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CLINICAL INVESTIGATIONS OF THE SALIVARY BUFFERING ACTION

by

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Some of the most frequent and important pathological conditions of the teeth and the oral cavity are strongly dependent on pH changes. Dental caries and erosion are both characterized, at least in their initial stages, by the dissolution of the inorganic salts of the hard dental tissues. This dissolution is mainly caused by a lowered pH value of the fluid layer adjoining the tooth surface. The formation of calculus, and of occasional stones in the salivary glands and ducts, is characterized by a precipitation of largely the same salts which are dissolved in the above-mentioned processes. Even if the role played by pH increases in this stone formation may be less dominating than the acidification is for the dissolution, there is still ample evidence that alkalinization is an important factor in calculus formation (*Næslund* 1926, *Sand* 1949).

The buffering power of the saliva is, by definition, its ability to counteract pH changes. It should then, theoretically, be of importance for the protection against the above-mentioned pathological processes. For obvious reasons most of the studies in this field have dealt with the relationship between caries and the salivary buffering in the pH range below neutrality, generally down to about pH 5. Table 1 gives a summary of published results.

It is seen from Table 1 that most investigators have found an inverse relationship between the salivary buffering power and the caries experience. A comparison with the results of studies of the relationship between other salivary factors and dental

Table 1. Previous investigations into the relationship between salivary buffering power and dental caries incidence. RS = saliva collected during rest. SS = saliva collected during stimulation.

Investigators, year	Material	Method	Results	Remarks
Röse, 1905	SS, collected for 45 min. 219 children, 12—14 years, from different towns; 42 children, 13 years, from one town.	Titration with 0.1-N HCl to colour shift of litmus.	Strong negative correlation caries rate-buffer value.	
Pickerill, 1924	50 caries-free Maori children, 50 susceptible European children.	Titration to methyl orange endpoint.	Approx. 6 times greater neutralizing action in Maori children.	Experimental procedure not detailed.
Karshan, Krasnow & Krejci, 1931	RS and SS. Students, 19—25 years. 17 immune, 6 arrested caries, 21 active caries (About 80 sets of determinations).	Titration with 0.02-N HCl to methyl orange endpoint.	No correlation in RS. Insignificant negative correlation in SS.	
Krasnow, 1932	RS and SS. Students, 19—21 years. 11 caries-free, 14 caries-active. 52 determinations.	Titration with 0.01-N HCl to methyl orange endpoint.	No correlation in RS. Negative correlation in SS (no statistics!).	Higher buffer values in afternoon samples than in morning samples (on rising).
Hubbell, 1933	SS. Children, 9—16 years. 15 caries-free, 17 with active caries.	Carbon dioxide capacity (Van Slyke & Cullen method). Titration of 5 ml. portions with 0.02-N HCl to methyl red endpoint.	Carbon dioxide capacity 44 % higher, alkalinity 24 % higher in caries-free children.	Strong correlation between the results of the two methods.

Grove & Grove, 1934	SS. 7 caries susceptible, 7 caries immune.	2 ml saliva + 4 ml 0.01-N HCl, back-titration to phenolphtalein with 0.01-N NaOH. "Alkaline reserve" = ml HCl — ml NaOH.	Average for susceptible group 0.4 ml, for immune group 1.6 ml.
White & Bunting, 1936	RS, SS. 12 caries-free, 13 caries-susceptible children.	Carbon dioxide capacity (Van Slyke & Cullen method).	In caries-free 5.1 % higher capacity in RS, 14 % higher capacity in SS. No statistics.
Karshan, 1936	SS (8 ml portions). 78 subjects, 13—42 years. 22 caries-free, 15 arrested caries, 41 caries-active, 27 miscellaneous.	CO_2 capacity (Van Slyke & Neill). Saliva sat. with alveolar air.	Strong negative correlation.
Hanke, 1937	SS (25 ml portions). 48 subjects, 7—65 years. 10 "immune", 12 non-susceptible, 26 susceptible.	pH measured after addition of 1 cc 0.1-N HCl to 10 cc saliva. Determ. of acid quantity required to obtain pH 4.5. Sampling time standardized.	Differences between "immune" + non-susceptible and susceptible statistically significant.
Karshan, 1939	RS, SS (8 ml portions). Subjects 10—41 years. RS: 21 caries-free, 25 caries-active. SS: 33 caries-free, 55 caries-active.	CO_2 capacity (Van Slyke & Neill). Saliva sat. with alveolar air.	Strong negative correlation, especially in SS.
Fosdick & Campaigne, 1939	SS. 10 caries-free, 10 caries-susceptible subjects.	Carbon dioxide capacity (Van Slyke & Neill method).	Averages for 3 ml: 42.2 for caries-free, 24.5 for susceptible. Difference strongly significant.

Table 1 (contd.)

Investigators, year	Material	Method	Results	Remarks
Dreizen & al., 1946	SS, 30 ml from each of 50 adult patients.	Titr. with 0.1-N lactic acid pH 7—6.	Good correlation with clinical findings, as well as with lactobacillus count and Fosdick test.	
Messerli, 1946	SS. 29 "immune" Italian workers, 20—40 years. 26 susceptible Swiss children, 13—16 years.	Titration with 0.05-N HCl to bromcresolgreen end-point (pH 4.4).	Average for immunes 1.91, for susceptibles 1.34 ml/ 5 ml.	
Sellman, 1948	RS. (6—7 ml). 21 adult subjects.	Titration with 0.1-N HCl to pH 6.5 and 4.	Difference statistically very probable.	
Ericsson, 1949	RS, SS. 36 adults, div. into high & low caries groups. 23 children 8—9 years, div. into caries-free and high-caries groups.	Microtitr. with 0.1-N HCl to pH 5.0 and to desaturation pH for apatite in the individual's saliva.	Higher buffer values for low-caries groups. Differences significant only for SS in adults.	*Note:* test combined with rate of flow.
Sullivan & Storvick, 1950	SS. 572 freshmen students.	Titr. to methyl red. and phenolphtalein endpoints with 0.01-NHCl and NaOH, respectively. Titration sum multiplied by rate of flow.	Highly significant neg. correlation to DMF surface number.	

Dewar, 1950	SS, collected for 20 min. 86 subjects, 6—24 years.	Electrometr. titration with 0.1-N lactic acid from pH 7 to pH 6. Saliva kept under paraffin oil.	"Good or fair agreement with clinical caries picture in 88 per cent of cases."	
Turner, Scribner & Bell, 1954	RS. 315 children, 5—11 years.	Titr. to methyl red endpoint with 0.001-N HCl, and to phenolphtalein endpoint with 0.001-N NaOH.	Differences caries-free — caries-susceptible statistically significant.	
Muracciole, 1955	SS. 204 children.	Titr. with 0.01-N lactic acid to colour shift of clorophenol red.	Correlation clearly apparent; no statistical evaluation	
Lilienthal, 1955	SS. 65 children from a vegetarian orphanage with low caries prevalence.	Dewar's method.	Agreement with clinical findings in about 2 cases of 3. No statistical calculation.	
Turner & Anders, 1956	RS. 411 children, caries-free, with rampant caries, or intermediate.	In 213 cases, the same method as Turner & al., 1954. In 198 cases electrometric titration to pH 5.5 and 5.0, and to pH 8.5 and 9.0, respectively.	Differences caries-free — rampant caries and intermediate — rampant caries significant when titrated to pH 5.5 or 5.0.	

caries justifies the statement that the buffering action has the best established connection with caries. Investigations by *Manly* (1954) of the acidity of artificial plaques in glucose — phosphate buffer solutions indicate one possible mechanism of this connection.

The physico-chemical mechanism of the salivary buffering has been the object of several previous studies (*Ericsson* 1949, *Sellman* 1950, *Wah Leung* 1951, *Lilienthal* 1955 a, b). It has been established that the CO_2-HCO_3 system accounts for the greatest part of the buffer capacity in the pH range about and below neutrality. With a constant concentration of the total carbonic acid system in the solution the chemical buffering capacity of this system has its maximum at pH 6.3, at the average ionic strength of saliva. However, both the CO_2 capacity and the actual salivary content of the total carbonic acid system increase with increasing pH. The actual chemical buffering of the carbonic acid system in the saliva therefore increases to about pH 7.7.

In addition, the carbonic acid system exerts a buffering action according to another mechanism. With a lowered CO_2 pressure or lowered pH, volatile CO_2 is liberated from the liquid phase. This escape of an acid substance constitutes the so-called phase buffering. Since the CO_2 pressure of the freshly secreted saliva is higher than that of the oral cavity, and even higher than that of the alveolar air (*Clark & Shell* 1927, *Sand* 1949) such a CO_2-liberation will always take place in the mouth. The presence in the saliva of the enzyme carbonic anhydrase may catalyze the transformation $H_2CO_3 \rightarrow CO_2 + H_2O$ (*Rapp* 1945, 1946, *Sand* 1949).

In comparison with the carbonic acid system the inorganic phosphates and the proteins play a minor role as buffer substances. This is evident from their comparatively low concentrations in the saliva. No correlation is found between the inorganic phosphate content of the saliva and its buffer effect (*Hubbell* 1933, *White & Bunting* 1936). The mandibular mucoid has practically no buffering action in the pH range below neutrality (*Oldfeldt* 1936). On the contrary, the mucoid is apt to contribute to the acidification of the saliva through the liberation of acid substances by the action of bacterial enzymes (*Rogers* 1948, 1949).

The experimental part of this investigation has had the following aims,

1. to establish a simple and practicable test method for the clinical estimation of the salivary buffering action,
2. to study the diurnal variation of the buffering,
3. to study the possible variation with some dietary factors.

I. METHODS OF ESTIMATION OF SALIVARY BUFFERING

The main method of estimation of the buffering action of the saliva within a certain pH range is titration with acid or alkali between two pH limits in this range. As can be seen from Table 1 a very common method has been the titration from the original pH of the saliva to the colour shift of methyl orange (about pH 4). This, however, is a rather indistinct endpoint, especially in flocculent saliva samples. Lactic acid is especially unsuitable for titration to this endpoint on account of its own diminishing dissociation in this pH region.

Electrometric titration has obvious advantages over the colorimetric method, but the endpoint is still somewhat difficult to define because of the slow escape of volatile CO_2. Some electrometric titration methods which have been used are indicated in Table 1.

Since the carbon dioxide capacity of the saliva is a function of its alkali reserve the determination of this capacity can be used as a buffer test. There is a strong correlation with the results of a titration method, as can be calculated from *Hubbell*'s parallel results with both methods (1933).

None of these methods is very precise. With a carefully performed micro-titration method *Ericsson* (1949) found about 6 % error. Simplified methods may therefore be used with little or no loss of accuracy. *Forbes* (1932) mixed 3 ml of saliva with 1+0.5 ml 0.02-N hydrochloric acid and measured the resulting pH electrometrically after each of these two additions of acid. The correlation of this test with the measurement of the carbon dioxide capacity was found to be good. *Ericsson* (1953), working with a similar method, found that the pH value was not stabilized until after 15—20 minutes' bubbling with air due to the gradual escape of the liberated carbon dioxide. Such an aeration procedure means a standardization of the phase buffering of the saliva samples.

For a clinical estimation of the salivary buffering the following method was first used by the author (clinical test I). Equal volumes of saliva and 0.01-N hydrochloric acid are mixed and bubbled through with air for 20 minutes. The resulting pH is determined, preferably electrometrically. The correlation between this test and the same author's microtitration method, mentioned above, is illustrated by Fig. 1.

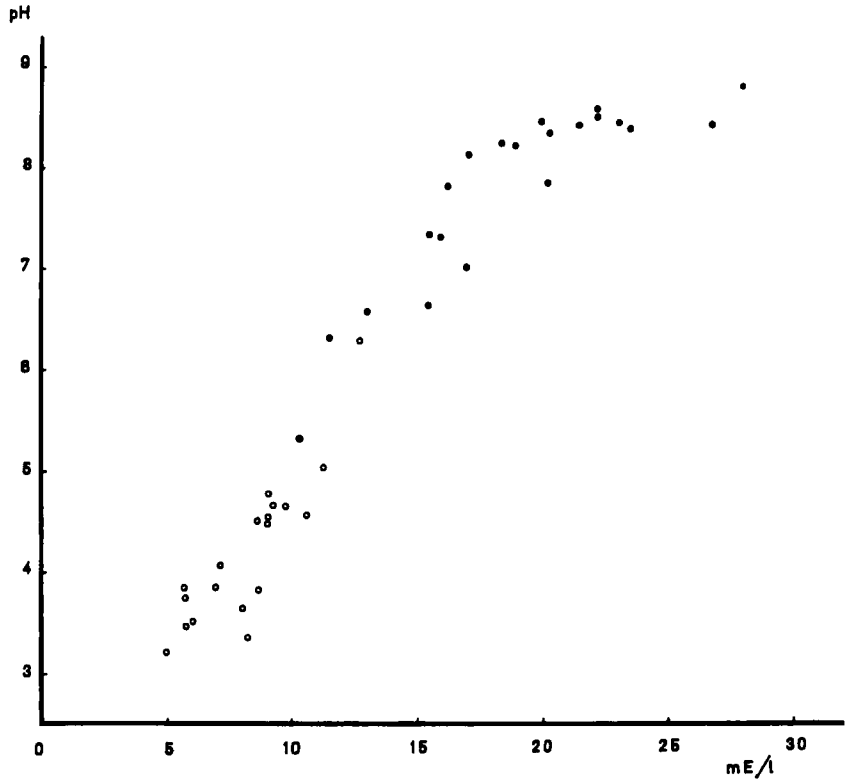

Fig. 1. Correlation of microtitration (abscissae) and clinical test I (ordinates).
° resting saliva . stimulated saliva
Note horizontal deviation for the strongest buffered salivas.

It appears from the diagram that this simplified test hardly differentiates between those stimulated salivas which, according to the microtitration, are most strongly buffered. While the method is still useful for clinical estimations, it can thus not be applied in more precise investigations of the stimulated saliva.

A revised method was therefore worked out in which the quantity of acid added to the stimulated saliva was increased 50 per cent. This method, which is here called the clinical test II, is described in the following.

Standardization of sampling

Throughout this investigation resting or unstimulated saliva (RS) was taken from subjects sitting in a relaxed position, with the lips closed and the head slightly bent forward, so that the saliva accumulated in the anterior part of the mouth floor, without any movements of the tongue or lips. The saliva was passively dripped through a funnel into an ice-cooled measuring glass which was then immediately sealed with wax.

Stimulated saliva (SS) was obtained by vigorous chewing of paraffin. It was collected in the same way as described above.

Tests were performed which showed that storage of the saliva markedly increases its buffer capacity, even at refrigerator temperature and with the addition of toluene (Table 2). The determinations were therefore performed immediately after the sampling.

Table 2. Effect of storage in refrigerator on salivary buffer capacity. Per cent increase of buffer effect as titrated down to pH 5.0 (average of 6 test series).

Type of storage	Saliva	3 hours	6 hours	24 hours
2 ml glass ampoule practically filled. 1 drop of toluene added	RS	4.4	0.8	14.1
	SS	11.0	14.5	17.9
2 ml saliva in 10 ml glass cylinder. No toluene	RS	3.5	5.2	30.0
	SS	10.5	12.3	11.7

Unstimulated saliva. 1 ml saliva + 2 ml 0.005-N HCl + 1 ml dist. water. One drop of capryl or octyl alcohol added to prevent foaming. Air bubbling through a capillary for 20 min. Electrometric determination of resulting pH.

Stimulated saliva. 1 ml saliva + 3 ml 0.005-N HCl; capryl or octyl alcohol; bubbling and pH determination as above.

With this method, lower pH values are obtained for stimulated saliva than according to the method illustrated by Fig. 1, and

consequently a clear differentiation is possible even between the most strongly buffered specimens, as appears from Fig. 3. On the other hand, comparisons between resting saliva and stimulated saliva are not possible. Stimulation by chewing paraffin generally increases the buffer capacity in this pH region by about 60—80 % *(Ericsson* 1949).

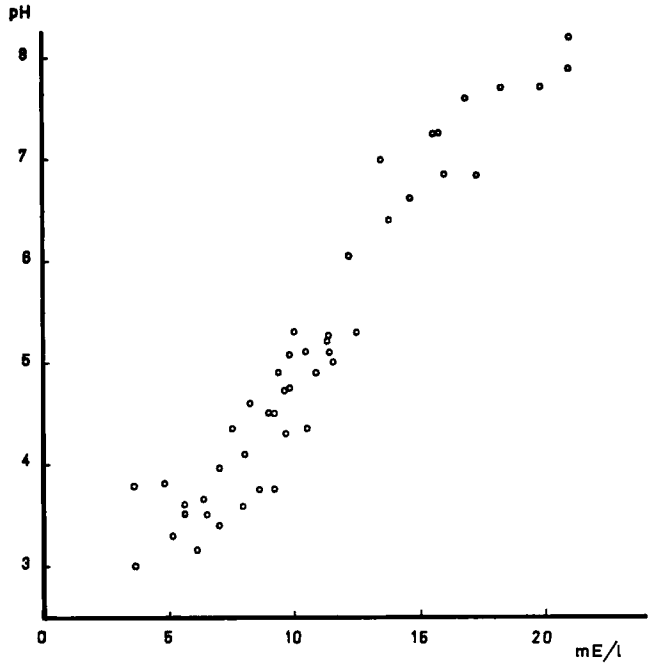

Fig. 2. Correlation of microtitration (abscissae) and clinical test II (ordinates). Resting saliva.

The diagrams, Figs. 2 and 3 show a strong correlation between this test and the author's microtitration method *(l.c.)*; the r values were found to be 0.94 for resting saliva and 0.96 for stimulated saliva. In another series of 190 individual cases where only the clinical tests were carried out the pH values found for resting saliva varied between about 2.75 and 6.75, for stimulated saliva between about 3.00 and 8.25. For resting saliva the average was found to be in the range 4.50—4.75, for stimulated saliva in the range 6.00—6.25.

On account of the linear relationship of this test to the titration results, and since the pH values obtained have shown a normal distribution in both RS and SS series, the figures have throughout been treated as a linear function in spite of their logarithmic nature.

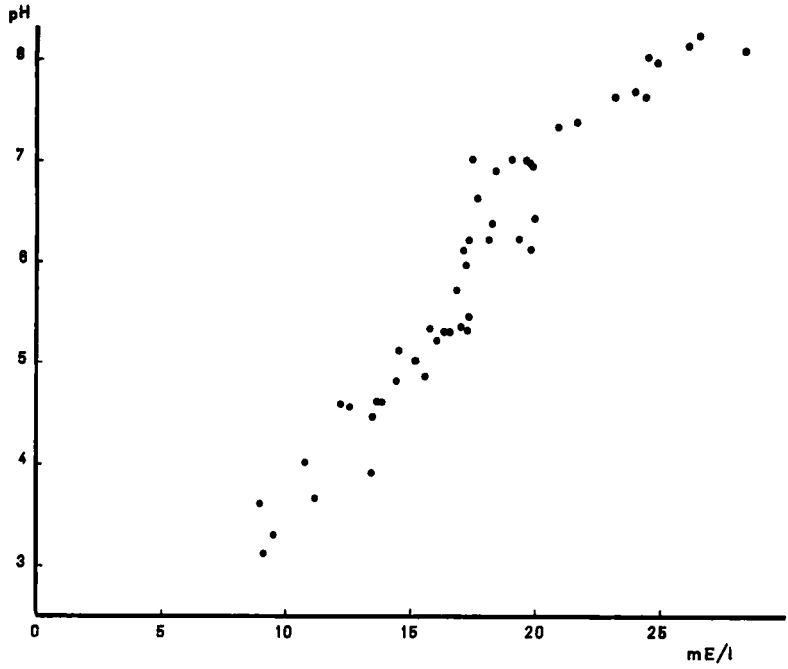

Fig. 3. Correlation of microtitration (abscissae) and clinical test II (ordinates). Stimulated saliva.

II. DIURNAL VARIATION OF SALIVARY BUFFERING

Little work has been done on the diurnal variation of the buffering action of saliva. *Krasnow* (1932) found a higher neutralizing power in the afternoon than in the morning. *Forbes* (1932) found a decrease in its neutralizing action after a light meal; this was not due to the chewing per se, since chewing of paraffin or gum did not produce the same decrease. *Van der Molen & Offringa* (1909) found, in 2 tests with resting saliva, the highest buffer capacity in the morning, the lowest one hour after breakfast and lunch, rising towards the following meals. *Külz* (1887),

in a single test, found a decrease in the salivary content of bound carbonic acid 1—3 hours after a meal.

The salivary pH values, which have a strong relationship to the bunffering action, have been studied more extensively. *Starr* (1922) found an inverse relationship of the salivary pH to the alveolar CO_2 pressure after the midday meal. On the other hand, *Anderson* (1949) found no influence of meals on the salivary pH measured "immediately afterwards". The same author found a statistically significant increase in the pH value 2 hours after lunch. *Henderson & Millet* (1927) found low pH values on rising in the morning, slightly increasing during the day. This course was interrupted by each meal which involved some chewing: a sharp rise in salivary pH immediately after the meal was followed by a depression ½—¾ hour afterwards.

When judging the results of pH and buffering measurements following a meal one must consider, in addition to the systemic changes produced by the food intake per se, the prolonged effect of the stimulation of the salivary flow and the effect of acids produced locally from foods containing carbohydrate. Fig. 4, from

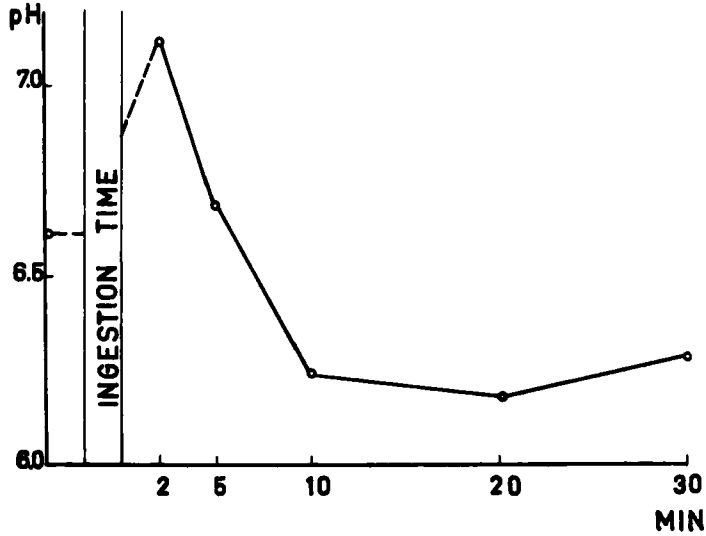

Fig. 4. pH changes of mixed saliva after candy ingestion. Average of 25 subjects. Saliva samples collected, without intentional stimulation, between time intervals 1½—2½ min (= 2 min sample), 4½—5½ min (= 5 min sample), etc.

unpublished work by *Ericsson & Öberg* (1954), illustrates both these effects. During the minutes immediately following the ingestion the salivary pH is considerably elevated because of the stimulation. After 10—15 minutes there is a pH decrease of an order which may well be explained by the acids formed in the oral cavity (*Ericsson* 1949, *Ericsson & al.* 1954, *Neuwirth & Summerson* 1951). These pH changes, which seem to have been unknown to some of the previous authors in this field, must evidently also influence the buffer effect.

Experimental

In 5 normal, healthy, young, adult subjects the diurnal variation was studied in the following way.

Samples of the mixed saliva secretion were taken immediately on rising in the morning, without previous brushing of the teeth, and at the following time intervals after breakfast, lunch and dinner: 15 min., 30 min., 1 hour, 2 hours, 3 hours. The teeth were brushed without dentifrice for 2 min. immediately after each meal. No smoking and no in-between meals were allowed on the test days.

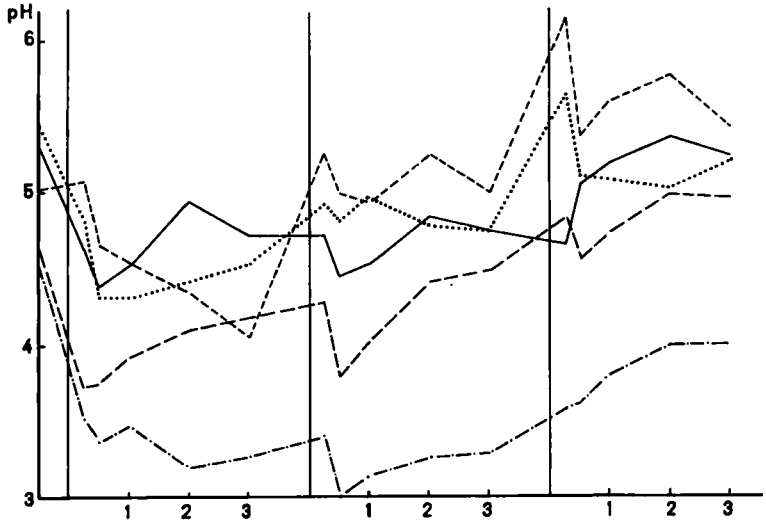

Fig. 5. Diurnal variation of the buffering power of resting saliva. 3-day averages of 5 subjects. Vertical lines denote end of meals, abscissae the following hours.

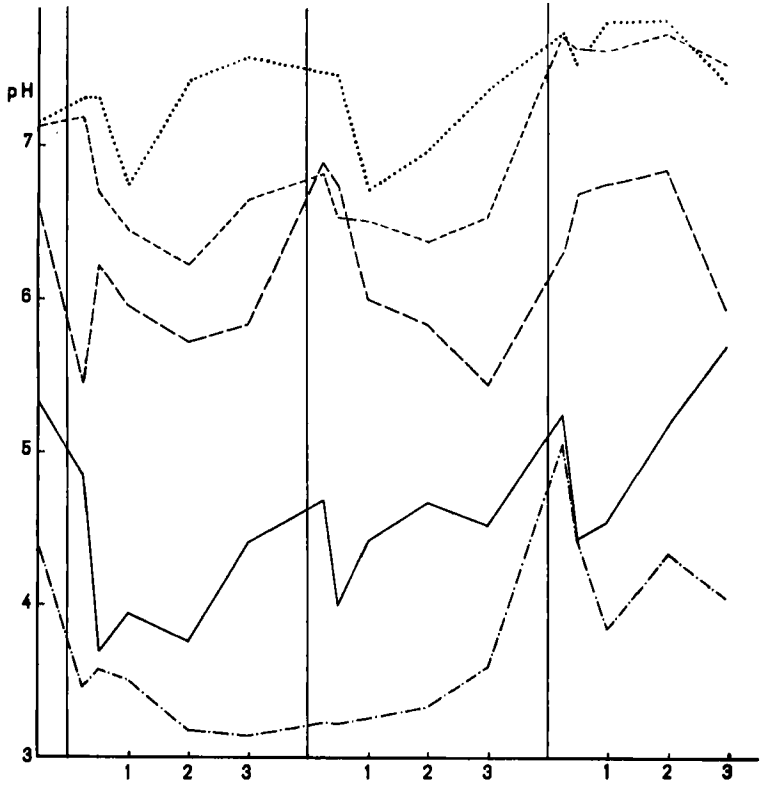

Fig. 6. Diurnal variation of the buffering power of stimulated saliva. The same subjects and diagram construction as in Fig. 5.

The saliva was collected and immediately pipetted for the buffer test which was performed according to the technique previously described (clinical test II).

Resting saliva was tested in this way for three days, paraffin stimulated saliva for three subsequent days.

Results

The averages for the 5 subjects are given in Figs. 5 and 6.

Fig. 7 gives an example of the single tests of resting saliva in one of the subjects.

In spite of the well-known general variation of the composition and quantities of the saliva the following features can be discerned.

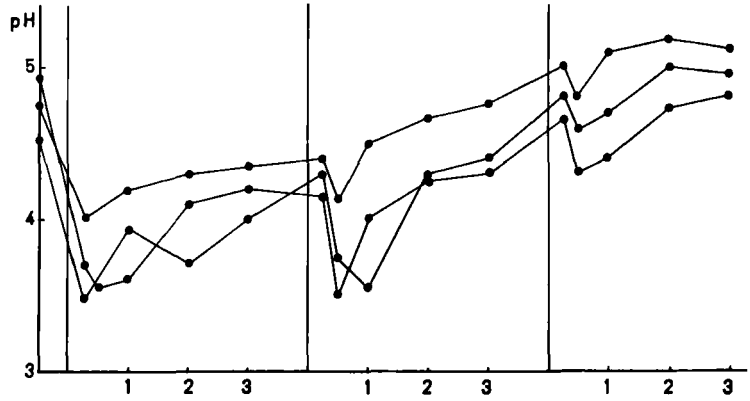

Fig. 7. Variation of the buffering power of resting saliva of one subject on three consecutive days.

1. A higher buffer capacity immediately on rising than after breakfast.

2. An increased buffer capacity ¼ hour after the meals.

3. In most cases a drop of the buffer capacity ½—1 hour after each meal.

4. A general trend towards increasing buffer capacity during the whole day after breakfast, with a downward tendency in most cases in the evening.

Discussion

It is well known that even slight nervous stimuli have an immediate effect on the salivary secretion, *inter alia* increasing its pH value *(Schmidt-Nielsen,* 1946, and others). This might be the reason for the high morning values obtained for the buffer effect, since rising from the bed involves a considerable irritation. However, *Henderson & Millet,* as pointed out earlier, found especially *low* salivary pH values on rising. Some difference in sampling technique may account for this apparent discrepancy.

Hastings & Eisele (1940) found the following changes in blood HCO_3 content during the day: a rise of about 2 millimoles during the forenoon (as compared with the morning value); a return to the morning value in the afternoon; an increase of approximately 1 mM 1.5—2 hours after a meal. It may be significant that these changes are directly opposite those of the salivary

buffering. The acidity of the saliva has likewise been found to vary inversely with that of the urine (*Starr* 1922) and that of the gastric juice (*Reindel* 1940).

Further, *Browne & Vineberg* (1932) found that an increased CO_2 content of the plasma caused a rise of both the volume and the acidity of the vagus-induced secretion of gastric juice. Diamox inhibition of carbonic anhydrase has been found to decrease the acidity of gastric and renal secretion (review: *Maren & al.* 1954), and to decrease the buffer capacity of the saliva (*Niedermeier & al.* 1955). All these data seem to fit the assumption that salivary alkalinity is influenced by a non-nervous mechanism also, which would have an opposite effect on the gastric and urinary secretions.

The stimulation of the buffer effect by a meal and the subsequent reaction indicate that saliva sampling for the determination of the buffer capacity for diagnostic purpose should be done at least one hour after a meal in order to obtain as stable results as possible. Tests for inter-individual comparisons should be done either before or after lunch.

III. INFLUENCE OF FOODS ON THE SALIVARY BUFFER CAPACITY

Nervous stimulation is well known to influence the rate of salivary secretion, and the blood supply to the glands also has an effect on the secretion. The established influence of water loss and thirst may be assumed to be exerted via the blood supply. Since there is a strong positive relationship between the secretion rate and the buffer capacity, the latter is evidently also dependent on the factors mentioned.

Regarding the possible influence of dietary constituents on the salivary buffer capacity there is much less information available. Only *Wills & Forbes* (1939) seem to have studied this question systematically, and on a very limited material. They found an increased capacity of the saliva to neutralize acid with a diet rich in protein and vegetables, while a diet rich in carbohydrates lowered the neutralizing power. The buffer capacity might further be slightly increased by the increased phosphate content which, according to some investigators, follows the administration of phosphate (*Eddy & al.* 1933, *Bleser* 1939, *Eggers Lura* 1946).

A. Influence of proteins, carbohydrates and vegetables on the salivary buffering

5 subjects were chosen for this investigation, all laboratory workers or persons familiar with this work, and all about 20 years of age and healthy.

The experimental period comprised 4 consecutive weeks during which the following dietary regimens were kept Monday—Friday: (1) the subjects' customary diet; (2) a protein-dominated diet; (3) a vegetable-dominated diet; (4) a carbohydrate-dominated diet.

During the *protein week* each lunch comprised eggs with bacon or sausages, or fish, and one small slice of bread with butter. Dinner comprised meat or fish and ice cream. No coffee or tea with these meals. Milk and cheese were included in every meal, i.e. also breakfast and occasional evening meals.

During the *vegetable week* lunch and dinner consisted of vegetables with the quantities of fat necessary for the preparation. The beverage was fruit juice. One small slice of bread with butter was included in the lunch. The dinner dessert was fruit. No coffee or tea with these meals. Proteins were avoided at breakfast and evening meal.

During the *carbohydrate week* every lunch comprised corn flakes with lingonberry jam and milk. Dinner consisted of flour-rich food, without meat, fish or eggs. The beverage was lemonade, and bread, butter and marmelade were included in each meal.

Samples of resting saliva and stimulated saliva were taken according to the standard technique half an hour and two hours after the completion of each lunch, Tuesday—Friday.

Results

The results of the buffer tests are condensed in Table 3.

It is seen that proteins and vegetables increase the buffer capacity, the strongest effect being obtained with proteins, while carbohydrates tend to decrease the buffer action. All the differences obtained point in this direction, while only part of the differences are statistically significant.

Discussion

The results of our tests agree with those of *Wills & Forbes,* although these authors found a greater buffering increase with vegetables than with meat. There are thus good reasons to as-

Table 3. Differences in salivary buffering action under various dietary conditions. Each figure constitutes the difference between the 4-day averages of 2 compared weeks.

Subject	Time after lunch	Proteins-Vegetables		Proteins-Carbohydrates		Vegetables-Carbohydrates		Proteins-Customary diet		Vegetables Customary diet		Carbohydrates Customary diet	
		RS.	SS.	RS.	SS.	RS.	SS.	RS.	SS.	RS.	SS.	RS.	SS.
	½ h.	1.08	—0.12	0.69	—0.08	—0.39	0.04	0.13	0.23	—0.56	0.45	—0.56	0.21
O. S.	2 h.	0.74	—0.20	0.17	1.15	—0.57	1.35	—0.02	0.55	—0.76	0.75	—0.19	—0.60
	½ h.	0.53	—0.90	0.79	0.18	0.26	1.08	0.48	—0.97	—0.05	—0.07	—0.31	—1.05
S. G. G.	2 h.	0.45	—0.04	0.73	0.63	0.28	0.67	0.92	0.25	0.47	0.29	—0.19	—0.38
	½ h.	0.80	0.97	1.00	1.51	0.20	0.54	0.80	1.75	0.00	0.78	—0.20	0.24
G. B. J.	2 h.	0.48	—0.70	1.06	0.99	0.58	1.69	1.04	0.75	0.56	1.45	—0.02	—0.24
	½ h.	0.14	0.70	0.46	1.11	0.32	0.41	0.72	1.04	0.58	0.34	0.26	—0.07
H. V.	2 h.	0.21	0.53	0.53	1.24	0.32	0.71	0.60	1.38	0.39	0.85	0.07	0.14
	½ h.	0.15	—0.13	0.41	0.15	0.26	0.28	0.54	—0.70	0.39	—0.57	0.13	—0.85
M. J.	2 h.	—0.11	0.07	0.42	0.74	0.53	0.67	0.49	0.99	0.60	0.92	0.07	0.25
	M	0.447	0.018	0.626	0.762	0.179	0.744	0.570	0.527	0.123	0.519	—0.094	—0.235
	t	3.92	0.10	7.03	4.52	1.54	4.70	5.58	2.10	0.79	2.90	1.23	1.57
	P	<0.01	>0.5	<0.001	<0.01	0.2—0.1	<0.01	<0.001	0.1—0.05	0.5—0.4	0.02—0.01	0.3—0.2	0.2—0.1

sume that the salivary buffering is one of the ways by which the different foods influence the caries process.

It is noteworthy that the alkalinity of the saliva is increased by proteins which are known to be acid-forming in the body, and to make the urine more acid. Part of the explanation might be the following. Proteins are acid-forming mainly through their content of sulphur which on oxidation yields sulphuric acid. While these acid groups are excreted with the urine to a great extent, very little sulphur is found in the saliva.

B. Influence of alkalosis and acidosis

There seem to be no investigations regarding the possible influence of alkalosis and acidosis on the salivary buffer capacity. The tests with different foodstuffs do not indicate any simple relationship to these factors; however, the foods may also have an influence through other mechanisms than their combustion residues.

Anderson (1949) found that peroral doses of ammonium chloride and sodium bicarbonate, sufficient to influence the urinary pH level, also significantly changed the salivary pH in the corresponding directions. With regard to the connection between pH and buffer action of saliva an investigation of the possible influence of alkalosis and acidosis on the latter thus seemed justified.

Experimental

The following tests were performed with 12 healthy adult subjects. The normal salivary values were obtained by analyses of samples of resting saliva and stimulated saliva taken 2 hours after breakfast and 2 hours after lunch for 4 days.

To test the influence of alkalosis 8 g sodium bicarbonate in gelatin capsules were swallowed together with 200 ml water one hour after breakfast on two consecutive days. Saliva sampling was performed two hours after breakfast and lunch on both days.

During two days of a following week the influence of acidosis was tested with 5 g ammonium chloride in the same way.

In addition to the buffer effect the secretion rate was determined, and in the stimulated saliva also calcium plus magnesium and inorganic phosphate. Ca+Mg was determined by titration

Table 4. Buffer effect and secretion rate in alkalosis and acidosis.
A. Resting saliva.

	Buffer effect						Secretion rate, ml/15 min					
	Fore-noon			Afternoon			Fore-noon			Afternoon		
	1. $NaHCO_3$	2. Control	3. NH_4Cl	4. $NaHCO_3$	5. Control	6. NH_4Cl	7. $NaHCO_3$	8. Control	9. NH_4Cl	10. $NaHCO_3$	11. Control	12. NH_4Cl
Max.	7.48	5.50	7.70	7.35	6.05	7.95	23.25	13.13	28.50	17.85	11.93	34.35
Min.	3.35	3.18	3.30	3.45	3.38	3.30	1.25	1.36	1.50	1.99	1.58	3.45
Average	4.97	4.52	4.73	4.91	4.76	4.89	5.89	5.61	7.49	5.21	5.68	8.31
Compared groups ..	1—2	3—2	4—5	6—5	7—8	9—8	10—11	12—11				
Average diff.	0.45	0.21	0.15	0.13	0.28	1.88	—0.47	2.63				
t	2.81	1.04	1.52	0.73	0.24	1.31	0.22	1.54				
P	0.01—0.02	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1				

Table 5. Buffer effect and secretion rate in alkalosis and acidosis.
B. Stimulated saliva.

	Buffer effect						Secretion rate, ml/15 min					
	Fore-noon			Afternoon			Fore-noon			Afternoon		
	13. $NaHCO_3$	14. Control	15. NH_4Cl	16. $NaHCO_3$	17. Control	18. NH_4Cl	19. $NaHCO_3$	20. Control	21. NH_4Cl	22. $NaHCO_3$	23. Control	24. NH_4Cl
Max.	7.63	7.35	7.75	7.30	8.00	7.80	48.53	46.73	48.40	49.13	47.45	47.25
Min.	3.80	3.45	3.19	3.75	3.45	3.40	12.15	12.08	12.90	13.75	9.78	11.85
Average	6.02	6.01	5.91	5.87	6.04	5.56	29.34	31.74	32.50	27.82	29.88	33.19
Compared groups ..	13—14	15—14	16—17	18—17	19—20	21—20	22—23	24—23
Average diff.	0.01	—0.10	—0.17	—0.48	—2.40	0.76	—2.06	3.31
t	0.53	0.46	—1.07	2.30	—0.93	0.34	0.99	1.83
P	>0.1	>0.1	>0.1	0.02—0.05	>0.1	>0.1	>0.1	0.05—0.1

Table 6. Mineral salts in alkalosis and acidosis. Stimulated saliva.

	Calcium + magnesium, mM/l						Inorganic phosphorus, mM/l					
	Fore-noon			*Afternoon*			*Fore-noon*			*Afternoon*		
	1. $NaHCO_3$	2. Control	3. NH_4Cl	4. $NaHCO_3$	5. Control	6. NH_4Cl	7. $NaHCO_3$	8. Control	9. NH_4Cl	10. $NaHCO_3$	11. Control	12. NH_4Cl
Max.	2.43	2.18	2.39	2.05	1.92	1.94	5.47	5.53	5.40	6.50	6.10	5.75
Min.	1.03	1.26	1.08	1.18	1.17	0.99	3.74	3.08	3.49	3.90	3.63	3.30
Average	1.64	1.62	1.62	1.58	1.52	1.45	4.48	4.13	4.27	4.99	4.57	4.34
Compared groups ..	1—2		3—2	4—5		6—5	7—8		9—8	10—11		12—11
Average diff.	0.02		0.00	0.06		—0.07	0.35		0.14	0.42		—0.23
t				2.29		1.35	3.88		0.66	1.22		2.92
P				0.02—0.05		>0.1	<0.01		>0.1	>0.1		0.01—0.02

with ethylene diamine tetraacetate according to a technique previously worked out (*Ericsson* 1955). P was determined photometrically after precipitation of the protein and mucoid at the isoelectric pH of saliva (*Ericsson* 1953).

Results and Discussion

A tendency towards an increase in buffer capacity and secretion rate in the resting saliva may be deduced from Table 4, as 7 out of 8 comparisons point in this direction. Since this tendency is found both in alkalosis and acidosis, and not in stimulated saliva, it may be due to the nervous irritation produced by the ingestion rather than to the alkalosis or acidosis *per se*. Compare the lack of correlation between plasma and salivary bicarbonate in *Sand's* experiments with acidosis (1951).

The content of inorganic phosphorus of the stimulated saliva was increased significantly one hour after the ingestion of bicarbonate, while the increase of the corresponding afternoon value was non-significant. The mechanism of this apparent effect must remain an open question.

III. INFLUENCE OF THE INGESTION OF BONE SALTS ON SALIVARY BUFFER CAPACITY

Several investigators have found a mitigating influence of the salts contained in bones and teeth on the development of animal or human caries or on the acid decalcification of dental enamel in vitro. *Schröder* (1941) reported that 1 % calcium phosphate added to flour prevented enamel dissolution if teeth were exposed in vitro to fermentation of this flour in saliva. The addition of calcium phosphate to a cariogenic diet had a caries-protective effect in animal experiments. *Harootian* (1943) obtained a caries reduction in human subjects through the administration of bone salts. *Strålfors* (1956, 1957) found similar caries reductions both in hamsters and school children through the addition of 2 % calcium phosphate to the food. *Barnard & Johansen* (1958) could confirm this in rat experiments.

While a direct local retention of such salts with a protecting action against enamel dissolution would be the simplest and perhaps also most probable explanation of this effect, a systemic

Table 7. Possible influence of bone meal ingestion on saliva. Resting saliva.

	Buffer effect				Secretion rate, ml/15 min			
	Fore-noon		Afternoon		Fore-noon		Afternoon	
	Test Days	Control Days	Test Days	Control Days	Test Days	Control Days	Test Days	Control Days
Max.	4.15	4.13	4.80	4.10	6.60	8.40	7.80	7.80
Min.	3.23	3.13	3.17	3.27	5.00	4.51	3.80	4.64
Average	3.63	3.59	3.88	3.72	5.80	6.17	5.30	5.38
Average diff.	0.04		0.16		—0.37		—0.08	
t	0.70		1.10		1.05		0.29	
P	>0.1		>0.1		>0.1		>0.1	

Table 8. Possible influence of bone meal ingestion on saliva.
Stimulated saliva.

	Buffer effect				Secretion rate, ml/15 min				Calcium+magnesium, mM/l				Inorganic P, mM/l			
	Fore-noon		Afternoon		Fore-noon		Afternoon		Fore-noon		Afternoon		Fore-noon		Afternoon	
	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days	Test Days	Contr. Days
Max.	8.13	8.40	7.43	7.67	39.02	44.50	40.30	43.77	1.853	1.71	1.85	1.62	4.73	4.83	5.63	5.67
Min.	4.10	4.47	3.00	4.30	20.45	17.00	20.31	15.31	1.44	1.40	1.34	1.37	3.23	3.53	2.43	3.63
Average	5.99	6.62	5.55	6.43	32.31	30.74	30.09	28.91	1.64	1.56	1.62	1.49	4.02	3.94	4.15	4.30
Average diff.	—0.63		—0.88		1.57		1.18		0.08		0.13		0.08		—0.15	
t	1.66		1.64		0.76		1.06		1.98		2.72		0.61		0.65	
P	>0.1		>0.1		>0.1		>0.1		0.05—0.1		0.02—0.05		>0.1		>0.1	

influence on the saliva may also be visualized. The following experiments were designed to test this supposition.

Experimental

In principle the experiment consisted of daily analysis of the salivary secretion rate, buffer capacity, and contents of calcium plus magnesium and phosphorus during a week with a low dietary calcium phosphate intake, supplemented for the last three days with bone meal tablets.

Seven normal healthy subjects between 15 and 45 years of age kept their ordinary dietary during the test week, with the exclusion of milk, cheese and eggs. Resting saliva and stimulated saliva were collected according to the standard method 2 hours after breakfast and 2 hours after lunch on the 2nd—7th day of the experiment.

Beginning with dinner the 4th day, 0.3 g bone meal tablets were taken before every meal, 3 tablets before breakfast and lunch and 4 before dinner. The tablets contained 100 mg Ca, 46 mg P, 0.5 mg Mg, 0.15 mg F, 0.03 mg Si, and small quantities of sorbitol, mannitol, cocoa, etulose, talcum and chocolate aroma. The tablets were chewed and swallowed with some water.

The analyses which were made according to the methods previously described comprised secretion rate and buffer effect in resting saliva and stimulated saliva, and calcium + magnesium and inorganic phosphorus in stimulated saliva.

Results

The results are condensed in Tables 7 and 8. The only apparent effect of the bone meal ingestion was on the calcium plus magnesium content of the saliva which was slightly elevated.

IV. INFLUENCE OF FLUORINE INGESTION ON SALIVARY BUFFER CAPACITY

It has been suggested that the ingestion of moderate doses of fluorine would increase the rate of flow of the saliva and, thereby, its buffer capacity (*Rathje* 1952, *Knappwost* 1952, *Bishop & al.* 1955). On the other hand, it has been reported that the ingestion of down to 10 mg sodium fluoride causes acidosis of the

blood and saliva (*Yonezawa* 1957). To test the supposition that moderate doses of fluorine might influence the salivary buffer capacity and/or secretion rate the following experiment was performed.

Samples of resting saliva and stimulated saliva were taken from ten healthy subjects 2 hours after breakfast and 2 hours after lunch, according to standard technique. On the 3rd and 4th days the subjects swallowed 5 mg fluorine, as sodium fluoride, dissolved in 15 ml distilled water; this was taken 1 hour after breakfast. Some of the subjects meant to feel some very slight nausea from the fluoride ingestion while the others could not report any particular sensation. (With twice this dose of fluorine the author had a very vague feeling of nausea).

Results

The results of the determinations of secretion rate and buffer capacity are condensed in Table 9 where the individual analysis figures represent the averages of the two test days and the two control days, respectively.

It appears from the table that the average buffer effect and secretion rate was higher on the test days than on the control days in all comparisons except one. This increase was greater in the fore-noon (one hour after the fluorine ingestion) than in the afternoon in three cases out of four. Two of the fore-noon differences may be regarded as significant.

Discussion

It may be concluded that these doses of fluorine have shown a slight tendency to increase the secretion rate and buffer capacity of the saliva. The lack of definite significance makes it improbable, however, that the much smaller single doses of fluorine ingested with drinking water or other vehicles would markedly influence the salivary secretion and buffer effect.

GENERAL DISCUSSION

This investigation was undertaken with a view to the clinical determination of the salivary buffer effect as a constitutional, or at least endogenous, factor in the pathogenesis of dental caries.

Table 9. Possible influence of fluorine ingestion on saliva.

	Resting saliva								Stimulated saliva							
	Buffer effect				Secretion rate, ml/15 min.				Buffer effect				Secretion rate, ml/15 min.			
	Fore-noon		Afternoon		Fore-noon		Afternoon		Fore-noon		Afternoon		Fore-noon		Afternoon	
	F	Contr.	F	Contr.	F	Contr.	F	Contr.	F	Contr.	F	Contr.	F	Contr.	F	Contr.
Max.	6.55	6.05	6.05	6.90	23.25	17.10	19.25	20.10	7.75	7.73	7.75	7.55	81.65	58.5	72.75	57.80
Min.	3.29	3.00	3.60	2.85	1.65	1.72	1.81	1.67	3.89	3.99	4.15	3.39	14.75	10.12	14.00	10.00
Average	4.28	4.21	4.52	4.24	7.58	6.04	6.16	6.73	6.87	6.43	6.27	6.14	34.11	29.16	30.04	29.10
Average diff.	0.07		0.28		1.54		—0.57		0.44		0.13		4.95		0.94	
t	0.66		0.97		2.50		1.09		2.49		0.67		1.82		0.38	
P	>0.1		>0.1		0.02—0.05		>0.1		0.02—0.05		>0.1		>0.1		>0.1	

There is strong evidence that the salivary buffering does constitute such a factor, but it is also evident that the importance of the local interaction of the carbohydrates and the bacterial flora overshadow the buffering. Only very definite deviations from the average values for salivary buffer effect are, therefore, at present of any diagnostic or prognostic value. Since the secretion rate and the composition of the saliva varies with many temporary stimuli, only influences which are apparent even in small groups of subjects seem to be of definite clinical importance.

The test method which has been described and used in this investigation has also been used at the author's department in some hundred clinical cases since 1955. It is felt that it is of greater analytical value to estimate the different salivary factors separately — buffer capacity, secretion rate, bacterial flora, Ca and P content, etc. — than to use caries tests which combine two or more of these factors without any possibility to distinguish between them. Especially one feature has been persistent in our clinical cases: the so-called paradoxical cases of caries development, i.e. rampant caries without excessive external causing factors, or relative immunity in spite of the presence of such factors, have practically uniformly shown very low salivary buffer values in the susceptible cases, very high in the resistant. This appears to help to explain such cases, which have often thrown doubt on the chemico-parasitic caries theory, and to support the clinical value of the salivary buffer tests.

The diurnal variation which has been demonstrated emphasises the importance of a standardization of the sampling conditions also as regards the time of the day. 1—3 hours after breakfast or lunch may be regarded as the most stable periods.

The beneficial influence on the buffer capacity of proteins and vegetables in the diet indicates that these foods are of importance for the caries resistance not only through their relative freedom from fermentable carbohydrates but also according to some as yet unknown endogenous mechanism. This corroborates the earlier results of *Forbes & al.* and thus gives a new aspect on the dietary measures against dental caries. With regard to the local attack through the formation of acids it is of importance to restrict the *frequency* and *duration* of the carbohydrate in-

take while the quantities seem to play a minor part. The importance of the salivary protection makes it desirable also to emphasize to some extent the value of a limitation of the *quantity* of ingested carbohydrate with a simultaneous increase of the dietary proteins and vegetables.

The mechanism of the diurnal variation and dietary influences on the salivary buffer capacity is far from clear. In addition to the nervous regulation there are indications of metabolic influences with simultaneous effects on the renal and gastric secretions. These problems should be the object of further studies.

SUMMARY

Previous investigations into the buffering action of the saliva are reviewed with special regard to the relationship to dental caries.

A convenient method for the clinical estimation of the salivary buffering is demonstrated.

The diurnal variation of the buffering is studied with special regard to the influence of meals. The most stable periods, suitable for taking clinical samples, are found to be 1—3 hours after breakfast or lunch. The mechanism underlying the diurnal variations is discussed in the light of some data regarding variations in other secretions and in the blood CO_2 content.

The influence of dietary proteins and carbohydrates and of vegetables is studied under normal conditions of living. Proteins and vegetables are found to increase, carbohydrates to decrease the buffering action of saliva.

Acidosis or alkalosis, as produced by moderate peroral doses of ammonium chloride and sodium bicarbonate, respectively, are not found to influence the buffering action appreciably.

The ingestion of bone salts is found to increase the calcium plus magnesium content of the saliva, but has no demonstrable influence on the buffer value or rate of flow.

The ingestion of sodium fluoride, providing single doses of 5 mg fluorine, shows a tendency towards higher values for secretion rate and buffer capacity, especially when measured one hour after the ingestion.

The significance of the results for clinical caries analysis and for dietary caries prevention is discussed.

RESUME

RECHERCHES CLINIQUES SUR L'ACTION TAMPON DE LA SALIVE

Un résumé est donné des recherches antérieures sur l'action tampon de la salive, étudiée spécialement en ce qui concerne ses rapports avec la carie dentaire.

Une méthode simplifiée de détermination de l'action tampon de la salive est décrite.

La variation diurne de l'action tampon est étudiée en ce qui concerne l'influence des repas. Les périodes les plus stables, donc indiquées pour la prise de prélèvements cliniques, sont de 1 à 3 heures après le petit déjeuner ou le déjeuner. Le mécanisme des variations diurnes est discuté à la lumière de quelque données concernant les variations d'autres sécrétions et du contenu de CO_2 du sang.

L'influence des protéines et des hydrates de carbone alimentaires et des végétaux est étudiée dans des conditions de vie normales. Il est trouvé que les protéines et les végétaux augmentent l'action tampon de la salive, les hydrates de carbone la diminuent.

Ni acidose ni alkalose, produites par des doses modérées l'une de chlorure d'ammonium l'autre de bicarbonate de sodium, n'ont d'influence démontrable sur l'action tampon de la salive.

L'ingestion de sels d'os augmente la teneur de calcium plus magnesium de la salive mais n'exerce aucune influence démontrable sur l'action tampon ni sur le flux de la salive.

L'ingestion d'une dose de 5 mg de fluor, sous forme de fluorure de sodium, apporte une tendance à une accélération de la sécrétion et à une augmentation de la capacité tampon, en particulier une heure après l'ingestion.

Les résultats de l'étude sont discutés en vue de l'analyse clinique de la carie et de sa prévention diététique.

ZUSAMMENFASSUNG

KLINISCHE UNTERSUCHUNG ÜBER DIE PUFFERWIRKUNG DES SPEICHELS

Eine Übersicht früherer Untersuchungen über die Pufferwirkung des Speichels wird gegeben, wobei das Verhältnis zur Karies besonders berücksichtigt wird.

Eine vereinfachte Methode zur Bestimmung der Speichelpufferung wird beschrieben.

Die Tagesschwankung der Pufferung wurde studiert, wobei der Einfluss von Mahlzeiten besonders berücksichtigt wurde. Die stabilsten und für die klinische Probeentnahme geeignetsten Perioden sind 1—3 Stunden nach dem Frühstück oder Mittagessen. Der Mechanismus der Tagesschwankung wird an Hand einiger Daten über Schwankungen anderer Sekrete und des CO_2-Inhaltes des Blutes diskutiert.

Der Einfluss der Vegetabilien, Proteine und Kohlehydrate der Nahrung wurde unter normalen Lebensbedingungen studiert. Es wurde gefunden, dass die Speichelpufferung durch Proteine und Vegetabilien erhöht, durch Kohlehydrate erniedrigt wird.

Acidose und Alkalose, durch mässige Dosen von Ammoniumchlorid und Natriumbikarbonat hervorgerufen, üben keinen deutlichen Einfluss auf die Pufferung aus.

Die Zufuhr von Knochensalzen erhöht den Inhalt von Kalzium plus Magnesium des Speichels, hat aber keinen nachweisbaren Einfluss auf die Pufferung oder die Speichelflut.

Die Zufuhr von 5 mg Fluor als Einzeldose von Natriumfluorid zeigt eine Tendenz zu erhöhten Werten der Speichelabsonderung und Pufferkapazität, besonders bei Bestimmung eine Stunde nach der Zufuhr.

Die Bedeutung der Ergebnisse für die klinische Kariesanalyse und die Kariesverhütung durch die Nahrung wird besprochen.

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