide downwards. Provided the titration endpoint lies below pH 6 the effect of the physiologic CO_2 tension is insignificant, if we only want to ascertain the amount of acid necessary to press down the pH.

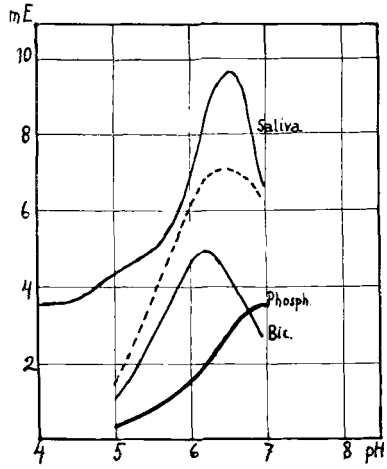

Fig. 9. Analysis of the buffer capacity, β, of the saliva 10. The "physiologic" pH is determined to 6.9, where the phosphate buffer has its maximum. The bicarbonate has its buffering maximum at pH 6.2. The broken line represents the resultant sum of the two protolytic systems with a maximum close to pH 6.5. The uppermost curve shows the empiric buffer capacity curve of the saliva in question. Cf. fig. 6.

If C_B means the amount of added base in milliequivalents per litre, the buffer capacity, β, at a particular pH level may be construed as

$$\beta = \frac{dC_B}{dpH} \text{ mE pr litre/pH unit}$$

β plotted as a function of pH will show a maximum at $pk_{(acid)}$ and minima at the equivalence points. The saliva 10 was analysed regarding the buffer capacity, β, of two proteolytic systems, viz. the phosphate and the bicarbonate. The total concentration of phosphate in this case is known to be 6.05 mM pr litre, the amount of bicarbonate on the other hand is variable and must be determined for a well-defined pH level. Taking pH 6.9 as a starting point (the calculated acidity by physiologic CO_2 tension) we find that one half of the phosphate exists as BH_2PO_4 ($pk = 6.9$). The bicarbonate must then be 7.05 mM pr l according to the Hen-

derson-Hasselbalch formula (pk' = 6.2, CO_2 = 1.4 mM). (The total CO_2 determined by means of the Van Slyke apparatus was 8.4 mM.) Fig. 9 gives a graphic representation of the calculated buffer capacity curve of the phosphate and the bicarbonate systems and the summed up resultant curve. The empirically estimated curve of the saliva is also depicted. The maximum buffering capacity of the saliva in question lies at pH 6.5 and agrees well with the theoretic maximum of the bicarbonate-phosphate constituents.

Clinical Part.

There are good reasons for the assumption that the resistance of the saliva against pH lowering acids is of importance as a caries inhibiting factor. The magnitude of this protection as related to other factors in the genesis of caries may be a matter of controversy, as well as there seems to be no indubitable evidence, that there is a real quantitative relation between the liability to caries and the buffering properties of the saliva. Recently DREIZEN et al. 1946 published a paper in which they maintain that there exists such a positive correlation as to make it possible to use the buffer value of a specific saliva as an individual susceptibility test. This test has also been used in an investigation (MANN et al. 1947). The reliability of the test, however, was among other things dependent on the nutritional status of the patients, so that it did not work so well in well-nourished persons as in mal-nourished. That fact must give rise to some doubt whether the method is satisfactory and serviceable. A good test should be comparatively insensitive to subsidiary circumstances but react promptly to changes in the principal facts.

As, however, the potentiometric titration of saliva is a rather simple procedure, it would be of considerable value, if the acid neutralizing power of saliva were an unequivocal gauge for the activity of caries. A study of the titration curves of caries active and caries resistant persons was therefore made, patients and students of the dental institute being test subjects. Hydrochloric acid, 0.1 N., was used (whereas DREIZEN titrated against lactic acid). The amount of saliva titrated was usually 6 ml, and 0.1 ml acid (in some cases 0.05 ml) was added between the pH readings. A glass electrode was used, adjusted to known buffer

solutions. Diagrams were plotted, the acid being recalculated to milliequivalents/lit.

As shown above there is no need of special precautions to prevent the escape of CO_2 dissolved in the saliva, if the titration is performed to an endpoint well below pH 6, the total amount of acid in any case being the same. The saliva should of course be

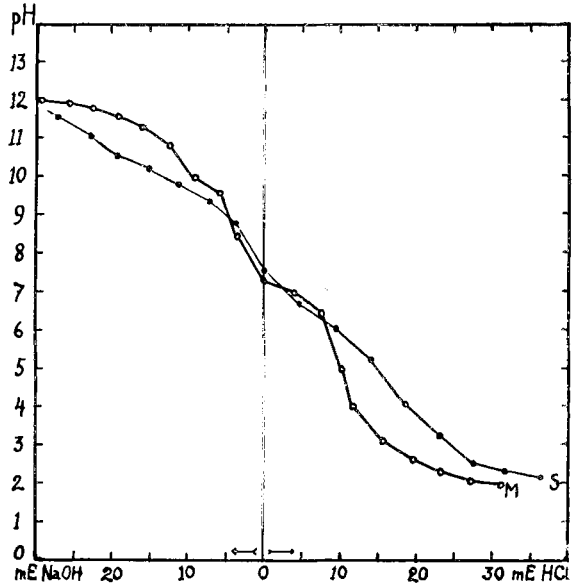

Fig. 10. Total buffer curves of two different salivas, M and S.

handled as soon as possible to avoid precipitation of insoluble lime salts, when the carbon dioxide escapes, and before fermentative processes by bacteria or tissue enzymes set in. The mode of collecting the saliva, however, may be open to discussion. MARSHALL 1916—1924 and many others have emphasized the difference between stimulated and non-stimulated saliva. The stimulated saliva has a greater acid neutralizing power than has the resting saliva, and MARSHALL founded his "salivary factor" on these conditions. DREIZEN titrated paraffin stimulated saliva. It seems however that the more or less stagnant resting saliva, that moistens the teeth between meals and in the night, should be of as great interest from the viewpoint of caries as the stimulated fluid that is secreted from the glands during mastication. The present tests were therefore performed with non-stimulated saliva,

but it must be borne in mind that the deliberate voiding of 6—7 ml saliva possibly implies a certain stimulation, the quality of which we know nothing about. All tests were made on saliva delivered at 11—12 o'clock, *i. e.* before lunch and certainly at least two hours after the preceding meal.

As it was not the purpose to perform a thorough analysis of the covariation to begin with, it was thought most expedient to

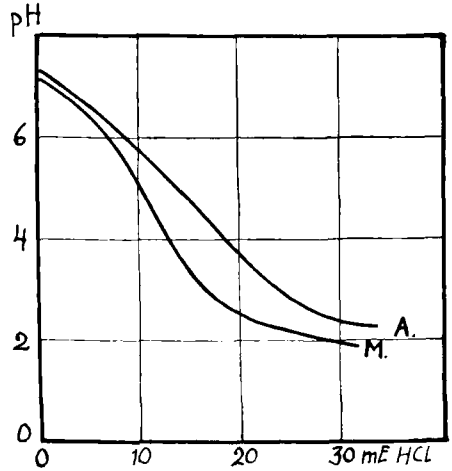

Fig. 11. Potentiometric titration curves. "A" caries resistant, "M" caries active.

compare two extreme groups, viz. one group of caries susceptible persons and one of caries resistant individuals. The caries resistant group was the most homogeneous as it consisted of mostly male students of approximately the same age and in the same social circumstances. There was no indication of actual decay going on, and the caries index was very low (0.0—0.15). The caries active group was more heterogeneous in as much as there were both students and male and female patients mixed. All had however rampant caries, and the caries index was extremely high (0.65—0.97 according to WESTINS index).

Several saliva determinations were made on each student, and it was evident that the titration curves of an individual are very stable and representative for that person. If plotted with the pH along the ordinate, and the amount of acid along the abscissa, some curves showed the form of a reversed S, while others were more straightlined. In many cases there is an inflexion just around pH 6, the curve becoming somewhat steeper. The total buffer

curve was determined for some salivas, *i. e.* the saliva was titrated both against 0.1 N. NaOH and against HCl. Such compound curves are shown in fig. 10. It does not seem impossible that the form of the curve might indicate the liability to caries. A straightlined curve, offering an evenly distributed resistance to the acid, might predominate among the caries resistant persons, whereas a more curved line, where the resistance suddenly (around pH 6) will

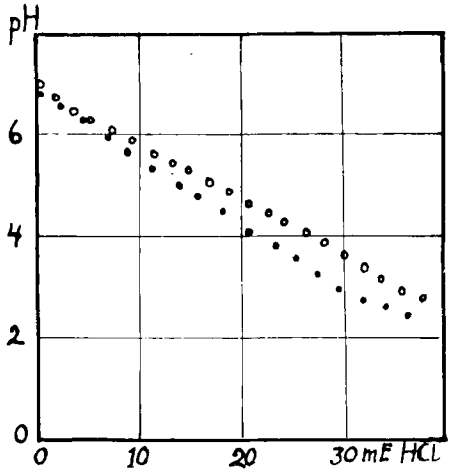

Fig. 12. Potentiometric titration curves, both equally straight-lined. One (○) from an absolutely caries free student, the other (dots) from a student with rampant caries.

diminish, might be prevalent in the caries active group. The material is not large enough to allow an analysis in this respect, but some examples will show that no such rule can be applied in routine work. Fig. 11 depicts the curves of a ten year old boy, caries free, and of his mother (caries active). They seem to conform to the above mentioned hypothesis, but if the curve of another carious patient (case 49), and that of an absolutely caries free student (case 37) are plotted together we find both equally straight-lined, fig. 12. On the other hand there are also S-shaped curves in both groups.

The amount of acid necessary to press down the pH to a particular level, viz. pH 6, 5, 4 and 3 respectively, is shown in table 6. The titration values computed as milliequivalents/litre are expressed for caries actives and caries resistants separately, and the means ($\bar{x}$) of each group are given in the table. The means are

Table 6.

Amount of Acid (mE/l) necessary to lower the pH of caries active and of caries resistant salivas.

Caries resistant.

Case	age	to pH 6 mE/l	to pH 5 mE/l	to pH 4 mE/l	to pH 3 mE/l
31	26	4.50	10.07	17.30	23.7
32	23	5.83	9.17	12.17	15.5
33	23	10.00	14.17	17.33	21.7
34	26	9.50	14.50	18.35	22.5
35	20	8.33	15.00	18.83	23.7
36	26	7.75	14.85	22.50	28.7
37	22	9.00	16.50	24.67	31.0
38	23	7.67	15.17	25.83	33.5
39	25	10.00	18.00	29.00	37.0
40	23	8.00	13.50	17.50	21.5
41	13	8.00	14.50	19.50	23.5
x		8.05	14.13	20.26	25.17

Caries active.

Case	age	to pH 6 mE/l	to pH 5 mE/l	to pH 4 mE/l	to pH 3 mE/l
42	23	10.5	15.0	18.8	23.0
43	23	4.0	9.0	15.5	21.0
44	18	5.0	10.0	16.0	22.0
45	18	5.0	9.5	15.5	22.5
46	18	7.0	15.5	17.0	22.0
47	13	8.5	14.0	19.0	23.0
48	36	3.0	10.0	16.0	23.5
49	21	4.5	10.2	16.7	23.3
50	26	4.0	9.5	15.5	21.0
51	38	7.0	10.7	10.3	16.5
x		5.85	11.34	16.03	21.78
Probability		0.05[x]	0.01[xx]	0.01[xx]	—

consistently lower for the caries active group but the divergences are not great. An analysis of variance results, however, in establishing a very probable statistical difference between the caries active group and the caries resistant one, if the titration is performed to pH 5 or below. There is no need to pursue the matter further with a larger material.

The inference to be drawn from these results is that the buffering properties of resting saliva may be a modifying factor in the origin of dental caries. It is true that there exists a very probable difference between the means of caries active and caries resistant

persons, but the dispersion of the individual values in each group is such, as not to allow a distinction between the values in respect to the liability to caries. This fact can not be invalidated by enlarging the number of test subjects as the degree of variation (*e. g.* measured as the standard deviation, σ,) scarcely can be influenced by the number of individuals.

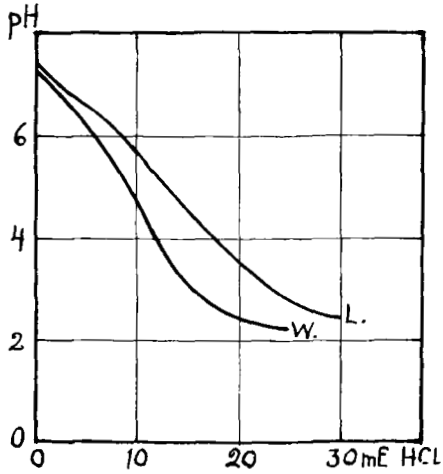

Fig. 13. Potentiometric curves. "L" extremely caries active, "W" caries resistant.

That the buffer value of saliva is a modifying, not a decisive factor in the origin of caries is also easily shown by analysis of the individual cases. The patient, case 46, a confectioner's apprentice, aged 18, had all liable surfaces of his teeth severely decayed, many of them beyond repair. There seems to be no doubt that the ravages of caries in this case mainly were due to his occupation. In spite of the rather good acid neutralizing power of his saliva, the occupational contact with sugar and fermentable carbohydrates (perhaps combined with intrinsic factors *e. g.* puberty) has predominated over the normal salivary protection and caused caries to occur. On the other side the poorly buffered saliva of case 32, caries resistant student, aged 23, has probably not been exposed to such stresses and consequently sufficed as a protection (fig. 13). It seems that only when the injuring factors are about equal with the protective ones, a variation or change in the acid neutralizing power might be of decisive significance to the origin of caries.

Summary.

There are reasons for the assumption that the resistance of the saliva against pH lowering acids is a caries preventive factor. Several authors have also used the buffer value of saliva as an individual activity test for caries.

By means of potentiometric titrations and of determination of the "CO_2 capacity" with the Van Slyke apparatus it could be shown that

1) the CO_2 absorption curve of saliva is in good agreement with that of a mixture of bicarbonate and phosphate. A theoretical deduction of this curve is given.

2) At "physiologic" pH levels the bicarbonate and the phosphate are the principal buffers, at lower pH values they are replaced by the proteins.

3) As the inorganic phosphate of the saliva is not influenced to any high degree by stimulation, the growth of the total CO_2 and of the neutralizing power in paraffin-activated saliva must be attributed to an increased production of bicarbonate in the salivary glands.

4) Because of the changeable ratio bicarbonate/carbon dioxide, the saliva is very unstable from the viewpoint of physical chemistry, and pH determinations should be made with due consideration to this fact.

5) Potentiometric titrations of "resting" saliva from two extreme groups, one caries inactive and the other caries active, showed a statistically very probable difference in potential alkalinity between the groups. The degree of variation within each group, however, was of such magnitude as not to allow the use of the buffer value as an absolute test on caries activity.

Zusammenfassung.

Man hat allen Grund anzunehmen, dass die Widerstandsfähigkeit des Speichels gegen pH-senkende Säuren einen kariesvorbeugenden Faktor darstellt. Mehrere Autoren haben auch die Pufferwirkung des Speichels als eine individuelle Aktivitätsprobe auf Karies verwendet.

Durch potentiometrische Titrierung und Bestimmung der "CO_2-Kapazität" mit dem Apparat von Van Slyke konnte man nachweisen, dass:

1. Die CO_2-Absorptionskurve des Speichels mit derjeningen einer Mischung von Bikarbonat und Phosphat gut übereinstimmt. Eine theoretische Herleitung der Kurve wird gebracht.

2. Bei "physiologischen" pH-Werten stellen das Bikarbonat und das Phosphat die Hauptpuffer dar, bei niedrigeren pH-Werten werden sie durch die Eiweissubstanzen ersetzt.

3. Da das anorganische Phosphat des Speichels nicht in höherem Grade durch Stimulierung beeinflusst werden kann, so muss die Zunahme der Gesamt-CO_2 und der Neutralisierungsfähigkeit des paraffinaktivierten Speichels einer vermehrten Bildung von Bikarbonat in den Speicheldrüsen zuzuschreiben sein.

4. Wegen seines wechselnden Gehalts an Bikarbonat und Kohlensäure ist der Speichel in physikalisch-chemischer Hinsicht sehr unstabil, und dieser Umstand ist bei pH-Bestimmungen zu berücksichtigen.

5. Potentiometrische Titrierungen an "Ruhespeichel" von zwei extremen Gruppen, die eine kariesinaktiv und die andere kariesaktiv, ergab einen statistisch sehr wahrscheinlichen Unterschied der potentiellen Alkalinität dieser zwei Gruppen. Der Grad der Schwankungen innerhalb jeder Gruppe war jedoch so bedeutend, dass der Pufferwert nicht als absolutes Mass der Kariesaktivität verwendbar ist.

Résumé.

Il y a des raisons d'admettre que la résistance de la salive vis-à-vis d'acides abaissant le pH est un facteur préventif de la carie. Divers auteurs ont aussi utilisé la valeur tampon de la salive à titre de test individuel de l'activité de la carie.

Par des titrations potentimétriques, et par la détermination de "capacité en CO_2" avec l'appareil de van Slyke, on a pu montrer:

1) Que la courbe d'absorption du CO_2 par la salive s'harmonise bien avec celle d'un mélange de bicarbonate et de phosphate. L'auteur présente une déduction théorique de cette courbe.

2) Qu'à des niveaux "physiologiques" du pH le bicarbonate et le phosphate sont les principales substances tampon, et qu'à des niveaux inférieurs elles sont remplacées par les protéines.

3) Qu'étant donné que le phosphate inorganique de la salive n'est pas influencé de facon tant soit peu importante par les excitations, il faut attribuer l'augmentation du CO_2 total, et celle du pouvoir neutralisant dans la salive activée par la paraffine,

à une production accrue de bicarbonate dans les glandes salivaires.

4) Qu'à cause du rapport variable existant entre le bicarbonate et l'acide carbonique la salive est très instable au point de vue de la chimie physique, et que les dosages du pH ne doivent être faits qu'en tenant dûment compte de ce fait.

5) Que les titrations potentiométriques de salives "au repos" provenant de groupes extrêmes, l'un de ceux-ci appartenant à la carie inactive, l'autre à la carie active, ont montré qu'il existe très probablement une différence, démontrable statistiquement, entre le potentiel d'alcalinité des groupes. Cependant, les variations n'étaient pas d'un ordre de grandeur suffisant pour permettre de se servir de la valeur tampon comme d'un test absolu mesurant l'activité de la carie.

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